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Niina Koivula

Temporal Perspective of Humification of Organic Matter

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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

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Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
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ABSTRACT

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Dead organic matter is stabilised in the humification process to humic substances (HS). Functionally the most important group of HS is humic acids (HA), which have essential functions in soil, sediments and water. Humification involves both biological and abiotic processes, but the (bio)chemical synthesis as well as the structures of HA are unknown. Numerous theories of humification have been presented over the years. Some of these emphasise aromaticity as the structural unit of HA, while others emphasise aliphaticity.

In this work, humification and the structure of HA were studied from a temporal perspective. HA samples were grouped according to age: early, intermediate and end stages of humification; and the respective samples were compost, peat and coal HA. Composting of cellulose with lignin-free bulking agent produced HA, indicating that humification and formation of HA can occur without a lignin contribution. Use of ash as a composting additive enhanced the rate of mineralisation and the formation of HA, indicating abiotic effects on humification during composting. HA was extracted from the above-mentioned matrices. The amount of HA extracted was greatest from peat samples and less from composts and coal samples. Degradation of HA by alkaline hydrolysis produced hydrophilic and hydrophobic phases. Structural study of HA was focused on the degradation products in the two phases, and on the importance of carbohydrate structures to HA. The significance of studying both phases was that more organic matter could be analysed. Compounds obtained in hydrophilic phase were mainly aliphatic, while compounds obtained in hydrophobic phase were mainly aromatic. Yields of aliphatic compounds were clearly higher than yields of aromatic compounds. Easily degradable carbohydrates are rapidly utilised by microbes during humification, but microbes then generate new polysaccharides. Compounds of carbohydrate origin are found throughout the coalification series. Carbohydrates are thus recycled during humification, which means that carbohydrates, too, are recalcitrant in humification, but in a different way than lignin. Lignin persists through being slow to decompose, while carbohydrates persist through recycling. The contribution of lignin to humification can also be discussed from an evolutionary perspective. Non-vascular plants were forming soils long before vascular plants had evolved. Conceivably the humification process did not change radically after lignin was evolved; rather, lignin was adopted into an existing humification and degradation system. From this it follows that, the humification process does not depend upon a lignin contribution. Cellulose compost HA produced aromatic degradation products.

Key words: Coal; compost; humic substances; hydrophilicity; hydroxycarboxylic acids; peat; phenolic compounds.

N. Koivula, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FIN-40014 University of Jyväskylä

Author´s address

Niina Koivula
Department of Biological and Environmental Science
P.O. Box 35
FIN-40014 University of Jyväskylä
Finland
E-mail: niikoiv@bytl.jyu.fi
Tel: +358-(0)14-2604214
Fax: +358-(0)14-2602321

Supervisor

Associate Professor Kari Hänninen
Department of Biological and Environmental Science
P.O. Box 35
FIN-40014 University of Jyväskylä
Finland
E-mail: kahannin@bytl.jyu.fi
Tel: +358-(0)14-2602313
Fax: +358-(0)14-2602321

Reviewers:

Professor Takao Katase
Nihon University
College of Bioresource Sciences
Laboratory of Chemistry
1866 Kameino, Fujisawa
Kanagawa, 252-8510 Japan

Docent Klaus Niemelä
Oy Keskuslaboratorio – Centrallaboratorium (KCL) Ab
P.O. 70
FIN-02151 Espoo
Finland

Opponent:

Professor Ingrid Kögel-Knabner
Lehrstuhl für Bodenkunde
Technische Universität München
D-85350 Freising-Weihenstephan
Germany
E-mail: koegel@wzw.tum.de
Tel: +49-00 49 8161/71 5174
Fax: +49-00 49 8161/71 4466

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. The papers were planned together with my tutor, Associate Professor Kari Hänninen. I performed a significant part of the experimental work and writing of the papers.

I Koivula, N., Hänninen, K. & Tolvanen, O. 2000. Windrow composting of source separated kitchen biowastes in Finland. Waste Manage Res. 18: 160-173.

II Koivula, N., Räikkönen, T., Urpilainen, S., Ranta, J. & Hänninen, K. 2004. Ash in composting of source-separated catering waste. Bioresource Technology 93: 291-299.

III Koivula, N. & Hänninen, K. 1999. Biodeterioration of cardboard-based liquid containers collected for fibre reuse. Chemosphere. 38(8): 1873-1887

IV Koivula, N. & Hänninen, K. 2001. Concentrations of monosaccharides in humic substances in the early stages of humification. Chemosphere. 44(2): 271-279.

