

Dynamics of water-soluble carbon substances and microbial populations during the composting of de-inking paper sludge

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Abstract

Composting is an alternative method to dispose of de-inking paper sludge (DPS). Today, few studies have investigated the water-soluble carbon (WSC) substances as indicators of the decomposition process and the microbial changes taking place during the composting of DPS. Accordingly, the goal is to study their dynamics during the composting of DPS at three nitrogen levels, 0.6%, 0.7% or 0.9% total N, using mechanical turning. The changes in WSC substances, microbial biomass carbon (MBC) and, total and DPS microbial populations were monitored during 24 weeks. Also, microorganisms were identified and tested for the production of selected enzymes. Regardless of N treatments, the dynamic of WSC substances indicated that cellulose and hemicellulose fractions of DPS fibers were mainly biodegraded during the first 8 weeks while the more resistant carbon (C) fractions were biodegraded thereafter. MBC also evolved regardless of N treatments but was correlated to WSC substances. Its high values decline mostly after 12 weeks indicating the exhaustion of this source of C energy for microbial growth and the stabilisation of DPS organic matter. The dynamic and identified microorganisms were comparable to those observed in other composting processes. However, the results pointed out that those mostly implicated in the hydrolysis of DPS fibers were the thermophilic actinomycetes and fungi and, by comparison to the 0.6% or 0.7% N treatment, they decreased in presence of the 0.9% N treatment. Most microorganisms were hemicellulolytic bacteria, while actinomycetes and fungi were capable of degrading a wide variety of substrates. Overall, dynamics of WSC substances and microbial populations indicated that during composting, DPS decomposition obey a two phase decay while, contrary to the lowest N treatment, the 0.9% N treatment has slowed down this process by harming the important microbial populations implicated in the degradation of DPS fibers.

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1. Introduction

In Quebec, papermills have been recycling used papers since the early nineties. This industrial process generates a residue called de-inking paper sludge (DPS). It consists mainly of wood fibers too short and weak to be reused in the papermaking process (Bellamy et al., 1995). DPS is similar to sludge derived from virgin paper production, i.e., primary paper sludge, in its high carbon (C) and poor nitrogen (N) content, but differs in

its content in residual inks, clay fillers, coatings and other chemicals such as detergents or surfactants used to remove inks, etc. (Beauchamp et al., 2002; Bellamy et al., 1995). Today, instead of burning or landfilling papermills sludge, new avenues such as composting are actively being developed in order to dispose of these residues in a more natural preservation manner. Composting is defined as the microbial degradation of organic solid material that involves aerobic respiration and passes through a thermophilic stage (Mustin, 1987). By this process, many advantages are reached such as sanitation, mass and bulk reduction, decrease of carbon to nitrogen ratio (C:N) in addition to upgrading the organic waste into a useful end-product called compost.

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Several studies have mentioned the importance of early stage control over different physico-chemical parameters such as temperature, moisture, pH, aeration, particle size and nutrient balance (Crawford, 1983; Golueke, 1992; Mustin, 1987). For DPS, the nutrient balance is the main factor that hinders its composting since this residue has a wide C:N ratio that can vary between 100:1 and 300:1 across papermills and years of production. A concomitant study (Charest and Beauchamp, 2002), in which an industrial-like scale was used to study the composting of DPS with poultry manure showed that 0.6% N treatment was the best to enhance DPS composting compared to 0.7% and 0.9% N treatments. In the 0.6% N treatment, nitrogen losses were lower while total weight, cellulose and hemicellulose losses were higher giving the lowest C:N ratio after 24 weeks of composting. Temperature–time profiles were similar in showing efficiency of the composting process regardless of the N treatments (Charest and Beauchamp, 2002). Initially, the temperature of compost piles was about 30 °C, rose to 40 °C within 3 days and then reached 60 °C within 2 weeks. Thereafter, the temperature declined slowly to about 50 °C and remained there for at least four months before dropping to ambient temperature.

The present study is more specific in its investigation of the available carbon substances and microbial changes taking place during DPS composting. The microorganisms are the principal actors of composting during which fresh organic matter is converted into rich humus compost. Many studies have reported on the dynamics of total populations of microorganisms during the composting of materials having C:N ratios above 50, such as straw (Chang and Hudson, 1967; McDonald et al., 1998; Waksman and Cordon, 1939) or wood bark (Chamuris et al., 2000; Davis et al., 1992; Kurtböke et al., 1993). However, to our knowledge, only one study reports on these biological changes during primary paper sludge composting (Atkinson et al., 1997). During this composting process, which was realized in bench-scale aerobic reactors, the level of microorganisms detected increased fourfold and microorganisms with the potential to degrade protein, starch and cellulose were present. Currently, knowledge on the microbiological aspects of composting related specifically to DPS is lacking.

Studies on the decomposition of DPS amended to soils (Chantigny et al., 2000a,b; Fierro et al., 1999) reveal that DPS residues obey a two phase decay process: a rapid decrease in cellulose followed by a slow decrease in recalcitrant carbon such as lignin. Also, Chantigny et al. (2000b) showed the presence of microbial monosaccharides in hot water carbon extracts indicating that during decomposition, part of added DPS was resynthesized as new microbial carbohydrates released in soil. Under composting conditions, it is also known that

cellulose and hemicellulose fractions of DPS decrease, whereas its lignin fraction rises over time (Brouillette et al., 1996; Charest and Beauchamp, 2002). These degradation patterns are probably linked to microbial enzymes (Atkinson et al., 1997; Tiquia, 2002; Tiquia et al., 2002) altering DPS fibers as well as to the release of available carbon substances supporting microbial growth and multiplication. It is also possible that new carbohydrates of microbial origin are released since composting is also a synthesis process. However, actually, decomposition of DPS during composting by following changes in water-soluble carbon substances have never been studied.

Hence, the first objective of this study was to investigate the dynamics of water-soluble carbon substances, microbial biomass carbon, total and DPS populations of culturable microorganisms in DPS composting piles differing in their N treatments during 24 weeks. The second objective of this study is to identify the microflora implicated in DPS degradation and evaluate their capacity to produce selected enzymes.

2. Methods

2.1. Compost materials and microbial analyses of DPS

Raw DPS were obtained from the Daishowa Inc. papermill located in Quebec City (now Stadacona, QC, Canada). Used papers involved 80% newsprint and 20% magazines. The poultry manure and chicken broiler floor litter were obtained from a poultry farm close to St-Henri-de-Lévis (QC, Canada). Physical and chemical properties of raw DPS, poultry manure and chicken broiler floor litter and its compost have already been described (Charest and Beauchamp, 2002). Microbial populations present in raw DPS were quantified as described in Section 2.5.2, and they are presented in Table 1.

2.2. Composting processes

The mixtures in compost piles and their handling over time have already been described (Charest and Beauchamp, 2002). Briefly, the experiment was carried out on the industrial site of Les Composts du Québec, Inc. (St-Henri-de-Lévis, QC, Canada) for 24 weeks within the permit conditions of Quebec Environment Ministry. Industrial-like compost piles of 100 m³ were formed by mechanically mixing together 10–20% poultry manure, 10–20% chicken broiler floor litter and 80–60% raw DPS. On a dry matter basis, these mixtures gave total N concentrations equivalent to 0.6%, 0.7% and 0.7% respectively in compost piles nos. 1, 2 and 3 which corresponded, respectively, to initial C:N ratios of 64, 54 and 54. These C:N ratios were above the optimum 25–30 suggested for most types of wastes, but appeared

Table 1

Logarithm of the most probable number of thermophilic and mesophilic microorganisms in the raw de-inking paper sludge (DPS) grown on DPS and total standard agar (Total)^a media, and their ratio

