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Characterisation of source-separated household waste intended for composting

Cecilia Sundberg [a,*], Ingrid H. Franke-Whittle [b], Sari Kauppi [c], Dan Yu [c], Martin Romantschuk [c], Heribert Insam [b], Håkan Jönsson [a]

[a] Department of Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, SE-750 07 Uppsala, Sweden
[b] University of Innsbruck, Institute of Microbiology, Technikerstr. 25, A-6020 Innsbruck, Austria
[c] University of Helsinki, Department of Environmental Sciences, Niemenkatu 73, 15140 Lahti, Finland

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ABSTRACT

Large-scale composting of source-separated household waste has expanded in recent years in the Nordic countries. One problem can be low pH at the start of the process. Incoming biowaste at four composting plants was characterised chemically, physically and microbiologically. The pH of food waste ranged from 4.7 to 6.1 and organic acid concentration from 24 to 81 mmol kg^{-1}. The bacterial diversity in the waste samples was high, with all samples dominated by Gammaproteobacteria, particularly *Pseudomonas* and Enterobacteria (*Escherichia coli, Klebsiella, Enterobacter*). Lactic acid bacteria were also numerically important and are known to negatively affect the composting process because the lactic acid they produce lowers the pH, inhibiting other bacteria. The bacterial groups needed for efficient composting, i.e. *Bacillales* and Actinobacteria, were present in appreciable amounts. The results indicated that start-up problems in the composting process can be prevented by recycling bulk material and compost.

1. Introduction

Large-scale composting of source-separated household waste has expanded rapidly in recent years in the Nordic countries and this expansion is expected to continue, although at a slower rate. One important factor behind the slowdown in the expansion is the fact that many plants receive numerous complaints from the public about disturbing odours. This has been attributed to low pH of the incoming waste (Sundberg and Jönsson, 2008). The pH can be so low that it severely inhibits the composting process, and due to limited bacterial activity, the pH remains low for a long time (Sundberg and Jönsson, 2008; Kurola et al., 2010; Partanen et al., 2010). Extended duration of the early stages of composting is problematic, both because a lot of odour is generated during these stages and because the capacity of the composting plant is severely reduced by the limited activity when the pH is low. In order to optimise the process in both existing and new plants, knowledge on the characteristics of the incoming biowaste is important.

The climate and the lifestyle are relatively similar in all the Nordic countries and this leads to similarities in the source-separated household biowaste collected. Throughout the year, it consists mainly of food waste, and for a large part of the year there is no fresh garden waste in the biowaste. Furthermore, in the winter the collected waste is often frozen or just a few degrees above 0 °C upon arrival at the composting plant, which can significantly delay the start of the decomposition process.

Previous studies of source-separated biowaste have shown average pH values of 4.9 for food waste collected in plastic bags in Uppsala, Sweden (Eklind et al., 1997) and 5.5 for waste from five municipalities in Norway that contained food waste and sometimes garden waste (Norgaard and Sorheim, 2004). Lactic acid and acetic acid tend to be the dominant acids, with reported concentrations of 0.39 and 0.14% (fresh weight), respectively, in the Swedish study cited above and 0.80 and 0.20% in the Norwegian study. Similarly, an average pH of 4.8 has been reported for biowaste collected in winter in Finland (Hultman et al., 2010; Partanen et al., 2010). Internationally, low pH (about 5, and even below 4) has often been found in source-separated food waste, as reported by e.g. Adhikari et al. (2008) and Fung and Wong (2006).

A combination of pH below 6.0 and temperatures above 40 °C has also been shown to seriously inhibit degradation during composting (Smårs et al., 2002; Sundberg et al., 2004). Sundberg et al. (2004) showed that a low pH in combination with a temperature of 46 °C induced a positive feedback loop. The degradation that occurred within this loop lowered the pH and thus prolonged the period of low pH, instead of increasing the pH, as was the case when the initial pH was higher. This mechanism can explain why many large-scale biowaste composting plants in the Nordic countries, where the incoming waste often consists mainly of food waste and has a low pH, struggle with an extended period of low microbial activity (Sundberg and Jönsson, 2008), as well as high odour in the initial composting stage.

* Corresponding author. Tel.: +46 18 67 18 11; fax: +46 18 67 35 29.
E-mail address: cecilia.sundberg@et.slu.se (C. Sundberg).

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The microbial diversity in biowaste has been reported to be high, and to decrease during the early phases of the composting process (Ryckeboer et al., 2003). However, certain bacterial and fungal phyla appear to dominate in the waste. A thorough investigation of a narrow range of waste and compost samples from southern Finland showed an abundance of lactic and acetic acid bacteria (Partanen et al., 2010) and of yeasts (Hultman et al., 2010). These group were found to increase in numbers during the early phases of composting. Pathogens were also detected in biowaste, including species of the genera *Salmonella* and *Yersinia* (Partanen et al., 2010). An important function of composting is to eliminate such pathogens, as well as plant pathogenic microbes and opportunistic pathogens such as Clostridia. Most plant and animal pathogenic microbes are mesophilic, and are efficiently destroyed at proper composting temperatures. However, the removal of viruses and the killing of weed seeds may require higher temperatures and/or longer exposure times.