V Koivula, N. & Hänninen, K. Time-dependent trends in humification. Part 2. Humic acids produced during peat and coal formation process. Organic Geochemistry. Submitted

VI Koivula, N. & Hänninen, K. Time-dependent trends in humification. Part 1. Humic acids produced by composting of bleached pulp and biowaste. Organic Geochemistry. Submitted

ABBREVIATIONS

Ara	L-Arabinose
cfu/g	Colony forming units per gramme
CM I	Compost mix containing 0% ash
CM II	Compost mix containing 10% ash
CM III	Compost mix containing 20% ash
CTMP	Chemi-thermo-mechanical pulp
dm	Dry matter content
FA	Fulvic acid
Fru	D-Fructose
Fuc	L-Fucose
Gal	D-Galactose
GC/FID	Gas chromatography with flame ionisation detector
GC/MS	Gas chromatography with mass spectrometry detector
Glc	D-Glucose
HA	Humic acid
HWE	Hot water extract
LPB	Liquid packaging board
Man	D-Mannose
OM	Organic matter content
ppm	Parts per million
PTFE	Polytetrafluoroethylene (Teflon)
REF	Recovered fuel
Rib	D-Ribose
UF	Unfractionated
v	Volume
w	Weight
Xyl	D-Xylose

1 INTRODUCTION

1.1 The humification process

Humification is a stabilisation process for dead organic matter. As recently noted by Kögel-Knabner (2002), compounds synthesised by plant and animal cells during the degradation of dead organic matter are important in the formation of humic substances. Kumada (1987) has stated that the humification process in the broad sense is controlled by such soil-forming factors as climate, parent material, vegetation and time. The development of humic structures is a slow time-dependent process in which residual organic carbon becomes increasingly resistant to decomposition; it turns into humus (Stevenson 1994). Humification can be divided into several processes:

Biodeterioration. When the ambient conditions are suitable for microbial degradation, the biodeterioration of dead organic matter begins immediately after the death of the organism. Biodeterioration, or decomposition, takes place slowly in soil. Earthworms and soil animals play an important role in the removal of plant and animal residues. Bacteria, actinomycetes, fungi and other microbes also participate in the process, from the beginning, and contribute in a major way at the final stage in the decomposition of the more resistant plant parts, such as lignin (Kumada 1987, Stevenson 1994, Brussaard & Juma 1996).

Composting. During the composting process, carbonic and nitrogenous compounds from biowaste are transformed through the activities of successive microbial populations into more stable and complex organic forms, which chemically and biologically resemble humic substances. The process involves interactions between organic waste, microbes, moisture and oxygen. In attacking the organic matter, microbes reproduce and liberate CO_2, water, other organic products and energy. The extra energy is given off as heat (Biddlestone et al. 1985, Epstein 1997, Paré et al. 1998, Veeken 2000). The basic composting process is depicted in Fig. 1.

Microbes associated with composting include psychrophiles, mesophiles and thermophiles. As temperature increases the growth of organisms

accelerates (Epstein 1997). The decomposition rate has been measured by oxygen uptake, which is a function of microbial activity. The supply of oxygen to the mass is vital to the microbes and to the composting process.