Medium	Thermophilic			Mesophilic		
	Bacteria	Actinomycetes	Fungi	Bacteria	Actinomycetes	Fungi
Log MPN (g) ⁻¹ dw						
DPS	1.62 ± 0.02	0.38 ± 0.01	0.25 ± 0.01	1.74 ± 0.03	0.25 ± 0.01	0.25 ± 0.01
Total	1.79 ± 0.03	0.71 ± 0.03	0.28 ± 0.08	1.81 ± 0.01	0.66 ± 0.02	0.38 ± 0.01
Ratio (%)	91	54	89	96	38	66

^aTotal standard agar media were: 10% Tryptic Soy Agar (TSA) for bacteria, Actinomycetes Isolation Agar (AIA) for actinomycetes and Rose Bengal Agar (RB) for fungi (Difco, Détroit, Michigan, USA). Results are mean of quadruplates ± standard error of mean.

permissible based on the DPS nature, some exploratory results (Charest and Beauchamp, 2002) and field research (Fierro et al., 2000). In fact, exploratory results showed that C:N ratios of industrial-like compost piles of DPS mixed with poultry manure, decreased more importantly over time when initial N doses were above 0.5%. There were also some indications that the largest dose (1% N) meant an important volatilization of N. Therefore, to decrease N volatilization and improve DPS biodegradation, a treatment where N concentration increased over time, through urea additions, was included. Indeed, total N content of compost pile no. 3 was increased on average to 0.9% after receiving sequential urea (46% N) additions of 74 kg each at weeks 8 and 16 of composting.

2.3. Compost sampling

Samples within each N treatment were collected as previously described (Charest and Beauchamp, 2002). Briefly, compost piles were mechanically turned at weeks 0, 4, 8, 12, 16, 20 and 24 with sampling after each time. At each sampling time, four 500 g were collected, 90° apart, at depth level of roughly 30 cm from the top of the pile and were put in sterile plastic bags. Samples were kept at 4 °C and analyzed within 48 h.

2.4. Compost water-soluble carbon substances analyses

The water-soluble carbon (WSC) substances were extracted using the method of Chantigny et al. (1996). The extracted matter was considered to be representative of easily available organic carbon. The WSC was determined on 5 g of humid compost extracted with 40 ml of distilled water and shaken on a reciprocal shaker for 40 min before centrifugation (685g, 10 min). The supernatants were recovered and filtered through Whatman no. 42 filter papers. These procedures were performed at room temperature. The compost WSC extracts were quantified through dry combustion using an infrared C analyzer (Shimadzu Model TOC-5050, Shimadzu; Kyoto, Japan).

Seven selected neutral sugars (arabinose, fucose, galactose, glucose, mannose, rhamnose and xylose) were identified and quantified in WSC extracts according to Martens and Frankenberger (1991). Briefly, WSC extracts were purified through solid-phase extraction columns (Supelclean LC-SAX and LC-SCX, Supelco, Bellefonte, PA) and then analyzed by liquid anion-exchange chromatography with pulsed amperometric detection (Dionex DX-500, Dionex Corporation, Sunnyvale, CA) equipped with a CARBOPAC1 column (Dionex Corporation, Sunnyvale, CA). The eluant was NaOH 21 mM and the chromatography time was 18 min. The neutral sugars were quantified by using PeakNet Software (Dionex Corporation, Sunnyvale, CA) and arabinose, xylose and glucose values were corrected as filter papers contributed small amounts of these sugars.

2.5. Compost microbiological analyses

2.5.1. Microbial biomass carbon

Microbial biomass carbon (MBC) was determined by the fumigation-extraction method of Vance et al. (1987). Briefly, 5 g of humid compost were fumigated with ethanol-free chloroform (CHCl₃) for 24 h at air temperature in a desiccator. Organic carbon content was extracted from the fumigated and non-fumigated compost extracts after mixing them using a reciprocal shaker with 40 ml of 0.25 M K₂SO₄ (Angers et al., 1995) for 20 min. After centrifugation (685g, 10 min), the supernatants were passed through Whatman no. 42 filter papers. Organic carbon in compost extracts was quantified through dry combustion (Shimadzu Model TOC-5050, Shimadzu; Kyoto, Japan). In addition, the fumigated extracts were analyzed using gas chromatography in combination with mass spectrophotometry (GC-MS, UI Hewlett, Packard) in order to detect residual chloroform, which did not account for more than 4% of the carbon present in these extracts. A proportionally constant of 0.45 (Wu et al., 1990) was used to calculate MBC.

2.5.2. Estimation of culturable microorganisms

Culturable bacteria, actinomycetes and fungi were enumerated by the plate-dilution frequency method described by Harris and Sommers (1968). Briefly, for bacteria and fungi, 10 g of humid compost were placed in 90 ml of a sterile phosphate saline buffer (Wollum, 1982). For actinomycetes, 10 g of humid compost were first dried at air temperature during 18 h and at 65 °C for an additional 4 h (McCarthy and Williams, 1990), before being mixed in the phosphate buffer. Suspensions were shaken using a reciprocal shaker for 20 min before 10-fold serial dilutions were performed. For bacterial enumerations, dilutions of 10^{-1} – 10^{-8} were used compared to a range of 10^{-1} – 10^{-6} for fungi and actinomycetes. Eight drops of 10 μ l of each dilution were distributed on solid culture media.

Commercial standard agar media proposed by Wollum (1982) were used for counting total populations of microorganisms. These were: (1) 10% Tryptic Soy Agar medium (TSA, Difco, Detroit, Michigan, USA) supplemented with 50 μ g ml $^{-1}$ of cycloheximide and 20 μ g ml $^{-1}$ of benomyl for bacteria; (2) Actinomycetes Isolation Agar medium (AIA, Difco, Detroit, Michigan, USA) for actinomycetes and (3) Rose Bengal medium (RB, Difco, Detroit, Michigan, USA) supplemented with 12.5 μ g ml $^{-1}$ of chloramphenicol for fungi.

The modified cellulose agar medium proposed by Wollum (1982) was used for counting DPS populations of microorganisms. The modification involves replacing the cellulose content by DPS as the sole source of carbon. The DPS amended medium contained per liter of distilled water was as follows: 12 g of 2 mm air-dry ground DPS, 0.5 g of NaNO $_3$, 1 g of K $_2$ HPO $_4$, 0.5 g of MgSO $_4$ ·7H $_2$ O, 0.01 g of FeSO $_4$ ·7H $_2$ O and 15.0 g of agar. The pH was adjusted to 7.0 with 1 M HCl. The same concentrations of fungicides (20 μ g ml $^{-1}$ of benomyl, 50 μ g ml $^{-1}$ of cycloheximide) and antibiotic (12.5 μ g ml $^{-1}$ of chloramphenicol) were used in this medium for the selection of bacteria and fungi.

Plates were incubated at 50 and 27 °C for growth of thermophilic and mesophilic microorganisms, respectively. The 50 °C temperature was chosen since there is no strict division between thermophilic and mesophilic microorganisms, especially for actinomycetes (Greiner-Mai et al., 1987). Based on the total number of drops showing growth on plates for each dilution and on the statistical table of Harris and Sommers (1968), the most probable number (MPN) of total bacteria and fungi was determined after 7 days of incubation while MPN of total actinomycetes and of DPS populations of microorganisms were determined after 21 days of incubation. Results of microbial estimation were expressed on a log $_{10}$ basis per gram of dry weight (MPN g $^{-1}$ dw).