It is clear that a rapid start to the composting process is essential in all situations, but is often difficult to achieve. Reliable predictions of the behaviour of waste at early phases of composting are needed for correct adjustment of process parameters. This in turn requires thorough knowledge of the incoming waste. This is particularly true in the Nordic countries, where problems are common and where process parameters from warmer climates cannot be used.

The objective of this work was to determine the chemical, physical and microbiological characteristics of source-separated biowaste collected in Finland, Norway and Sweden. Such information can be used to assess the suitability of biowaste for composting and to enable the composting process and plant operation to be optimised.

2. Methods

2.1. Waste and mixtures

The incoming source-separated biowaste at four composting plants located in Sweden, Finland and Norway was investigated. At all plants, the waste was composted in a static process with the turning interval ranging from 3 to 30 days. At plants A, B and C, samples were taken from incoming biowaste as well as from the biowaste mixed with structural amendments (hereafter called substrate mixtures). At plant D, a mixture of biowaste and amendments prepared for a laboratory composting experiment (not reported here) was analysed. Sampling was carried out once at plant D and twice at each of plants A, B and C, at two different times of the year. Samples of the incoming biowaste at plants A-C and the waste mixture from plant D were analysed chemically (pH, organic acids, C_{tot}, N_{tot}, NH_4-N and NO_3-N) and by microbiological methods (Table 1). The number of replicates of each sample used in microbial analyses varied between 1 and 6 (Table 1). These replicates are denoted by Roman numerals in the text.

At plant A, the biowaste consisted of household food waste collected in starch bags plus unpackaged garden waste (Table 1). Plant B received food waste from households packed in starch bags and a large proportion of food waste from commercial sources. Plant C received food waste in paper bags, as well as unpackaged commercial food waste. Two types of biowaste arriving at plant C were analysed: incoming food waste (*f*) and the solid phase of processed food waste (*p*). Processing involved first mixing with water and then pressing the liquid phase out (for anaerobic digestion). The solid phase (*p*) was composted. The biowaste at plant D consisted of food waste from households packed in plastic bags, which were removed by air separation before composting. At the other plants, the bags were composted with the waste.

Table 1
Description of biowaste samples taken for chemical and microbial analysis.

Sample	Date	Waste type	Number of microbial samples
A1	2005-12-15	Food waste with garden waste	2
B1	2006-01-31	Food waste	6
C1f	2006-02-10	Food waste	2
C1p	2006-02-10	Food waste, pressed	2
D1	2006-03-07	Food waste mixed with several amendments, see text	3
A2	2006-05-12	Food waste with garden waste	2
B2	2006-08-29	Food waste	2
C2f	2006-11-14	Food waste	1
C2p	2006-11-14	Food waste, pressed	1

At plant D, the biowaste was sampled after plastic bag removal. Impurities were removed by hand and the food waste was milled, mixed and stored at -20 °C. On five occasions, a substrate mixture was prepared from thawed food waste; two fractions of structural material – fine (<3 mm) and coarse (3–19 mm) crushed wood waste; mature compost from the composing plant; and water (wet weight proportions food waste:coarse structure:fine structure:compost:water = 20:8:2:1:1). The substrate mixture was finely milled and mixed by grinding through a screen plate with approximately 20 mm holes.

Samples of the substrate mixtures from plants A-C were taken for physical analysis of dry matter (DM), bulk density and gas-filled pore volume (Table 2). At plant A, the biowaste was mixed with crushed wood waste with a particle size of up to 80 mm, as well as recycled structural materials screened at 40 mm after processing (when available). Therefore two different mixtures were analysed (Table 2). At plant B, screened crushed wood waste was used as a structural amendment, which on the sampling occasion in January 2006 was mixed with crushed stumps and roots (Table 2). Plant B also used garden waste, mainly coarser fractions, and recycled structural material screened at 25 mm. At plant C, food waste was amended with crushed garden waste, or with the fine fraction (<100 mm) from the sieving of mixed household waste.

2.2. Sampling

At plants A-C, sampling was conducted using the following procedure: A half to one front loader scoop of waste from each of 3–5

Table 2
Substrate mixtures taken for physical analyses.

Sample number	Date	Structure material and mixing conditions
$A1a_{mix}$	2005-12-15	Food and garden waste:crushed wood waste, volume proportions 1:1
$A1b_{mix}$	2005-12-15	Food and garden waste: recycled structure:crushed wood waste, volume proportions 3:2:1
$B1_{mix}$	2006-01-31	Food waste:wood chips with stumps:recycled structure:garden waste, mixing proportions 20 ton:7 m^3:7 m^3:7 m^3
$C1_{mx}$	2006-02-10	Food waste:garden waste, volume proportions approx.3:1
$A2a_{mix}$	2006-05-12	Food waste: recycled structure:crushed wood waste, volume proportions 3:2:1
$A2b_{mix}$	2006-05-15	Food waste: recycled structure:crushed wood waste, volume proportions 3:2:1
$B2_{mix}$	2006-08-29	Food waste:wood waste:recycled structure:garden waste, weight proportions 15:1:1:1
$C2a_{mix}$	2006-11-14	Pressed food waste:screen residue, volume proportions 1:1
$C2b_{mix}$	2006-11-14	Food waste:garden waste:screen residue, mixing proportions unknown

biowaste trucks was gathered into a heap. The heap was mixed and ground twice with an ALLU screener-crusher (www.allu.net).The heap was then spread out using the front loader to approx. 40 cm height, and separated into 3–4 parts with the help of the loader scoop. Three buckets (10 or 20 l each) were filled with material taken from each cut surface, one cut per bucket. From these buckets, samples for physical, chemical and microbiological analyses were taken by hand, one sample per analysis from each bucket. Thus, samples from three buckets from the same heap represented triplicates for each sampling occasion.