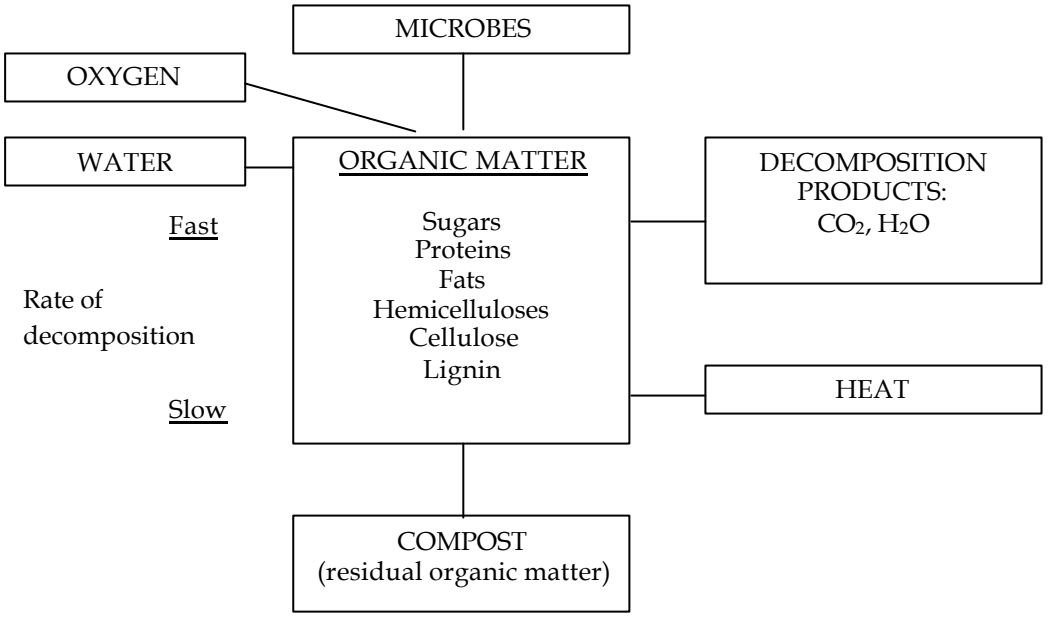


FIGURE 1 The composting process (revised from Epstein 1997).

Characteristics of the aerobic process are rapid decomposition, high temperatures and lack of objectionable odours (Biddlestone et al. 1985). Microbes require carbon as a source of energy and need nitrogen to synthesise protoplasm. Up to two-thirds of the carbon consumed can be given off as CO_2, while the other third is combined with nitrogen in living cells or remains as fixed carbon in the organic matter (humus).

After composting, the product can be used as organic fertiliser or soil conditioner in agriculture. Among other things, the application of compost to soil affects the quality of organic matter in the soil, the physical, chemical and biological characteristics of the soil and the nutrient yield of plants. The application of compost improves physical properties of soil such as porosity, water-retention capacity and bulk density. It also improves soil buffering capacity and increases the percentage of organic matter and the cation exchange capacity. According to Hayes et al. (1989), the positive effects of mature compost on soil and crops are associated with the humic substances.

Peat formation. Peat is a heterogeneous material, which is usually formed first under natural aerobic conditions and then in an anaerobic humification process. According to Waksman (1936), the following four stages are involved in peat formation: 1) relatively rapid decomposition of water-soluble substances, 2) slow decomposition of plant constituents, 3) gradual accumulation of resistant constituents and 4) synthesis of microbial cell substances.

Carbohydrates are an important class of compounds during peat formation. They comprise not less than 70% of all mass of the original peat

forming plants. Peat ranging in age from 5,000 to 8,000 years may contain up to 40% of carbohydrates. Even buried peat of the last interglacial period, more than 60,000 years in age, very often contains considerable quantities of cellulose and easily hydrolysed carbohydrates (Rakowski 1959).

According to Manskaya & Drozdova (1968), true lignin is not present in algae and mosses. Although it does not contain true lignin, sphagnum moss, which is the main peat forming plant in Finland (Sarkanen & Ludwig 1971), does contain a phenolic polymer, known as sphagnol, in its cell walls (Czapek 1899). Sphagnol is constructed of phenolic units (mainly p-hydroxyphenyl) and associated with the carbohydrate matrix (Farmer & Morrison 1964, Engmann 1971).

Since the last deglaciation occurred some 9,000 years ago, almost all the peatland in Finland is at most a few thousand years old. Isolated pockets of older peat from the interglacial period occasionally occur in deeper soil layers.

Coalification of peat. Coalification begins when peat becomes buried and exposed to elevated temperatures and pressures. With the loss of relatively small molecules like water, CO_2 and/or CH_4, chemically active sites are generated, which allow cross-linking between polymer molecules. Rakowski (1959) proposes that, chemically, the moment of transition from peat to ɷal occurs when carbohydrates have disappeared from the mass. The formation of black coal, in particular, is primarily a tectonic, geochemical process under the influence of high pressures and temperatures (Given 1984).

Fuchs (1930) demonstrated the aromatic nature of black coal experimentally by producing benzenecarboxylic compounds through $KMnO_4$ oxidation. An aromatic, "honeycomb-like" structure was produced, and is now generally accepted as the structure of the black coal molecule.

1.2 Humic substances

Humic substances have been defined as ”a category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterised as being yellow to black in colour, of high molecular weight, and refractory” (Aiken et al. 1985). They represent a heterogeneous mixture of molecules, which, in any given soil or sediment, may range in molecular weight from as low as around 2,000 to perhaps over 300,000 (Stevenson et al. 1971, Wolf et al. 2001).