2.5.3. Identification of culturable microorganisms isolated from DPS composts

Bacteria, actinomycetes and fungi, with different colony morphology growing on DPS media at 50 or 27 °C were isolated regardless of compost N treatments and of time of composting, since these manipulations were time consuming. The colonies were purified before identification. Bacteria and actinomycetes were identified through measures of fatty acid methyl-esters using the MIDI Microbial Identification System (MIS) libraries (Bacterial Strain Identification and Mutant Analysis Service, Department of Plant Pathology, Auburn University, Auburn, Alabama, USA). Unknown microorganisms which matched a bacterial profile with no second choice and with a similarity index (SI) \geq 0.2 were identified up to the species level, and up to the subspecies level, whenever SI \geq 0.5. Those which matched a bacterial profile with a SI \leq 0.2 were considered to be similar to the genus level of microorganisms listed in the database or had no match at all. Thermophilic fungi were identified by Agriculture and Agri-Food Canada (National Identification Service, Centre for Land and Biological Resources Research, Central Experimental Farm, Ottawa, Ontario, Canada). Mesophilic fungi were identified by Dr C. Richard (Centre de recherche et de développement sur les sols et les grandes cultures, Ste-Foy, Québec, Canada).

2.5.4. Enzymatic profiles of microorganisms isolated from DPS composts

The enzymes (amylase, protease, lipase, pectate lyase, polygalacturonase and chitinase) produced by microorganisms isolated from DPS composts were detected on specific agar media as described by Hankin and Anagnostakis (1975). The xylanase enzymes were detected on Williams and Withers (1983) basal medium supplemented with 2 mg l $^{-1}$ of birch xylan (Sigma, Ontario, Canada). Hydrolysed zones were visualized by flooding this medium with Gram's iodine solution. The endoglucanase enzymes were detected on Teather and Wood (1982) culture medium supplemented with 0.1% CMC (carboxymethylcellulose, Sigma, Ontario, Canada). The hydrolysed zones were visualized by flooding CMC medium with congo red. For bacteria and actinomycetes, the pH of each culture medium was adjusted to 7.0 while for fungi the pH was adjusted to 6.0. The culture media were dot inoculated with needle for bacteria and actinomycetes grown previously on TSA and AIA culture media, whereas mycelial plugs coming from the edge of fungal colonies grown on Potato Dextrose Agar (PDA, Difco, Detroit, Michigan, USA) were used as inoculum. In general, microbial hydrolyzed zones were measured after their appearing, i.e., for plates incubated at 50 °C, they were measured after 3 days, and for plates incubated at 27 °C, they were measured after 2 days for bacteria and actinomycetes, and after 3 days for fungi.

The results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolyzed zones by the estimated diameter of the colony growth (cm/cm). Here, estimated diameter means the average measurements on two perpendicular axes.

2.6. Statistical analyses

As described in Charest and Beauchamp (2002), the experiment was set up as a nested design (Stilwell, 1993). Briefly, four replicates were nested within N treatments and seven measurements over time were carried out. The repeated measures model of Milliken and Johnson (1992) was used to determine the effect of N treatments on changes of water-soluble carbon and microbial parameters over time. When treatment means were significantly different, contrast analyses were performed for the linear and non-linear time responses of measured compost parameters to N treatment. The relationships between the different composting parameters were measured using Pearson's correlation coefficients. Correlation coefficients followed by one, two or three asterisks are significant at the 5%, 1% or 0.1% level, respectively. All these analyses were carried out using the Statistical Analysis System procedure (SAS, 1988).

3. Results

3.1. Compost water-soluble carbon substances characteristics

The WSC substances content of compost piles increased over the first four weeks and significantly decreased thereafter ($P < 0.001$) irrespectively of the N treatments (Fig. 1(a)). The WSC substances were also negatively correlated with time ($r = -0.59^{***}$, 0.6% N treatment; $r = -0.87^{***}$, 0.7% N treatment; and $r = -0.66^{***}$, 0.9% N treatment). Among the seven neutral sugars measured, glucose and xylose were the most present in compost extracts. Their content in DPS compost piles generally increased over the first eight weeks and significantly decreased thereafter ($P < 0.001$), regardless of N treatments (Fig. 1(b) and (c)). Glucose and xylose were also negatively correlated with time (glucose: $r = -0.40^*$, 0.6% N treatment; $r = -0.40^*$, 0.7% N treatment; and $r = -0.44^*$, 0.9% N treatment; xylose: $r = -0.33$, 0.6% N treatment; $r = -0.48^{**}$, 0.7% N treatment; and $r = -0.38^*$, 0.9% N treatment).

3.2. Compost microbiological analyses

3.2.1. Microbial biomass carbon

The changes in the MBC of compost piles fluctuated from 5000 to 12000 $\mu\text{g g}^{-1}$ for the first 12 weeks of composting before stabilizing between 300 and 3000

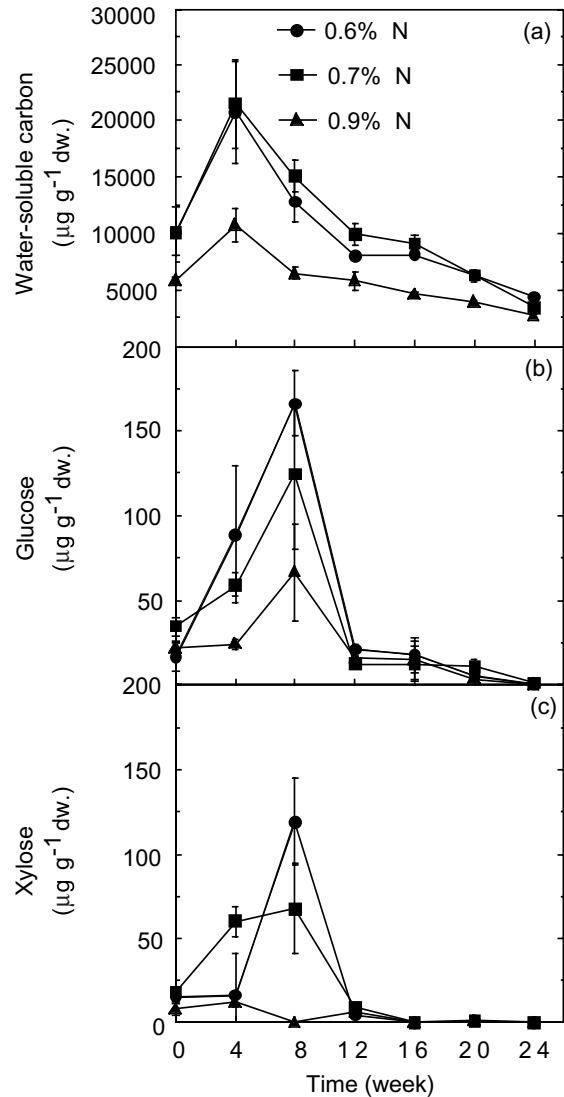


Fig. 1. Changes in water-soluble carbon (a), glucose (b) and xylose (c) content in composting piles of DPS differing in their N treatments: 0.6% total N, 0.7% total N and 0.9% total N treatments. Error bars show standard errors of the means ($n = 4$).

$\mu\text{g g}^{-1}$, regardless of N treatments (Fig. 2). Also, the MBC of compost piles was not correlated to the microbial estimations, but rather positively to WSC substances (WSC: $r = 0.62^{***}$, 0.6% N treatment; $r = 0.69^{**}$, 0.7% N treatment; and $r = 0.52^{**}$, 0.9% N treatment, glucose: $r = 0.63^{***}$, 0.6% N treatment; $r = 0.20$, 0.7% N treatment; and $r = 0.48^{**}$, 0.9% N treatment and xylose: $r = 0.63^{***}$, 0.6% N treatment; $r = 0.51^{**}$, 0.7% N treatment; and $r = 0.42^*$, 0.9% N treatment).