The same procedure was performed separately on incoming waste and on the compost substrate mixtures, although grinding was excluded for the mixtures. However, at plant B, samples of the substrate mixture were taken when falling from the conveyor belt, in three 20-litre buckets.

2.3. Chemical analysis

For the analysis of pH and organic acids, waste samples were mixed with de-ionised water in the proportions 1:5 (usually 30 g biowaste and 150 g water). The mixture was stirred and allowed to rest for 1 h, after which time pH was measured in the liquid phase. HPLC (high-pressure liquid chromatography) was used for the analysis of organic acids (acetic acid, lactic acid, butyric acid and propionic acid) in the liquid after filtering through a 0.45 μm PVDF filter. An ion-exchange column (HC-75, Hamilton, USA) at 60 °C and refractive index detection at 40 °C were used. The mobile phase was 0.05 M sulphuric acid (H_2SO_4) and the flow rate was 0.5 ml/min. The retention times were 13.6, 15.8, 18.3 and 22.0 min respectively for lactic, acetic, propionic and butyric acid and the detection limit was 0.1 g/l. Dry matter was determined on 10–100 g samples of the biowaste. Samples were dried at 105 °C for 22–24 h. Samples of the substrate mixture weighing approximately 1 kg were dried at 105 °C for 24–72 h depending on the oven. Volatile solids (VS) were determined by ignition at 550 °C for 4 h. Total carbon (C_{tot}) and total nitrogen (N_{tot}) were measured in dried (50 °C) and milled samples using a Leco CNS-2000 analyser (Michigan USA). Ammonium and nitrate nitrogen (NH_4-N and NO_3-N) were analysed in frozen samples, which were milled to 5–10 mm, shaken in 2 M KCl overnight and centrifuged. The liquid phase was analysed colorimetrically by a TRAACS 800 AutoAnalyser (Seal Analytical, UK). Correlations were tested with simple linear regression in Minitab 15 (Minitab Inc. 2007).

2.4. Physical analysis

Bulk density (volumetric weight) was measured by filling buckets with a known volume (10 or 20 l). The buckets were then tapped on the floor four times from a height of approximately 10 cm and again filled to the brim before being weighed (Aasen and Lystad, 2002). Each bucket was then filled to the brim with water. The volume of water used was assumed to be equivalent to the gas-filled pore volume in the sample.

2.5. Clone library analysis

Major bacterial species present in the samples (Table 1) were determined by analysis of the 16S ribosomal RNA gene diversity. Total DNA was isolated from homogenised compost samples using the Qiagen DNA isolation kit for soil samples as described previously (Hui et al., 2009). The approximate amount and purity of the DNA isolated were determined by gel electrophoresis, by comparing the extracts with concentration standards run in the same gels. Primers Mf341and Mr907 (Muyzer et al., 1998) were used to amplify a 566 base pair region, including the variable regions V3-V5. DNA extracts were cloned using the Qiagen cloning kit (PCR CloningPlus, Qiagen, Hilden, Germany), as described by Hui et al. (2009). Forty insert-containing vectors from each sample were isolated, reamplified using vector primers, and sequenced in one direction using capillary sequencing as described by Hultman et al. (2008). The sequences were assembled with the Staden package (Staden, 2000), and annotation was achieved using the EMBL Fasta 3 database (http://www.ebi.ac.uk/fasta33/).

Species membership was determined by homology of at least 97%, and genus membership was set at 94% homology. By the sequencing of just 40 clones, only the major bacterial species were targeted. Bacteria representing less than about 2.5% of the bacterial DNA were not expected to have been revealed.

2.6. Microarray analysis

The COMPOCHIP microarray, spotted with 369 probes targeting microorganisms that have been reported previously in the composting process, as well as plant, animal and human pathogens and plant disease suppressive bacteria, was used to analyse the compost samples. The array was spotted with at least three probes for most target organisms, and all the probes included in COMPOCHIP were designed to have similar melting temperatures. Probe sequences ranged in length from 17 to 25 nucleotides and they are reported in Franke-Whittle et al. (2009). The specificity of all probes was assessed *in silico*, using the ARB program (Ludwig et al., 2004) and was tested with DNA extracted from bacteria grown in axenic cultures. Fluorescence labelling of target DNA, hybridisation on aldehyde-coated epoxy microscope slides, scanning and image analysis were conducted as described by Franke-Whittle et al. (2005, 2009). Principal component analysis (PCA) of the total signal-to-noise ratio