Humic substances can be divided into humic acid (HA), fulvic acids (FA) and humins on the basis of their solubility in acid and alkali. HAs are soluble in alkali and precipitated in acid (pH 1-2), whereas FAs are soluble and humins are insoluble in water at all pH values. These terms do not refer to pure compounds, but rather the definitions are operational and each fraction consists of a highly complex and heterogeneous mixture of organic substances (Hayes et al. 1989). These fractions may also contain non-humic materials, which are

defined as "compounds belonging to known classes of biochemistry, such as amino acids, carbohydrates, fats, waxes, resins, organic acids, etc" (Stevenson 1994). Without specific information on chemical structures it becomes difficult to estimate the amounts of humic and non-humic substances in a certain fraction of humic substances (Saiz-Jiminez 1996; Hayes & Wilson 1997). For example, there is evidence to suggest that cellulose fibres can dissolve in alkaline solution to some extent (III).

Humic acid and related substances are widely distributed in terrestrial soils, natural waters, marine and lake sediments, peat bogs, shales and brown coals. It has been concluded that the amount of organic carbon in the earth present as HAs (60×10^{11} tons) greatly exceeds the amount that occurs in living organisms (7×10^{11} tons) (Stevenson 1994, Andreux 1996). Many of the functions of humus are known, including its effect on soil structure, chelating of heavy metals, and adsorption of pesticides and other toxic pollutants. However, the mechanisms involved will remain obscure until more is known about the structural chemistry of humic and fulvic acids (Stevenson 1994).

Norwood (1988) has presented an overview of chemical and physical methods that are extensively applied in the structural study of humic substances. These methods are based on the degradation of complex macromolecules (oxidative and reductive degradation; hydrolysis and pyrolysis) or on nondegradative methods (spectrometric methods: IR, NMR, EPR, UV/VIS; X-ray analysis; electron microscopy; electron diffraction; measurement of viscosity; surface tension measurements and titrimetric methods). According to Hänninen & Niemelä (1991), alkaline hydrolysis preserves the aliphatic structures of HA, and hydrolysis has been applied as a degradation method for humic substances in soil, peat, sediment and water (Neyroud & Schnitzer 1975, Tsutsuki & Kuwatsuka 1979, Bourbonniere & Meyers 1983, Kumke et al., 2002). Hänninen & Niemelä (1991, 1992) and Grasset & Amblés (1998) have investigated both hydrophilic and hydrophobic phases of the hydrolysis products of HA.

Stevenson (1994) has listed the functional groups commonly identified in humic substances. These include carboxylic acids, phenolic-OH, enolic-OH, quinone, hydroxyquinone, lactone, ether and alcoholic-OH. Hayes et al. (1989) state that the heterogeneity of the components of humic substances presents a formidable obstacle to structural determinations, which otherwise might assist our understanding of humus formation.

It is generally agreed that humic substances originate from the degradation of plant remains (Hatcher & Spiker 1988), but the synthesis of the chemical structure of humic substances is still an unknown process. Formation of humic substances is understood to be mainly a biological reaction, but abiotic reactions are involved as well. Catalysis by abiotic soil constituents (e.g. OH ions, Fe and Mn oxides) causes reactions with organic compounds, analogous to enzyme-mediated transformation, yielding humic substances (Kumada 1987, Liu & Huang 2002).

Hedges (1988), Hatcher & Spiker (1988) and Stevenson (1994) have reviewed theories that attempt to explain the synthesis of humic substances. Hedges (1988) and Hatcher & Spiker (1988) categorised these theories according to whether the reactions directly responsible for humic substance formation are assumed to have a net degradative (lowered molecular weight) or aggregative (increased molecular weight) effect. The hypothesis in the first category is that the refractory plant biopolymers (e.g. lignin, cutins, suberins, melanins), and possibly some small molecules linked to this biopolymer during humification, are modified by partial degradation to form the central core of humic substances. The hypothesis in the second category is that humic substances are formed by condensation, or repolymerisation of small reactive organic molecules that have been generated by essentially complete breakdown of bonds between structural units in the original biopolymer.

Stevenson (1994) summarises four major pathways to humus formation. The classical theory suggested that humic substances are formed through certain modifications of lignin. According to this theory, microbes partially degrade lignin and the residuum becomes part of the soil humus. In two other theories, quinones are the major building blocks forming humic substances. Quinones are formed from polyphenols, which are either products of biodegradaton of lignin, or products synthesised by microbes from non-lignin carbon sources. In the presence or absence of amino compounds, quinones polymerise to form humic-like macromolecules. The fourth theory suggests a partial or even predominantly carbohydrate origin. Carbohydrates can produce brown nitrogenous polymers through the condensation of reducing sugars and amines formed by sugar amine condensation; this mechanism is called the browning reaction or Maillard reaction (Cheshire 1979, Ikan et al. 1986, Cheshire et al. 1992). Although these polymers can develop from low-molecular-weight substances, it seems more likely that a large proportion of them arise from the transformation of polysaccharides derived from plants and microbes (Cheshire et al. 1992).