3.2.2. Estimation of culturable microorganisms

Total thermophilic bacteria were present in high numbers throughout the 24 weeks of the process, and regardless of N treatments (Fig. 3a). In contrast, DPS thermophilic bacteria decreased significantly over time,

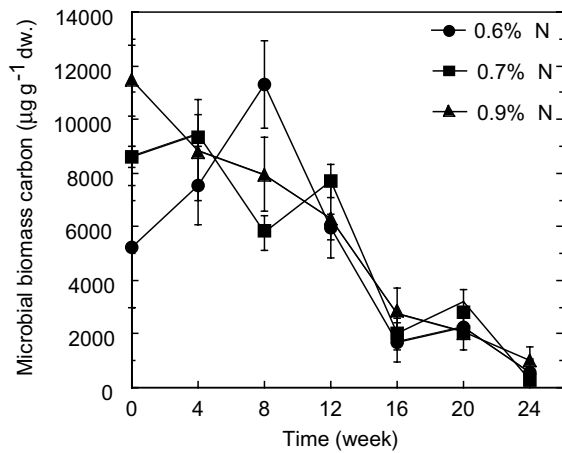


Fig. 2. Changes in microbial biomass carbon content in composting piles of DPS differing in their N treatments: 0.6% total N, 0.7% total N and 0.9% total N treatments. Error bars show standard errors of the means ($n = 4$).

regardless of the N treatments ($P < 0.01$; Fig. 3b). Also, DPS thermophilic bacteria were negatively correlated with composting temperature ($r = -0.81^{***}$, 0.6% N

treatment; $r = -0.68^{***}$, 0.7% N treatment and, $r = -0.59^{**}$, 0.9% N treatment).

Total and DPS mesophilic bacteria were also present in high numbers throughout the 24 weeks of the process (Fig. 3(c) and (d)). However, for the 0.9% N treatment, these bacterial populations were significantly larger at weeks 8 and 16, and smaller at weeks 12 and 20 compared to bacterial populations of 0.6% and 0.7% N treatments ($P < 0.01$). The evolution of these bacterial populations did not follow a linear relationship over time.

Total and DPS thermophilic actinomycetes increased sharply during the first four weeks of composting before decreasing slightly afterwards, regardless of N treatments (Fig. 4(a) and (b)). However, DPS thermophilic actinomycetes were significantly higher in the 0.9% N treatment compared to the 0.6% N treatment ($P < 0.05$; Fig. 4b). Again, the evolution of these populations did not follow a linear relationship over time, a similar situation than for the DPS mesophilic bacteria. In addition, total thermophilic actinomycetes were positively correlated with temperature ($r = 0.69^{***}$, 0.6% N treatment; $r = 0.73^{***}$, 0.7% N treatment; and, $r = 0.83^{***}$, 0.9% N treatment), as well as for DPS thermophilic

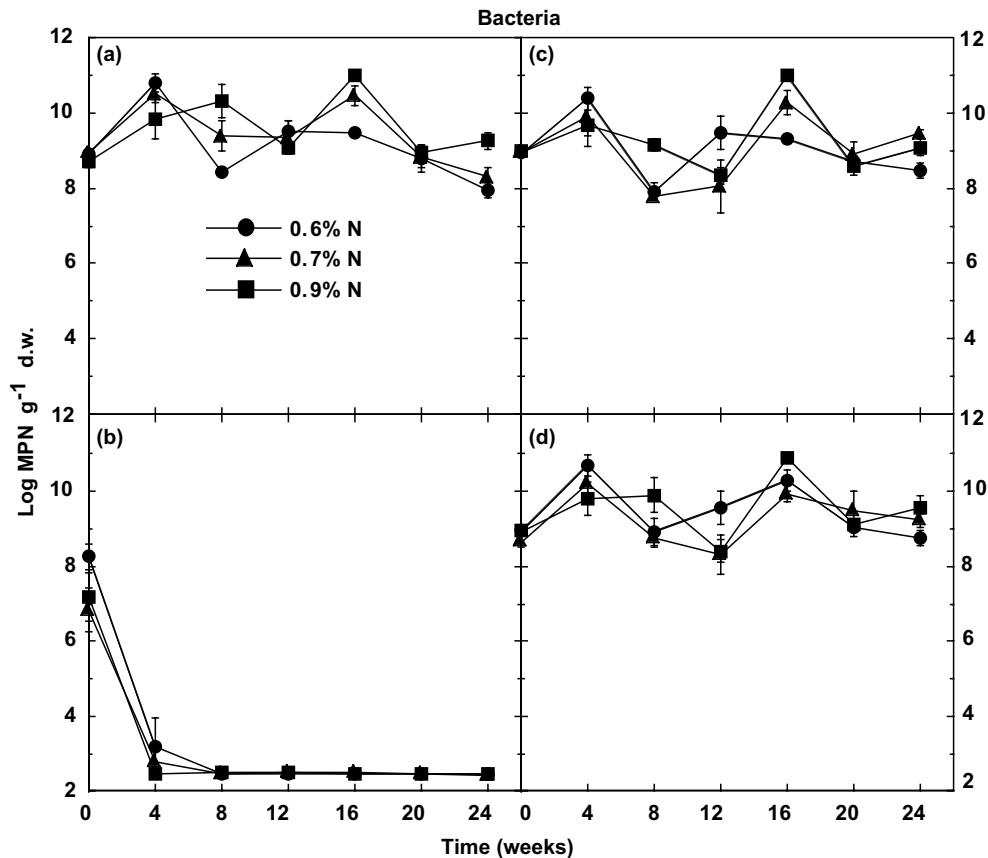


Fig. 3. Changes in total and DPS populations of thermophilic and mesophilic bacteria in composting piles of DPS differing in their N treatments: 0.6% total N, 0.7% total N and 0.9% total N. Standard errors of means ($n = 4$) may be hidden by symbols: (a) total thermophilic; (b) DPS thermophilic; (c) total mesophilic; (d) DPS mesophilic.

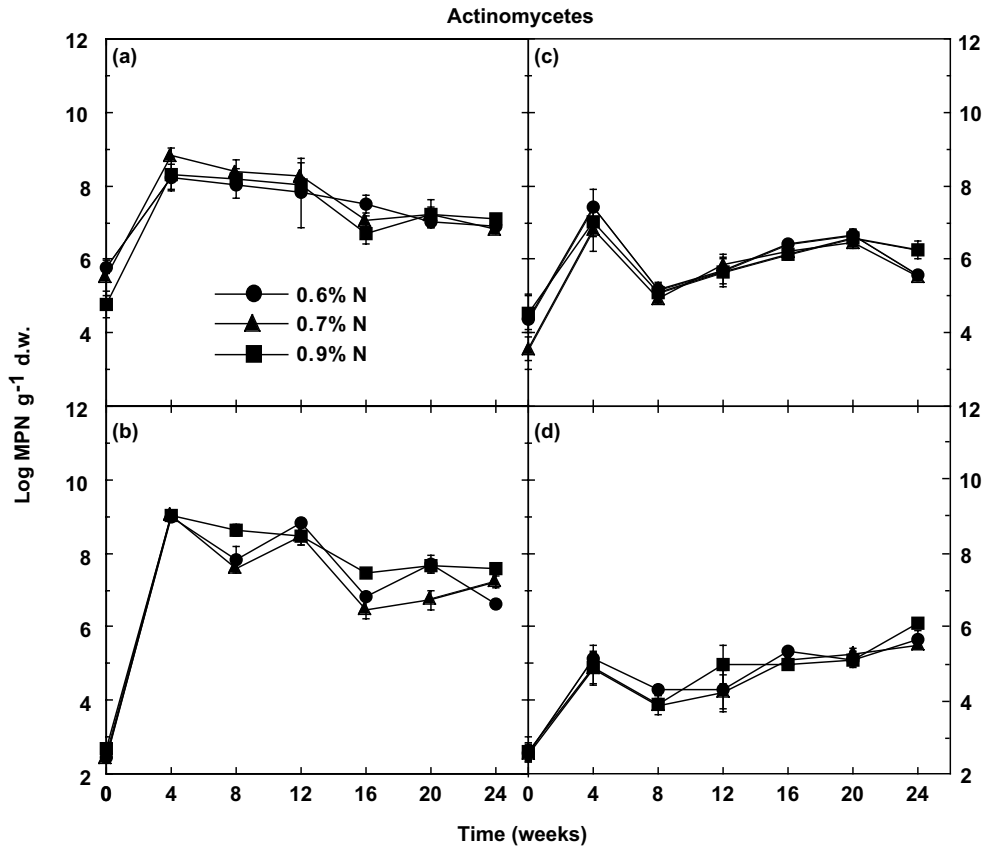


Fig. 4. Changes in total and DPS populations of thermophilic and mesophilic actinomycetes in composting piles of DPS differing in their N treatments: 0.6% total N, 0.7% total N and 0.9% total N. Standard errors of means ($n = 4$) may be hidden by symbols: (a) total thermophilic; (b) DPS thermophilic; (c) total mesophilic; (d) DPS mesophilic.

actinomycetes ($r = 0.88^{***}$, 0.6% N treatment; $r = 0.81^{***}$, 0.7% N treatment; and, $r = 0.76^{***}$, 0.9% N treatment).