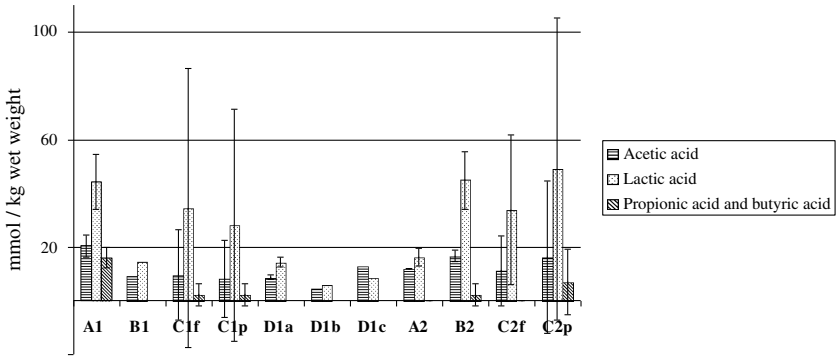

Fig. 1. Organic acids detected in the waste samples. Error bars indicate the standard deviation of the total acid concentration. Triplicate samples, except D1a (duplicate) and B1, D1b, D1c (single values).

Table 3
Chemical characteristics of incoming biowaste from composting plants A–C and the substrate mix from Plant D; means and standard deviations of triplicate samples.

Sample	DM (%)	pH range	C/N-ratio	C_{tot} (% of DM)	N_{tot} (% of DM)	NH_4-N (mg/kg DM)	NO_3-N (mg/kg DM)
A1	38.4 ± 3.8	5.2–5.9	17.6	43.5 ± 3.4	2.5 ± 0.6	720 ± 190	85 ± 19
B1	28.9 ± 1.2	5.9*	18.5	47.0 ± 0.1	2.5 ± 0.3	810 ± 60	220 ± 50
C1f	30.6 ± 0.4	4.9–6.1	20.6	48.1 ± 0.9	2.3 ± 0.1	970 ± 40	100 ± 40
C1p	29.0 ± 1.1	4.7–5.9	24.3	48.7 ± 0.4	2.0 ± 0.1	590 ± 240	71 ± 7
D1a	45.3 ± 1.7	5.9*	27.2	44.3 ± 0.9	1.6 ± 0.1	590 ± 30	160 ± 40
D1b	45.0 ± 1.2	5.8*	29.6	44.9 ± 0.3	1.5 ± 0.1	540 ± 20	118 ± 3
D1c	44.3 ± 1.3	5.8*	27.8	43.3 ± 0.9	1.6 ± 0.1	590 ± 40	114 ± 2
A2	47.8 ± 1.7	5.9–6.0	19.8	32.7 ± 1.5	1.7 ± 0.2	1700 ± 1100	98 ± 18
B2	28.9 ± 1.2	4.8–5.1	14.3	44.4 ± 0.6	3.1 ± 0.9	1100 ± 160	130 ± 50
C2f	29.7 ± 4.4	5.0–5.5	16.6	47.7 ± 3.1	2.9 ± 1.0	860 ± 220	69 ± 50
C2p	32.3 ± 2.2	5.4–5.6	21.4	48.8 ± 0.6	2.3 ± 0.5	400 ± 50	52 ± 7

* Single samples.

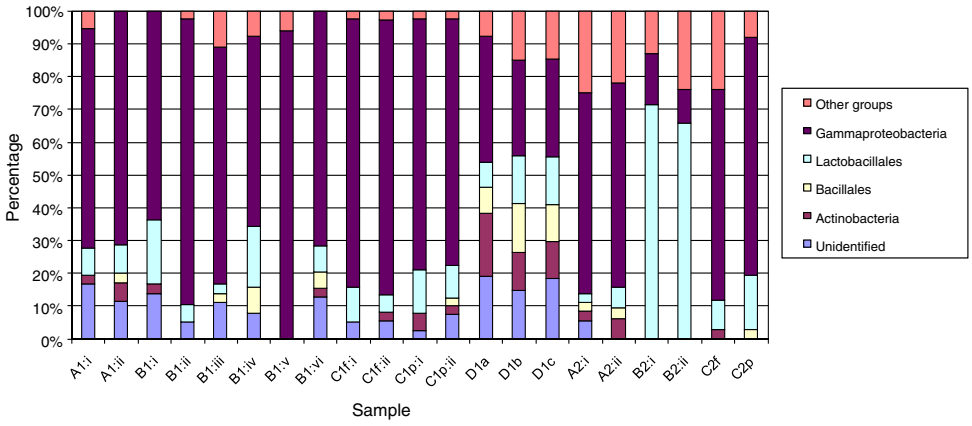

Fig. 2. Bacterial diversity in waste samples determined by sequencing of cloned 16S rRNA genes. Sequenced clones with a similarity >94% are included. Only four major classes are included. 'Other groups' denotes bacterial sequences with similarity to classes other than the four major bacterial classes identified here. 'Unidentified' denotes bacterial sequences with no close similarity to sequences in the nucleotide database. The identity of the samples is given in Table 1. Replicate samples are indicated by Roman numerals.