While the significance of carbohydrates in humification has been noted, this theory is not so widely accepted as the polyphenol theory. At present, according to Stevenson (1994), common opinion supports the polyphenol theory as the formation pathway for humic substances.

Recent and more precise analyses have suggested new model structures for HAs (Schnitzer 2000, Bruccoleri et al. 2001, Piccolo 2002). Hayes & Clapp (2001) argue that there is little point in seeking definitive structures of humic molecules, but it is important to have an awareness of the types of structures in the mixtures and of the types of associations that these can form.

On a global basis, vascular plant tissue makes up the largest living pool of actively cycling organic matter on earth and the starting materials for humification. Lignin, a primarily ether-linked phenylpropanoid terrestrial biopolymer found in wood cells and in small amounts in foliar tissue, is the second most abundant biopolymer after cellulose (Filley et al. 2002). Cellulose comprises the major structural components of the cell walls of lower and higher

plants and also occus in algae and fungi (Sjöström & Westermark 1999, Kögel-Knabner 2002).

Lignin is generally considered to be recalcitrant towards humification. Direct evidence for the transformation of lignin in soils nevertheless exists, and it is hard to believe that, invariably, refractory lignin components are selectively preserved. More likely this preservation is not directly related to recalcitrance of lignin components but rather to environmental conditions that are unfavourable for continuous microbial activity (Saiz-Jimenez 1996).

Certainly one group of fungi, white-rot fungi, is able to completely decompose lignin to CO_2. Other fungi induce structural changes in lignin. Probably, under aerobic conditions in soil, consortia of decomposer microbes mediate lignin degradation (Phelan et al. 1979, Colberg & Young 1982, Falcón et al. 1995, Kögel-Knabner 2002). Aerobic lignolytic microbes have the ability to solubilise polymeric lignin to fragments of reduced molecular size, and if these fragments are of sufficiently small molecular size (i. e., MW < 1400), and become available in anoxic zones, anaerobic microbial communities may be able, in part, to degrade them to CO_2 and CH_4 (Colberg & Young 1982, Young & Frazer 1987, Colberg 1988).

Carbohydrates have been estimated to constitute from 5 to 25 per cent of the soil organic matter. Plant remains contain simple sugars, hemicelluloses, and cellulose and these are utilised by soil microbes. Carbohydrates have been classified, therefore, as compounds that are rapidly degraded during composting and humification (Stevenson 1994, Stott & Martin 2001). The rate of the biodegradation of carbohydrates is dependent on the length and branching of the carbohydrate chain (Stott & Martin 2001) It is claimed that cellulose, in turn, decomposes slowly under aerobic conditions in soil (Kögel-Knabner 2002).

1.3 Objectives of the research

There were three main objectives of the research:

1. To carry out composting of source-separated biowaste, cellulose and ash
    a. to produce, synthetically, humic acids in the early stages of humification,
    b. with addition of ash, to evaluate abiotic effects in the humification process,
    c. in the case of cellulose, to produce humic acids from lignin-free raw materials
2. To study samples (composted materials in the early stages, peat in intermediate stage and brown and black coals in the end stage) able to provide information on humification in temporal perspective
    a. to determine the amounts of humic substances in relation to sample age,

   b. to apply alkaline degradation to the samples, and to study the hydrophilic and hydrophobic phases formed in the alkaline hydrolysis of samples of different age
3. To study the contributions of lignin and carbohydrates to humification
   a. to evaluate the importance of lignin and carbohydrates in humification
   b. from the evolutionary point of view

2 MATERIALS AND METHODS

A summary of the analyses and the samples studied in publications I-VI is presented in Table 1.

2.1 Biodeterioration of cellulose (III, IV)

Waste liquid packaging board (LPB) was collected and delivered from Germany to Finland for processing as recycled fibre and as plastic reject for incineration. LPB board is composed of pulp and chemi-thermo-mechanical pulp (CTMP), polyethylene and aluminium. Biodeterioration of LPB bales (1 m^3) was studied under different conditions during storage of 18 months (III). Biodeterioration of recovered fuel (REF) was studied during storage of three months. REF is defined as source-separated, dry and solid waste, which is suitable for incineration. Dry wastes, which are collected from municipalities and enterprises, are processed into REF and used as fuel (III, Kallunki et al. 2002).
Eight LPB bales were stored in four different types of conditions. One badly deteriorated old bale and unused LPB were studied as reference samples (Table 2). Measurements were done in two different periods: from February 1995 to September 1996 and from September 1996 to October 1997. The storage shed was available only during the first period. Temperature, humidity and concentration of CO_2 were measured in the bales. Samples of LPB were taken after 6 and 18 months storage. Moisture content of board was measured and board samples were air-dried and homogenised through a 0.5 mm sieve for measurement of pH, conductivity and ash content (III).