Total and DPS mesophilic actinomycetes increased slowly over time, regardless of N treatments (Fig. 4(c) and (d)). Also, total mesophilic actinomycetes increased over time ($r = 0.23$, 0.6% N treatment; $r = 0.38^*$, 0.7% N treatment; and, $r = 0.41^*$, 0.9% N treatment). The same applies to DPS mesophilic actinomycetes ($r = 0.67^{***}$, 0.6% N treatment; $r = 0.78^{***}$, 0.7% N treatment; and, $r = 0.72^{***}$, 0.9% N treatment).

Total and DPS thermophilic fungi remained relatively stable throughout the DPS composting process, regardless of the N treatments (Fig. 5a and b). However, the population of DPS thermophilic fungi under the 0.9% N treatment was significantly higher than the population from the 0.6% N treatment ($P < 0.01$; Fig. 5b). Again, for the 0.9% N treatment, this populations was larger at weeks 8 and 16, and smaller at weeks 12 and 20 compared to the population of 0.6% N treatment, exhibiting a non-linear evolution over time.

The populations of total and DPS mesophilic fungi were minimal during the first 12 weeks before increasing until the end of the composting process, regardless of N

treatments (Fig. 5(c) and (d)). Also, these fungal populations increased over time ($r = 0.43^*$, 0.6% N treatment; $r = 0.24$, 0.7% N treatment; and, $r = 0.64^{***}$, 0.9% N treatment), as well as for DPS mesophilic fungi ($r = 0.72^{***}$, 0.6% N treatment; $r = 0.76^{***}$, 0.7% N treatment; and, $r = 0.67^{***}$, 0.9% N treatment).

3.3. Identification of culturable microorganisms isolated from DPS composts and their enzymatic profile

No thermophilic bacteria were isolated. However, 18 mesophilic bacteria with different morphologies were selected from the DPS medium and identified (Table 2). Their enzymatic profile indicated that most were able to degrade either starch (amylase), protein (protease) or lipids (lipase), while among the more complex substrates tested, most degraded hemicellulose (xylanase), a few degraded cellulose (endoglucanase), two degraded chitin (chitinase) and one degraded pectin (pectate lyase and polygalacturonase; Table 2).

Four thermophilic and 16 mesophilic actinomycetes were identified as being different species or subspecies (Tables 3 and 4), and five isolates had no match. Their enzymatic production profile show that most possess the

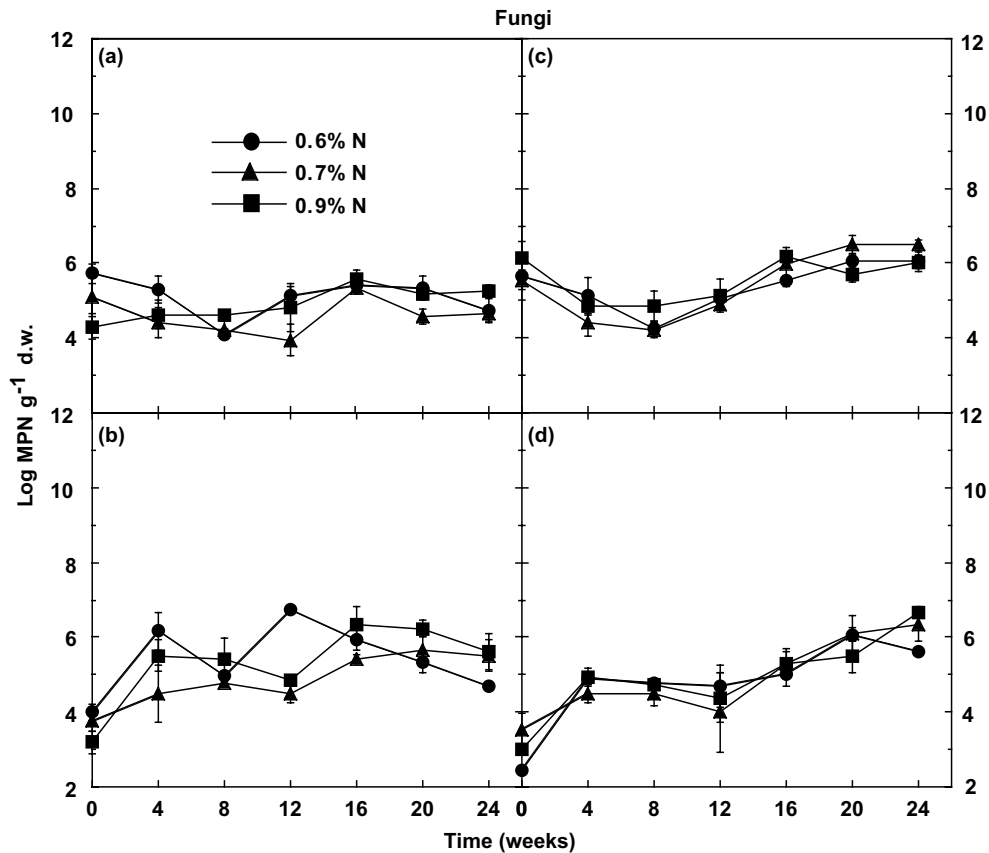


Fig. 5. Changes in total and DPS populations of thermophilic and mesophilic fungi in composting piles of DPS differing in their N treatments: 0.6% total N, 0.7% total N and 0.9% total N. Error bars show standard errors of the means ($n = 4$): (a) total thermophilic; (b) DPS thermophilic; (c) total mesophilic; (d) DPS mesophilic.

enzymes to degrade either simple (starch, protein, lipid) or complex compounds, such as hemicellulose and cellulose. Pectin and chitin were degraded only by the genus *Streptomyces* (Tables 3 and 4).

Eleven thermophilic and 23 mesophilic fungi were identified at the species or subspecies level (Tables 5 and 6). Their enzymatic production profiles showed that the majority had several enzymes required to decompose either the simple or the complex C compounds tested (Tables 5 and 6). However, most of these fungi were rarely chitinolytic.

4. Discussion

4.1. Compost water-soluble carbon substances

This study shows that for DPS composting and regardless of N treatments, the dynamic of WSC substances indicated that DPS decomposition obeys a two phase decay process during composting, a situation similar to the one observed under soil conditions (Chantigny et al., 2000a,b; Fierro et al., 1999). Peaks in WSC substances, mainly glucose and xylose, the chief

constituents of cellulose and hemicellulose (Cheshire, 1979), indicated that these WSC substances were bio-degraded during the first 8 weeks of composting. In fact, peaks in WSC substances coincided with the severe fiber losses of cellulose and hemicellulose measured (Charest and Beauchamp, 2002), even though a proportion of new glucose or other WSC substances may have originated from microorganisms since composting is also a synthesis process. Thereafter, the drop in WSC substances indicated that the microorganisms used these sources of C energy not only to grow and multiply, but also to degrade and alter the most resistant carbon fractions of DPS fibers. Contrary to hemicellulose and cellulose fractions, lignin increased during the composting process (Charest and Beauchamp, 2002), and thus probably forms the core of DPS humic substances.