(SNR) microarray data of the study was conducted, using CANOCO for Windows 4.5 (Ter Braak and Smilauer, 2002).

3. Results and discussion

3.1. Organic acids, pH and lactic acid bacteria

The incoming biowaste, food waste with or without garden waste, from the different plants was characterised by low pH (between 4.7 and 6.1), in combination with moderate to high concentrations of organic acids (11 and 81 mmol kg^{-1} waste fresh weight), particularly lactic acid (Fig. 1, Table 3). This confirms the findings of previous research (Eklind et al., 1997; Norgaard and Sorheim, 2004). In all samples, lactic acid was the dominant acid, often followed by acetic acid. Butyric acid and propionic acid were also detected in certain samples. Acid concentrations and pH were not highly correlated, but the five samples (B1, D1a-c and A2) that had the highest pH values also had lower acid concentrations than other samples. The DM content was between 28 and 48%, with most of the samples below 40% (Table 3). The pH and DM were found to be correlated ($p = 0.006$), with higher pH at higher DM concentrations.

Lactic acid bacteria were found in significant proportions in the different waste samples by the cloning approach (Fig. 2), as well as with the microarray (in particular *Lactobacilli*; Fig. 3). Some of the microorganisms in these phylogenetic groups are known to be heat-tolerant (up to 50 °C). Lactic acid bacteria are common in the environment and in dairy products, and they are detrimental to the composting process since they produce organic acids that lower the pH of the waste so that other bacteria are inhibited (Partanen et al., 2010). This effect is especially strong if the pH is decreased below 6.0 and the temperature is above 40 °C (Smårs et al., 2002; Sundberg et al., 2004). The low pH and high acid concentrations, in combination with the presence of lactic acid bacteria, indicate that process problems could be expected if the temperature in the composting process were to increase rapidly to above approx. 40 °C (Sundberg et al., 2004).

In the B2 samples, the pH was very low and the concentrations of organic acids high (Table 3), and the proportion of lactic acid bacteria was extremely high (Fig. 2). That can perhaps be explained by the collection procedure in the plant B region, where compostable waste is collected in starch bags and only from multi-storey houses, and therefore does not contain any garden waste. With collection in plastic bags (ordinary plastic or starch plastic) the material does not dry out as much as with collection in paper bags. This, as well as the higher temperatures in the summer, may have increased acid formation and thus lowered the pH in the waste. As pH and DM were correlated in the waste samples investigated (Table 3), it would appear that food waste intended for composting should be collected together with drier materials also suitable for composting, such as pot plants, tissue paper, garden waste and cat litter.

3.2. Microbiology

The bacterial diversity in the waste samples was rather high. The most numerous group in all waste samples except B2 was

Fig. 3. Visualisation of microarray results for all wastes. The probe names are listed in each row, while columns represent the different samples. The SNR values for all probes were normalised to the UNIV 1389 probe, and attributed a value between 0 and 1 (UNIV 1389 = 1). The relative intensity of the normalised SNR signal of probes is indicated by the depth of shading. Only probes which had a maximum signal for all hybridisations above the threshold value of 2 were included in the table.

the Gammaproteobacteria (Fig. 2). Among the Gammaproteobacteria, *Pseudomonas* and the Enterobacteria (*E. coli, Klebsiella, Enterobacter*) were the most dominant genera (results not shown). Another important group in the incoming waste was the lactic acid bacteria (Lactobacillales), as discussed above.

Bacillales were found in 11 of the 21 samples and Actinobacteria in 13 of the 21 samples. In the samples from plant D (substrate mixtures including old compost), the proportions of Actinobacteria and *Bacillales* were higher than in the samples from the other plants (incoming biowaste only).

The hybridisation results of probes for which the highest SNR for all samples tested was above the threshold value of 3 are shown in the heat map in Fig. 3. Darker shading indicates a higher SNR value. Bacteria belonging to the genus *Acinetobacter* were found in high numbers in the waste samples from A1, B1, C1 and D. Probes targeting *Acinetobacter lwoffii* and *A. calcoaceticus* gave strong signals. Signals above the detection threshold were obtained upon hybridisation for most of the *Lactobacillus* probes with many of the waste samples. The microarray revealed high signals with the Enterobacteriaceae probe KO246 in all samples. High levels of *Salmonella* and *Pantoea* were also found in all waste samples. Probes targeting the genus *Pseudomonas* revealed high levels of these organisms in the different waste samples. Similarly, probes targeting *Stenotrophomonas maltophilia* and the genera *Xylella/Xanthomonas/Stenotrophomonas* yielded signals with waste samples from all plants.

The results of cloning and sequencing were generally in good agreement with those of the microarray analysis. However, the utility of these two methods is different. While the sequencing approach roughly determines the bacterial community composition, the microarray technique measures whether selected genera of interest are present in the sample. Thus, the sequencing complements the chemical analysis in characterising the waste composition, while the microarrays reveal the presence of a specific organism, for example a pathogenic bacterium. Despite the rather low number of sequences per sample observed, the total diversity in the waste was quite high, in agreement with earlier observations (Partanen et al., 2010). Polymerase chain reaction (PCR) was used to amplify target DNA from the waste samples prior to cloning and microarray hybridisation. PCR greatly increases the sensitivity, but it introduces a bias towards organisms whose DNA is more easily accessible and amplified (von Wintzingerode et al., 1997).