Board was fractionated by humic extraction. Elemental (C, H, N) and carbohydrate analyses were done from board and humic fractions (III, IV). Methods for both analyses are described in section 2.3.

TABLE 1 Summary of analyses made and samples studied in papers I-VI and the relevant literature.

	Analysis	Samples	Age of samples	Reference for methods:
I	Temperature, concentration of O_2, moisture and ash contents, pH, conductivity, volatile substances and microbiological, elemental (C, H, N, O), nutrient, heavy metal and humic analyses	Windrow compost	1 week to 1.5 years	Anon. 1993 Greenberg et al. 1985 DIN 51720 Hänninen et al. 1995
II	Temperature, concentrations of O_2, CO_2, CH_4, and H_2S, moisture and ash contents, pH, conductivity and nutrient, heavy metal and humic analyses	Pilot-scale drum-composted catering waste using ash as a composting additive. Compost mixtures: CM I 0% ash; CM II 10% ash; CM III 20% ash	0 day to 2 years	Anon. 1993 Hänninen et al. 1995
III	Temperature, humidity and concentration of CO_2 inside LPB bales. Moisture and ash contents, pH, conductivity, total carbohydrate content and elemental (C, H, N), humic and microbiological analyses,	Eight waste liquid packaging board (LPB) bales stored under different conditions. Badly deterioratirated LPB bale Unused liquid packaging board	Storage time 6 and 18 months	Hänninen et al. 1995 SCAN-P 14:65 SCAN-P 15:90 Safarik & Santruckova 1992
IV	Monosaccharide analysis by GC/FID, organic matter content	Unfractionated waste LPB and its humic extraction fractions. Unfractionated windrow compost and its humic extraction fractions	stored 6 and 18 months 1 week to 1.5 years	Safarik & Santruckova 1992 Cheshire 1977
V	Analysis of humic substances, organic matter content, alkaline hydrolysis, elemental analysis (C, H, N)	Cellulose, lignin, milled peat, interglacial peat (IGP), brown coal and black coal. HA fraction extracted from soil, milled peat, interglacial peat (IGP), brown coal and black coal	700 to 100,000,000 years	Hänninen et al. 1995 Hänninen & Niemelä 1991
VI	Analysis of humic substances, organic matter content, alkaline hydrolysis, elemental analysis (C, H, N), solubility experiment	Cellulose, lignin, cellulose compost and catering waste compost HA fraction of cellulose compost and catering waste compost	1 week to 3.8 years	Hänninen et al. 1995 Hänninen & Niemelä 1991

TABLE 2 Storage conditions for LPB bales (III).

Pair 1/Bale 1	Inside the storage shed, (temperature 20°C), watered 5 litres/week
Pair 2/Bale 2	Inside the storage shed (temperature 20°C)
Pair 3/Bale 3	Outside in the open air
Pair 4/Bale 4	Outside in the open air, shielded with plastic film
Old deteriorated bale	Outside in the open air

A biodeterioration experiment on REF was carried out between September and November 2001 at Mustankorkea Waste Station, Jyväskylä, Finland. REF was stored outdoors in one windrow (50 m^3) and two bales (1 m^3). One bale was unshielded, while the other was shielded with plastic film. Temperature was measured inside the windrow and inside the bales. Samples for chemical analysis were taken at the outset of the experiment and after two and three months. Moisture and ash contents, pH, conductivity, and elements C, H and N were determined (Kallunki et al. 2002).

2.2 Composting of catering waste and cellulose (I, II, IV, VI)

Composting of catering wastes comprising source-separated wet kitchen biowaste was studied by large- and pilot-scale composting in windrows and a drum, respectively. An important research objective in the drum composting experiment was to study the use of ash as a composting additive. Lignin-free compost was produced by composting of fully bleached pulp (cellulose) with polypropylene bottle caps as bulking agent. The efficiency of different composting processes was studied by temperature and gas measurements during composting and by determinations of moisture content, pH, conductivity, ash content, nutrients and heavy metals in the compost mass at regular intervals. Humic substances were quantified at different stages during the composting and they were studied by elemental (N, C, H) and carbohydrate analysis and by quantifying hydrophilic and hydrophobic degradation products of alkaline hydrolysis (I, II, IV, VI).