4.2. Compost microbiological analyses

4.2.1. Microbial biomass carbon

The general trend in MBC of compost, i.e. high values followed by a decrease, was similar to the one observed during the composting of pig manure (Tiquia et al., 1996), or ligno-cellulosic wastes (Kostov et al., 1991;

Table 2
Enzymatic production profile of mesophilic bacteria isolated from the DPS medium at 27 °C^a

Species	Amylase	Protease	Lipase	Pectate lyase ^b	Polygalacturonase ^b	Xylanase (birch)	Endoglucanase ^b	Chitinase ^c
<i>Acinetobacter johnsonii</i>	–	3.3	2.3	–	–	–	–	–
<i>Agrobacterium radiobacter</i>	–	–	–	–	–	1.8	–	–
<i>Aureobacterium esteraromaticum</i>	2.4	2.0	–	–	–	6.6	–	–
<i>Bacillus brevis</i>	4.8	5.0	–	–	–	7.5	–	–
<i>Brevibacterium iodinum</i>	1.0	2.4	–	–	–	–	–	–
<i>Corynebacterium bovis</i>	1.8	–	1.0	–	–	6.5	4.5	–
<i>Cellulomonas flavigena</i>	1.2	–	1.0	–	–	3.0	1.0	–
<i>Cytophaga flevensis</i>	5.3	–	2.1	2.0	1.9	10.2	2.8	–
<i>Flavobacterium mizutaii</i>	1.2	1.0	1.0	–	–	2.1	–	–
<i>Hydrogenophaga pseudoftava</i>	–	–	–	–	–	–	–	–
<i>Klebsiella pneumoniae</i>	0.9	–	–	–	–	2.5	1.3	–
<i>Methylobacterium fujisawaense</i>	5.3	–	2.1	–	–	10.2	2.8	–
<i>Micrococcus luteus</i>	1.0	5.3	1.0	–	–	–	–	–
<i>Pseudomonas aeruginosa</i>	–	2.6	1.0	–	–	1.0	–	2.0
<i>P. stutzeri</i>	–	–	2.1	–	–	–	–	–
Most similar to <i>Xanthomonas campestris</i>	1.0	2.2	1.0	–	–	4.0	1.6	–
Most similar to <i>Bergeyella/Weeksella</i>	0.4	–	1.0	–	–	2.9	3.6	1.4
Most similar to <i>Sphingomonas/Rhizomonas</i>	1.0	1.8	4.0	–	–	2.7	1.6	–

(–) No hydrolyzed zones measured.

^a Media had a pH of 7.0 and did not contain any fungicides. The hydrolysed zones were measured in general after two days of incubation at 27 °C. Results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolysed zones by the estimated diameter of the colony growth (cm/cm). Here estimated diameter means height plus width divided by 2.

^b Hydrolysed zones measured after three days.

^c Hydrolysed zones measured after seven days.

Table 3
Enzymatic production profile of thermophilic actinomycetes isolated from the DPS medium at 50 °C^a

Species	Amylase	Protease	Lipase	Pectate lyase	Polygalacturonase	Xylanase (birch)	Endoglucanase	Chitinase ^b
<i>Actinomadura yumaensis</i>	1.5	2.7	2.1	–	–	1.7	–	–
<i>Actinomadura viridis</i>	1.3	2.2	–	–	–	1.9	–	–
<i>Amycolatopsis orientalis</i>	–	7.7	1.5	–	–	1.8	–	–
<i>Streptomyces violaceusniger</i> subsp. <i>Violaceus</i> (3 ^c)	2.8	2.1	3.1	–	–	3.2	2.0	1.0
No match	1.4	5.7	2.9	–	–	4.8	4.9	–
No match	–	2.5	–	3.3	–	2.5	4.3	2.5
No match	2.0	1.4	3.4	–	–	12.4	–	1.4

(–) No hydrolysed zones measured.

^a Media had a pH of 7.0 and did not contain any fungicides and hydrolysed zones were measured in general after 3 days of incubation at 50 °C. Results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolysed zones by the estimated diameter of the colony growth (cm/cm). Here estimated diameter means height plus width divided by 2.

^b Hydrolysed zones measured after 14 days.

^c Number of isolates identified as that name.

Table 4
Enzymatic production profile of mesophilic actinomycetes isolated from DPS medium at 27 °C^a

Species	Amylase	Protease	Lipase	Pectate lyase ^b	Polygalacturonase ^b	Xylanase (birch)	Endoglucanase ^b	Chitinase ^c
<i>Gordona amarae</i>	1.6	–	2.4	–	–	2.3	–	–
<i>G. bronchialis</i> (2 ^d)	1.4	–	2.9	–	–	4.0	–	–
<i>Nocardia asteroides</i>	1.2	–	1.0	–	–	1.3	–	–
<i>Oerskovia xanthineolytica</i>	3.0	4.8	2.5	–	–	5.1	–	–
<i>Rhodococcus globerulus</i>	1.1	–	2.3	–	–	3.0	–	–
<i>R. rhodochrous</i>	1.5	–	2.7	–	–	3.6	–	–
<i>Streptomyces californicus</i>	2.8	2.7	2.2	2.0	1.9	4.6	2.4	–
<i>S. cyaneus</i> subsp. <i>chartreusis</i> (2)	3.3	3.1	1.0	–	–	9.2	1.9	1.2
<i>S. halstedii</i> subsp. <i>scabies</i> (3)	3.8	3.4	4.3	4.0	2.4	7.3	3.6	1.5
<i>S. halstedii</i> subsp. <i>olivaceus</i> (2)	2.7	2.5	3.1	2.3	1.0	5.1	2.6	2.2
<i>S. lavendulae</i>	2.5	2.2	2.8	1.8	2.0	5.3	2.7	1.1
<i>S. rochei</i> subsp. <i>rochei</i>	2.8	3.1	2.0	–	–	5.5	1.1	1.1
<i>S. violaceusniger</i> subsp. <i>Violaceusniger</i> (8)	3.8	2.7	2.5	1.5	1.7	4.8	3.6	1.1
<i>S. violaceusniger</i> subsp. <i>hygroscopicus</i>	–	–	–	1.5	1.7	3.7	2.9	–
<i>Streptoverticillium reticulum</i>	2.9	2.4	2.5	–	–	4.8	3.0	1.1
<i>S. cinnamoneum</i> subsp. <i>cinnamoneum</i>	3.7	2.7	4.4	1.7	2.0	3.4	3.3	–
No match	2.0	1.9	3.6	–	–	4.7	4.6	–
No match	2.1	4.5	3.0	–	–	8.0	4.1	2.2

(–) No hydrolysed zones measured.

^a Media had a pH of 7.0 and did not contain any fungicides and hydrolysed zones were measured in general after two days of incubation at 27 °C. Results are expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolysed zones by the estimated diameter of the colony growth (cm/cm). Here estimated diameter means height plus width divided by 2.

^b Hydrolysed zones measured after three days.

^c Hydrolysed zones measured after 14 days.

^d Number of isolates identified.

Mondini et al., 2002), and indicated the presence of abundant degradable organic matter during at least the first 12 weeks of composting of DPS. Afterwards, the decline of MBC of DPS compost was probably related to exhaustion of these readily decomposable substances caused by the intense microbial activity and by stabilization of DPS organic matter into humic substances. In fact, MBC of DPS compost was correlated with WSC substances and so, it is not surprising that its decline

coincided with that of these substances. These results support the hypothesis that WSC substances favored microbial growth and multiplication during the DPS composting process. Finally, MBC of compost was not correlated with microbial populations and this may be due to the fact that counts achieved on culture media allowed only the recovery of culturable microorganisms (Atlas and Bartha, 1987; Kloepper and Beauchamp, 1992).