3.3. Differentiating between samples

The differences in the chemical variables between seasons and composting plants were small. The analyses performed provided no support for any statement on general differences between seasons or plants, even though some tendencies could be seen. The lack of significant differences between plants and seasons could be due to the small number of samples investigated in the study. However, Hansen et al. (2007), who analysed 40 samples taken from five cities in Denmark throughout one year, only found significant seasonal variations in ash, sulphur and chloride content, and not in any of the other 13 variables investigated, indicating little seasonal variation in the composition of source-separated municipal biowaste.

Analysis with the COMPOCHIP 16S rRNA gene microarray was applied in this study in order to determine the microbial communities present in the different waste types, and to examine whether the waste samples from the different plants could be differentiated from each other (Fig. 4).The two axes explained 56.1% of the variance (33.4% and 22.7% respectively). The PCA loading plot showed that some probes were more influential in discriminating between the samples than others. The longer the arrow, the more influential the probe. Probes targeting the *Enterobacteriaceae*, *Pseudomonas*, *Salmonella*, *Actinomyces*, *Enterococcus/Lactobacillus*, *Clostridium* and *Listeria* were among the most significant probes in the differentiation of the different waste samples used in this study.

The various waste samples grouped differently in the PCA analysis. The A1, B1 and C1 samples were found to cluster separately from the A2, B2, C2 and D samples. Interestingly, the A1, B1 and C1 samples were all collected from their respective plants in winter, while the A2, B2 and C2 samples were collected in the summer and autumn months. This would indicate that either the temperature, which fluctuates significantly in the Nordic countries, or seasonal variations in the material collected plays a role in determining the microbial communities present in waste. This result was confirmed by the cloning approach. For most of the samples collected in the winter, the Gammaproteobacteria were found to dominate, while there was a lower proportion of microorganisms belonging to groups not dominating and grouped as 'Other' in Fig. 2.

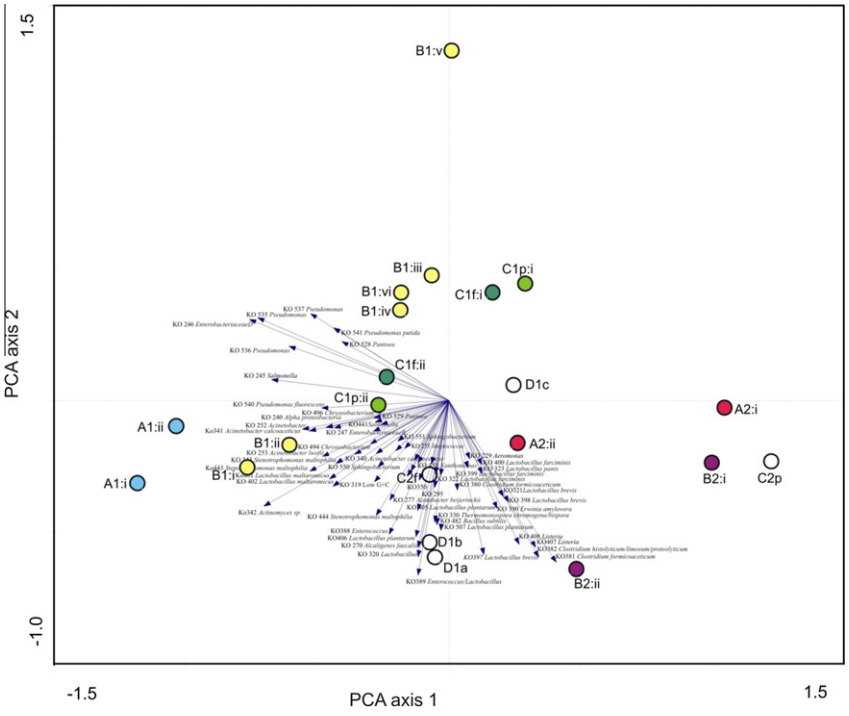

Fig. 4. Loading plot obtained by redundancy analysis, depicting the organisms responsible for community differences between the wastes. The two axes represent 56.1% of the explained variance. The vectors show the covariance structure of the probe signals.

The sampling method used in this study was developed with the aim of obtaining representative samples with minimal manual work. The resulting variation was small for some characteristics such as DM and C_{tot} (Table 3), but rather large for others, e.g. organic acids (Fig. 1). Representative sampling of heterogeneous materials such as household waste is difficult. The specific qualities of the waste and analytical methods are important in determining the appropriate sampling method for a particular waste sample. Thus methods developed for other types of materials, including other waste fractions, are not directly applicable to biowaste. A method for sampling biowaste involving several stages of extraction of small and large samples plus milling has been developed and analysed statistically by la Cour Jansen et al. (2004), but it was considered too resource-demanding to be applicable for this project.