Large-scale windrow composting (I)

Source-separated catering waste was composted in open windrows at Mustankorkea Waste Station in Jyväskylä, Finland, from June 1996 to December 1998. Catering waste was collected in the City of Jyväskylä. The initial composting area was a 3000 m^2 asphalt field, and later, in November 1996, a further 7000 m^2 of asphalt field became available. The area of the curing fields was 7000 m^2. Windrow composting was improved during the experiment by changing the process parameters (Table 3). All windrows were designated according to when they were piled up: month, first or second windrow of the month and year (e.g. March II (97)). Curing piles were designated with successive numbering (e.g. Curing pile 2).

The composting process was followed by measurement of temperature and, after September 1997, also by measurement of O_2 concentration inside the windrows. Samples for chemical and microbiological determinations were taken monthly or bimonthly for determination of moisture content, pH and conductivity and ash content.

Soluble and total nutrients and heavy metals were determined at a commercial laboratory (Viljavuuspalvelu Ltd, Mikkeli, Finland) during composting and maturation. Microbial determinations (Salmonella, faecal chain coccus and thermophilic faecal coliform bacteria) were determined in a commercial laboratory (City of Jyväskylä Environmental Laboratory, Finland). $ECEC_{om}$ analysis was carried out in two separate windrows, Jul III (97) (age 1 to 9 weeks) and Oct I&II (97) (age 2 to 9 weeks), and in Curing pile 5 (97) (age 24 to 58 weeks). Analysis was made at the University of Joensuu according to Saharinen, 1998.

Quantification of humic substances was done for samples of composts aged one week to 1.5 years. Elemental (C, H, N) and carbohydrate determinations were carried out on compost samples and their humic fractions. Alkaline hydrolysis was applied to humic acids extracted from composts matured one-week- and 1.5 years. Methods for these analyses are described in section 2.3.

TABLE 3 Modification of process parameters during the experiment.

Composting parameter	Phase 1 Jun. 1996 to Sept. 1996	Phase 2 Sept. 1996 to Jun. 1997	Phase 3 Jun. 1997 to Aug. 1998
Height of windrow (m)	2.5	1.5 – 2.0	1.0 – 1.5
Bulking agent	Wood chips and peat	Bark	Waste wood chips
Ratio of catering waste to bulking agent (w/v)	1 / 1	1 / 2	1 / 1
Turning frequency	After 4 weeks	At initial blending After 1 week After 3 to 10 weeks	Once a week during the first month ➔ 2 low windrows were combined to a larger pile
Piling up the windrow	With a wheel loader	With a wheel loader	Catering waste was crushed on the field ➔ blended with the bulking agent with a screener-crusher bucket.

Pilot-scale drum composting (II)

Use of ash as an additive during composting of catering wastes was an important factor for study in the drum composting experiment. Bottom ash was supplied from a small district heating plant at Virrat, Finland. The plant utilises

wood chips, sod peat, residues from the plywood industry, along with waste-derived fuel. Target value for the waste fuel was 10% of the total fuel value. The composting experiment was carried out in a pilot-scale drum composter (volume 10 m^3) at the facilities of Erkki Salminen Ltd Waste Management Company in Jämsänkoski in Central Finland between January and April, 2000.

Source-separated catering waste was collected from communities of Jämsä and Jämsänkoski in Central Finland. Peat and wood chips were used as bulking agent in ratio 1/1, and the compost mass was made by mixing source-separated catering waste with bulking agent in ratio 1/1. The ash comprised 0, 10 and 20 w% of the compost mass, with the obtained compost mixes (CMs) designated I, II and III, respectively. The CM I, without ash, was used as a reference compost.

A charge of about 3 m^3 of compost mix was fed to the drum once a week, with the loading of the drum kept at 6 m^3. The compost was aerated with air blown from a tube at the bottom of the drum and mixed once a day by turning of the drum hydraulically. Nine cubic metres of each compost mix was treated in the drum. After the drum-composting phase (2 weeks), three heaps were formed from masses of the three mixes.

Concentrations of O_2, CO_2, H_2S and CH_4 were measured by IR-gas analyser (GA 94, Geotechnical Instruments Ltd., England) during the drum composting phase, and the temperature was measured in both the drum composting and maturation phases. Samples for chemical analysis were taken before and after the drum composting phase and at regular intervals during the maturation phase. Moisture content, pH, conductivity and ash content were measured. Soluble and total nutrients and heavy metals were determined at a commercial laboratory (Viljavuuspalvelu Ltd, Mikkeli, Finland) during composting and maturation.