Table 5
Enzymatic production profile of thermophilic fungi isolated from the DPS medium at 50 °C^a

Species	Amylase	Protease	Lipase	Pectate lyase	Polygalacturonase	Xylanase (birch)	Endoglucanase	Chitinase ^b
<i>Aspergillus fumigatus</i> Fres.	1.2	1.4	1.4	1.2	1.2	1.2	1.2	1.0
<i>Aspergillus nidulans</i> (Eidam) Winter	1.0	1.7	1.0	1.1	1.0	1.2	1.4	–
<i>Chaetomium thermophile</i> La Touche	–	1.0	0.9	–	–	1.5	1.0 ^c	0
<i>Emericella nidulans</i> (Eidam) Winter ^c	–	–	–	–	–	–	–	–
<i>Humicola</i> sp.	1.0 ^d	1.0	1.2	–	–	4.9	1.0 ^e	–
<i>Humicola insolens</i> Cooney & Emerson	1.0 ^d	1.0	1.1	–	–	1.2	1.1 ^e	–
<i>Humicola grisea</i> Traaen var. <i>thermoidea</i>	1.4	1.3	0.7	–	–	1.7	1.9	–
<i>Paecilomyces</i> sp.	–	–	–	–	–	1.4	1.4	1.0
<i>Scytalidium thermophilum</i> ^f	–	–	–	–	–	–	–	–
<i>Talaromyces thermophilus</i> Stolk (3 ^f)	1	2	–	–	–	1.7	–	1
<i>Thermomyces lanuginosus</i> Tsiklinsky (2)	1	1	0.8	–	–	1.2	–	1.0

(–) No clearing zones measured.

^aMedia had a pH of 6.0 and did not contain any antibiotics. The hydrolysed zones were measured in general after three days of incubation at 50 °C. Results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolyzed zones by the estimated diameter of the colony growth (cm/cm). Here estimated diameter means height plus width divided by two.

^bHydrolysed zones measured after 14 days.

^cNo regrowth of the fungus avoiding its enzymatic production profile.

^dClearing zones measured after two days due to rapid growth of the fungus.

^eClearing zones measured after one day due to rapid growth of the fungus.

^fNumber of isolates identified as that name.

4.2.2. Culturable total and DPS populations

All bacterial populations thrived during all stages of the DPS composting process. This general trend was also observed in other composting systems (Miller, 1993). The populations of mesophilic bacteria were the highest compared to the populations of actinomycetes and fungi. The DPS thermophilic bacteria were rapidly inhibited after 4 weeks and never recovered thereafter. This inhibition was probably due to compost temperature since they were negatively correlated with it. Thus, this result clearly pointed out that thermophilic populations of bacteria are secondary decomposers of DPS fibers during the composting thermophilic phase.

In addition, these results indicated that there was ample C and N content for bacterial growth and multiplication at weeks 8 and 16 in the 0.9% N treatment compared to 0.6% and 0.7% N treatments. However, their decreases from weeks 8 to 12 and from weeks 16 to 20 support the possibility that a certain proportion of them were killed or negatively affected by urea additions. As we have shown in our concomitant study (Charest and Beauchamp, 2002) and by comparison to the two other N treatments, sequential urea additions in the 0.9% N treatment created peaks in N-NH_4^+ (>6000 $\mu\text{g g}^{-1}$ compared to a mean value of 2000 $\mu\text{g g}^{-1}$ for 0.6% and 0.7% N treatments) and in electrical conductivity (>6 dS m^{-1} compared to a mean value of 3 dS m^{-1} for 0.6% and 0.7% N treatments) at weeks 8 and 16, followed by sharp decreases in these parameters at weeks 12 and 20. Thus, the possibly excessive concentrations of free ammonia and soluble salts from urea additions may have slowed down the DPS composting process by harming hydrolysis of the fibers by these

mesophilic populations that are known (Siu, 1951) to contain the principal cellulolytic bacteria.

The populations of thermophilic actinomycetes increased sharply during the first 4 weeks of composting and decreased thereafter, where these populations were correlated with compost temperature. Thus, our results support the accepted knowledge that actinomycetes tend to develop in harsher conditions such as existing at peak temperatures where nutrient and competition levels are in general lower (Mustin, 1987). Again, the negative impact of urea additions on DPS mesophilic actinomycetes may have slowed down the DPS composting process by harming the biodegradation of its principal components. Nevertheless, the populations of thermophilic actinomycetes appeared to be important decomposers of DPS fibers during the thermophilic composting phase.

The populations of mesophilic actinomycetes gradually re-colonized DPS compost piles after the thermophilic composting phase, regardless of N treatments. This pattern is similar to other composting processes (Waksman and Cordon, 1939; Waksman et al., 1939a,b) and is probably related to compost temperature that declines especially after 16 weeks of composting (<40 °C). Thus, these populations of actinomycetes appeared to play a major role mostly during the cooling period of the DPS composting process.

The thermophilic populations of fungi remained relatively stable and survived throughout the DPS composting process that is in contrast with other composting studies (Chang, 1967; Thambirajah et al., 1995). This is probably due to the nature of the composting material and because the compost temperature never reached values higher than 60 °C (Charest and

Table 6
Enzymatic production profile of mesophilic fungi isolated from the DPS medium at 27 °C^a

Species	Amylase	Protease	Lipase	Pectate lyase	Polygalacturonase	Xylanase (birch)	Endoglucanase	Chitinase ^b
<i>Absidia corymbifera</i> ^c								
<i>Acremonium roseo-griseum</i>	2.1	2.5	2.3	–	–	2.4	2.2	1.1
<i>Arthrotrix</i> sp.	1.0	–	1.0	–	1.2	0.7	1.0	–
<i>Aspergillus niger</i> van Tieghem	1.0	–	1.8	1.0	1.0	1.1	1.2	–
<i>Aspergillus ustus</i> (Bain.) Thom & Church	1.4	1.8	1.0	1.2	1.4	1.6	1.7	–
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi (4 ^d)	2.6	2.7	3.1	1.7	1.4	2.5	2.9	0.5
<i>Aureobasidium</i> sp.	1.1	2.4	1.9	–	2.3	1.3	2.3	–
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	–	2.9	2.6	1.7	2.7	1.4	2.5	–
<i>Chaetomium globosum</i> Kunze ex Steud.	0.7	–	0.8	–	–	0.9	1.1	1.0
<i>Chrysosporium pannorum</i> (Link) Hughes	3.1	3.3	2.5	–	2.1	4.4	3.2	1.5
<i>Fusarium oxysporum</i> Schlecht.	–	–	1.0	1.1	1.1	1.0	1.1	–
<i>Humicola fuscoata</i> Traaen var. <i>fuscoatra</i> (2)	1.1	1.3	1.0	–	–	1.6	1.9	1.0
<i>Microascus trigonosporus</i> Emmons & Dodge	1.4	1.6	1.9	–	–	2.0	2.2	–
<i>Scopulariopsis fusca</i> Zach.	–	–	0.8	1.3	1.6	1.2	1.3	–
<i>Phoma pomorum</i> Thüm.	1.6	–	1.0	–	1.6	2.0	2.1	–
<i>Phoma</i> sp.	1.0	1.1	1.0	1.3	1.3	1.2	1.2	–
<i>Penicillium chrysogenum</i> Thom (2)	1.7	2.1	1.4	1.3	1.5	1.9	1.7	–
<i>Penicillium</i> sp.	1.2	–	1.3	1.3	1.7	1.6	1.5	–
<i>Penicillium</i> sp.	–	–	1.3	–	1.2	–	1.8	–
<i>Trichoderma hamatum</i>	1.1	1.1	0.9	1.1	–	1.0	1.0 ^e	–
<i>Trichoderma viride</i> Pers. ex Gray (2)	1.0	1.0	0.8	1.0	–	1.0	0.7 ^e	1.0
<i>Trichoderma polysporum</i> (Link ex Pers.)	0.7	0.7	0.6	–	–	1.0	0.6 ^e	–
<i>Verticillium nigrescens</i> Pethybridge	1.5	1.6	1.6	1.2	2.1	1.6	1.6	–

(–) No hydrolysed zones measured.