3.4. Waste mixtures

The DM content, bulk density and gas-filled pore volume of waste mixtures are presented in Table 4. The waste mixture at plant B was wetter and more compact than that at plant A and most of those at plant C. However, the most compact material was one of the waste mixtures at plant C ($C2b_{mix}$ in Table 4). There was a good correlation between bulk density and gas-filled pore volume (Fig. 5).

A large volume of gas-filled pores is essential in achieving a good flow of air through a compost matrix. The recommended gas-filled pore volume is at least 30% (Haug, 1993). All the waste mixtures analysed in this study, with the exception of C2b, thus theoretically had an adequate gas-filled pore volume, even though that in B2 was borderline. However, there is a risk of the material being compacted when it is piled several metres high during composting, which reduces the gas-filled pore volume (Aasen and Lystad, 2002). We did not investigate this aspect in our study.

Table 4
Physical characteristics of waste mixtures at composting plants A–C.

Sample	DM content (% of fresh wt.)	Bulk density (kg/m^3)	Gas-filled pore volume (%)
$A1a_{mix}$	0.38	502 ± 6	46.1 ± 1.0
$A1b_{mix}$	0.43	481 ± 13	47.4 ± 0.5
$B1_{mix}$	0.31 ± 0.02	577 ± 60	41.4 ± 5.6
$C1_{mx}$	0.36 ± 0.05	582 ± 33	45.4 ± 3.2
$A2a_{mix}$	0.54	433 ± 15	55.3 ± 0.8
$A2b_{mix}$	–	452 ± 37	57.5 ± 2.7
$B2_{mix}$	0.34	633 ± 32	32.1 ± 3.0
$C2a_{mix}$	0.39	545 ± 40	48.1 ± 3.0
$C2b_{mix}$	0.42	776 ± 99	27.2 ± 9.3

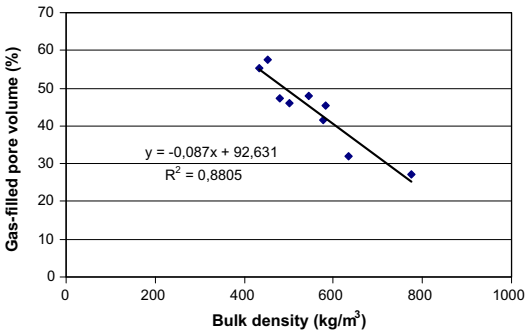

Fig. 5. Gas-filled pore volume as a function of bulk density for waste mixtures at the three plants A–C.

The C1 substrate mix had a considerably higher bulk density than the A2 substrate mixes, despite having a similar gas-filled pore volume (Table 3). This was probably due primarily to differences in the structural materials used. At plant A the materials were comprised mainly of dry wood waste, which is light, while at plant C, garden waste with a high content of gravel and soil was used.

3.5. Microbial suitability for composting

Microorganisms belonging to the *Bacillales* and Actinobacteria, known to be critical for an efficient composting process, were sufficiently abundant in the waste to inoculate the subsequent composting process. However, the numbers were found to be lower than those of the lactic acid bacteria in most samples. Since the commencement of efficient composting depends on a strong presence of thermotolerant microbes such as *Bacillus* species and Actinobacteria, the early stages of the composting process should be steered towards conditions that favour such organisms. The inclusion of recycled compost is one way to improve the conditions for composting, since it increases the proportion of Actinobacteria and *Bacillales* (see D samples in Fig. 2). Recycled bulk material is usually cheap, which means that ample amounts can be used and thus good structure and aeration attained.

An alternative treatment would be to anaerobically digest the biowaste. Lactic and acetic acids are two of the products of fermentation, one of the initial processes in anaerobic digestion. The low pH of the incoming material can thus be seen as an indication of the material being suitable for, and already entering, anaerobic digestion. Furthermore, as most anaerobic digestion plants use continuously stirred tank reactors, the negative effects of the low pH of the incoming substrate would be minimised by it being immediately mixed with large volumes of substrate at a suitable pH.

3.6. Pathogens

Several different pathogens were found in the different waste samples. The analysis by cloning performed in this study only allows reliable detection of bacteria that represent $\geq 2.5\%$ of the bacterial community, i.e. at least $\sim 2 \times 10^6$ cells per gram of waste. Thus, the presence of specific pathogenic species present below 2.5% may not be detected. The presence of *Salmonella enterica*-specific sequences in five of the samples indicates the presence of this pathogen in these particular samples, but the rather high detection limit precludes claims of their absence in the other samples. On the other hand, sequences representing *Escherichia* were present in all samples, but distinction between strains of the normal intestinal flora of mammals and pathogenic *E. coli* strains was not possible. It is clear from the cloning results that bacterial groups and classes that contain pathogens are common (Gammaproteobacteria), or at least occasionally present (Alpha- and Betaproteobacteria, Clostridia, Flavobacteria) in the waste samples. The Enterobacteriaceae family within the Gammaproteobacteria includes the genus *Salmonella*, most of which are pathogens, and *Escherichia*, which also includes pathogenic strains. Clostridia, which include human and animal pathogens, are anaerobic spore-forming firmicutes that produce noxious odours while fermenting proteins and lipids.

Various pathogenic microorganisms were also detected by the microarray. Probes targeting members of the *Enterobacteriaceae* as well as members of the genera *Pseudomonas*, *Salmonella, Enterococcus, Clostridium* and *Listeria* were most influential in differentiating the various samples from each other in the PCA analysis of the microarray results. These genera include species considered to be human pathogens, and have been reported previously to be present in compost samples (Ryckeboer et al., 2003; Danon et al.,

2008; Cayuela et al., 2009). Signals from the *Aeromonas hydrophila* probes were found in the A2, B2 and C2 samples and low levels of different species of *Clostridium* were found in the A2, B2 and D samples (Fig. 3). Furthermore, probes targeting *Neisseria meningitidis* yielded signals in the B2 and D waste samples. *Listeria* was detected in the A2, B2, C2 and D samples. The presence of these microorganisms in an end-product would indicate an insufficient composting process, and could pose a potential threat to compost users. In a well-managed composting process, animal, human and plant pathogens are eliminated through high temperature and through microbial succession in the composting process. However, an improperly managed composting process can allow the survival, proliferation and spread of pathogens. The observed presence of pathogens emphasises the importance of hygienisation through high temperatures at some stage of the composting process. For this reason, a minimum temperature and time period for composting is required in most European countries. In Sweden, these time and temperature requirements range from 24 h at 70 °C to 7 days at 55 °C.

Probes targeting members of the genus *Acinetobacter* yielded high signals in the waste samples from A1, B1, C1 and D. A link between *A. calcoaceticus* and bovine spongiform encephalitis has been made in the past (Ebringer et al., 2005), making the occurrence of this organism in the waste samples of some concern. Cayuela et al. (2009) found *A. calcoaceticus* primarily in composts that were produced with meat and bone residues as a N amendment. The A1, B1, C1 and D samples in the present study were collected in the winter months, which would again indicate a possible role of season, reflecting temperature, substrate or environmental effects.

3.7. Other microorganisms

In addition to the major bacterial groups identified in Fig. 2, the sector denoted as *Other groups* contained identified bacteria with a similarity of at least 94% to bacterial phyla in the databases. The sequences representing bacterial classes that were present as more than singletons, and the samples from which they were isolated, are listed in Table 5. Alphaproteobacteria are typically plant-associated and mostly aerobic Gram-negative rods. The sequences observed represented *Rhizobium leguminosarum* and several additional Rhizobiales sequences with a similarity below 97%. *Brevundimonas diminuta* and *Sphingomonas* sp. were also found. The phylum Bacteroidetes was represented mostly by members of the class Flavobacteria, e.g. *Wautersiella falsenii*, which is a human pathogen.

The phylum Bacteroidetes also contains the common anaerobic Gram-negative intestinal bacterial genus Bacteroides. None of these bacteria are typical compost bacteria, and all or most of them are likely to be killed in an efficient, high temperature composting process. However, members of this class, in particular *Dysgonomonas capnocytophagoides*, have been reported to be associated with volatile organic compound production in fresh biowaste (Mayrhofer et al., 2006).

Table 5
Microbial groups identified by clone library analysis, other than those in Fig. 2.

Bacterial group	Identified in sample No.
Alphaproteobacteria	B1:iv, C1f:i, C1p:ii, D1a-c
Bacteroidetes	C1f:ii, A2i, B1:iii, B1:iv, B2:ii, C2f, C2p
Betaproteobacteria	A1:i, B1:ii, B1:v, C1f:ii, D1a, D1b, A2:I, A2:ii, C2f, C2p, B2:i, B2:ii
Clostridia	B2:ii
Sphingobacteria	A2i

Betaproteobacteria were represented among the clones only by the order Burkholderiales, e.g. the fungal pathogen *Janthinobacterium* sp. and the plant pathogen *Acidovorax* sp. Pathogens such as *Neisseria* (detected with the microarray) are also found among the Betaproteobacteria. The class Flavobacteria consists of the single order Flavobacterium, which is common in environmental samples and includes opportunistic pathogens.

Sphingobacteria, found by cloning only in A2, are soil inhabitants with a broad degradation capacity, and members of this group are also regularly found in composts (Franke-Whittle et al., 2009). Up to 19% of all bacteria in waste samples remained unidentified, which is typical for environmental samples such as compost (Partanen et al., 2010).

The order *Bacillales* observed by cloning was represented by *Bacillus thermoamylovorans*, *Bacillus coagulans, Paenibacillus* sp. etc., which are not among the species targeted by the probes used in hybridisations. However, the results from microarray hybridisation indicated that only low numbers of a couple of genera of *Bacillus* were present in a few of the waste samples (as well as species of the genera *Listeria* and *Thermoactinomyces*, which were present at lower levels).

4. Conclusions

The quality of the wastes investigated in this study, with low pH, high organic acid content and lactic acid bacteria present, poses a serious challenge, which, unless properly met, can substantially delay a successful composting process. A wide variety of pathogens were found, which indicates the need for high temperature hygienisation during the process. For efficient composting, it is recommended that food waste be mixed with ample amounts of recycled bulk material and compost, provided that this has a pH well over 6. This helps to buffer the pH, as well as to increase the numbers of bacteria needed for a good composting process.

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