^a Media had a pH of 6.0 and did not contain any antibiotics. The hydrolysed zones were measured in general after three days of incubation at 27 °C. Results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolysed zones by the estimated diameter of the colony growth (cm/cm). Here estimated diameter means height plus width divided by 2.

^b Hydrolysed zones measured after 14 days.

^c No regrowth of the fungus avoiding its enzymatic production profile.

^d Number of isolates identified as that name.

^e Hydrolysed zones measured after 2 days due to rapid growth of the fungus.

Beauchamp, 2002), which is the upper temperature limit for growth of thermophilic fungi (Cooney and Emerson, 1964). Also, N treatments did not have a significant influence on the total populations of thermophilic fungi, but rather on the DPS populations of thermophilic fungi. Again, urea additions may have slowed down the DPS composting process by negatively affecting this population of microorganisms. Again, the populations of thermophilic fungi appeared to be important decomposers of DPS fibers during the thermophilic phase of the composting process.

The mesophilic populations of fungi evolved similarly to those of actinomycetes, i.e. they gradually re-colonized DPS compost regardless of N treatments, and were correlated with compost temperature. Thus, our results support the accepted knowledge that mesophilic populations increased after compost temperature decline (Mustin, 1987).

4.3. Identification and enzymatic production profile of culturable microorganisms isolated from DPS composts

The common genus *Bacillus* sp. and *Pseudomonas* sp. found in many composting residues (Miller, 1993), as well as two cellulolytic bacteria, *Cellulomonas flavigena* and *Cytophaga flevensis* were identified from DPS composts. *Acinetobacter johnsonii*, *Corynebacterium bovis*, *Cellulomonas* sp. and *Klebsiella pneumoniae* were isolated from compost made of papermills primary sludge (Atkinson et al., 1997). Vääänen and Niemelä (1983) reported that temperature above 55 °C reduced *K. pneumoniae* population. In our study, the temperature of compost reached 60 °C within 2 weeks (Charest and Beauchamp, 2002). Thus, *K. pneumoniae* was isolated from compost probably when temperature had not risen yet (week 0) or later on, from a cross-contamination from other piles present on this industrial site.

Among the identified mesophilic actinomycetes, some belong to the *Nocardiaceae* family (*Nocardia* sp., *Oerskovia* sp., *Rhodococcus* sp.), while most belong to the *Streptomyces* family (*Streptomyces* sp.). *Nocardia* sp., *Rhodococcus* sp. and *Streptomyces* sp. have also been isolated from peat (Croft et al., 2001) and from different composts (Miller, 1993). Three thermophilic actinomycetes belong to *Streptomyces*, *Maduromycetes* (*Actinomadura* sp.), or the *Pseudonocardiaceae* families (*Amycolatopsis* sp.). No species of genera frequently reported in self-heating materials such as *Thermomonospora* (Lacey, 1973; Waksman et al., 1939b), *Thermopolyspora* (Gregory et al., 1963; Lacey, 1973) or *Thermoactinomyces* (Gregory et al., 1963; Lacey, 1973; Waksman et al., 1939a) were isolated from DPS compost piles. The low number of isolates due to the selectivity of the DPS medium and the identification method used can explain these results. Identification of our actinomycetes isolates was in general successful but five of them had no match. Also, among our *Streptomyces* isolates, many were identified as belonging to the same species and subspecies. This can be due to a low diversity of isolates in compost or that the data banks are incomplete. More than 460 species of *Streptomyces* exist, but only 142 of them are actually known (Prescott, 1995), and only 20–30 different species can be identified through fatty acids data bank (John McInroy, personal communication). As mentioned by McCarthy and Williams (1990), actinomycetes identification to the genus or species level, and especially in large genera like *Streptomyces*, is very difficult without access to relatively specialized techniques. This also probably explains why *Streptomyces* isolates are frequently identified only to the genus level in many composting studies (Kurtböke et al., 1993; Lacey, 1973; Waksman et al., 1939b) that have used standard techniques and references. In future studies, the molecular method such as 16S rRNA gene sequences analysis should be used to confirm the species identification. Otherwise, actinomycetes isolated from DPS composts possess an enzymatic versatility that can allow them to decompose the resistant C fractions of DPS. It can explain why their population tends to increase during the maturation phase of DPS compost.

Most fungi belong to the *Deuteromycetes* family except *Absidia* sp. or *Emericella*, *Talaromyces* and *Chaetomium* sp. that belong, respectively, to *Phycomycetes* or *Ascomycetes* families. Among the *Deuteromycetes* family, the prevalent genera were *Aspergillus*, *Humicola*, *Penicillium* and *Trichoderma*. Many of these mesophilic fungi have also been isolated in other composts (Miller, 1993). In fact, *Trichoderma* isolates and several thermophilic fungi (*Humicola insolens*, *H. grisea*, *Scytalidium thermophilum* and *Chaetomium thermophile*) are very good cellulose decomposers (Chang and Hudson, 1967; Cooney and Emerson, 1964; Miller, 1993). As shown by

Waksman and Cordon (1939), *H. insolens* is capable of degrading greater quantities of hemicellulose and cellulose compared to a mixed population of thermophilic microorganisms.

Overall, the enzymatic activities suggest that isolated bacteria grew mostly at the expense of the hemicelluloses or the water-soluble products coming from the extracellular breakdown of DPS cellulosic fractions. In contrast, for the actinomycetes and fungi, their high enzymatic potential probably allowed them to decompose the resistant C fractions of DPS with efficiency.

5. Conclusions

Regardless of N treatments, the dynamic of WSC substances during composting indicated that DPS decomposed principally in two phase: a first phase where cellulose and hemicellulose fractions were intensively biodegraded during the first 8 weeks and a second phase where the more resistant carbon fractions such as lignin were biodegraded. To our knowledge, this is the first report to show the dynamic of sugar units under composting conditions as a useful indicator on the biodegradation of woody residues such as DPS.

Regardless of N treatments, the dynamics of MBC during composting of DPS indicated that microorganisms were abundant and could easily grow and multiply during the first 12 weeks since organic matter was also abundant and easily available. However, afterwards, the decline of MBC indicated that DPS organic matter stabilized into humic substances and could no more support microbial growth and activities. MBC was correlated with WSC substances and thus, these substances were mainly recognized as carbon and energy sources to support microbial growth and multiplication. It would be also very interesting to develop a maturity index based on WSC substances, MBC or C values under composting conditions.

The dynamic of microbial populations during the composting of DPS was in general comparable to those observed in other composting processes. Also, populations of bacteria were the highest followed by those of actinomycetes and fungi except for DPS populations of thermophilic bacteria. These bacterial populations were inhibited after 4 weeks due probably to the thermophilic temperature and thus never recovered. Therefore, results clearly showed that thermophilic populations of actinomycetes and fungi were those that were mostly active in the degradation of DPS fibers. Also, the results pointed out that, under the 0.9% N treatment, some key microbial populations (e.g. total and DPS mesophilic bacteria, DPS thermophilic actinomycetes and fungi), decreased after urea additions compared to the 0.6% or 0.7% N treatments. Thus, the 0.6% N treatment appears

to be the best to enhance DPS composting process by favoring the most microbial degradation of its fibers, microbial N immobilization and its recycling later on.

Microorganisms isolated from DPS composts were similar to those reported previously in the literature. Among bacteria and actinomycetes, most were *Pseudomonas* and *Streptomyces* while among fungi, many *Penicillium*, *Aspergillus*, *Humicola* and *Trichoderma* were identified. According to their enzymatic profiles, bacteria were mostly hemicellulolytic while actinomycetes and fungi were capable of degrading a wide variety of substrates and especially hemicellulose and cellulose.

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