Humic substances were extracted from compost mixes at regular intervals starting with the initial compost mixtures and ending with mixes that had matured for two years. The method is described in section 2.3.

Earlier, in Jyväskylä in 1995, a pilot-scale drum composting experiment was made for the composting of source-separated catering waste. Catering waste was composted with peat in a drum for two weeks and then allowed to mature in pile for seven years. Compost samples that had been matured for three and seven years were used in a study of humic substances (Kovanen & Hänninen 1996).

Cellulose compost (VI)

Fully bleached solid chemical pulp made from birch wood was collected from M-Real Äänekoski Paper, Finland in 1998. Solid pulp (dm 95%) is the starting material for paper and board manufacture. Residual lignin is removed from pulp during the bleaching process. The main constituents of fully bleached chemical pulps are cellulose and hemicelluloses; only traces of lignin (<0.1%) remain (Sjöström & Westmark 1999). Later in this text, fully bleached pulp is referred to as cellulose.

Cellulose was composted in 250-l composting equipment for 3.8 years. Dry pulp (20 kg) was shredded to 12 × 12 cm pieces and wetted in a nutrient solution. Nitrogen content of the solution was increased by the addition of urea to obtain a C/N ratio of 30. Plastic bottle caps made from polypropylene were used as bulking agent during composting. The compost mass was mixed at regular intervals; water, urea, and cellulose pieces were added as needed. The composting process was followed with measurement of temperature.

After composting of 1.8 and 3.8 years, samples were taken for further analysis. The compost mass was air-dried and polypropylene caps were removed before grinding of the mass through a 0.5 × 0.5 mm sieve. Humic substances were extracted and alkaline hydrolysis was carried out on the humic acid fraction of a cellulose compost matured 1.8 years. Methods for these analyses are described in section 2.3.

2.3 Humification study (I, II, III, IV, V,VI)

Samples for humification study (I, II, V, VI)

Composting was employed as a synthetic process for producing humic substances in their early stage. Humic substances were extracted from windrow composts, pilot-scale drum composts and cellulose composts of different age.

Milled peat was collected from the Haukineva mire in Peräseinäjoki, Western Finland, and botanical analyses were carried out at the University of Helsinki. Owing to milling and extensive humification, only ca. 30% of cells were identifiable. Of these, 70% were coniferous tree cells, 10% deciduous tree cells, 10% eriophorum cells, 6% moss (*Sphagnum*) cells and 4% carex cells. The degree of humification was estimated to be H_{6-7} by a modification of the von Post procedure developed by Puustjärvi for dry peat samples. Radiocarbon dating showed the peat to be older than 2000 years (Hänninen et al. 1993).

Interglacial peat (IGP) was collected from Vesiperä at Haapavesi, Finland. It was dug out from an organic deposit 30 cm thick, lying above the ground water table and beneath a 3-metre-thick layer of till. The peat was composed of rich forest mull soil with spruce twigs and fragments of alder and a considerable proportion of *Sphagnum* spores. Pollen analysis indicated that the soil was formed during the Eemian Interglacial ca. 100 000 years ago. Radiocarbon analysis of the mull, wood and root material defined the age of the deposit as between ca. 40,000 yr BP to 48,800 yr BP (Hänninen & Kankainen 1992).

Polish brown coal and Polish black coal samples were collected from bulk coal imported to Finland by Imatran Voima Ltd in 1987 for use in its energy plants (Hänninen & Paajanen 1989).

Sulphate lignin (lignin) precipitated from black liquor, collected from the Department of Applied Chemistry at the University of Jyväskylä in 1999, and

cellulose (fully bleached solid chemical pulp made from birch wood) were used as reference materials without extraction of humic acids. These reference materials were chosen to allow comparison of the contributions of carbohydrates and lignin in humification.

Extraction of humic substances (I, II, III, V, VI)

Humic substances (humic acid (HA), fulvic acid (FA) and humin) were extracted from samples of windrow composts (age 1 week to 1.5 years), pilot-scale drum composts with ash as a composing additive (age 0 weeks to 2 years), pilot-scale drum composts (age 3 and 7 years), cellulose composts (age 1.8 and 3 years) and waste liquid packaging board (age 6 and 18 months) according to Hänninen et al. (1995) (Fig. 2, I, II, III, VI). The extraction method for peat and brown coal samples was similar but without the hot water extraction. The black coal sample was heated at 200 °C in a heating box and then twice with 1.0 M NaOH in an autoclave for four hours at 170 °C in order to obtain the regenerated humic acid (V, Hänninen & Paajanen 1989).

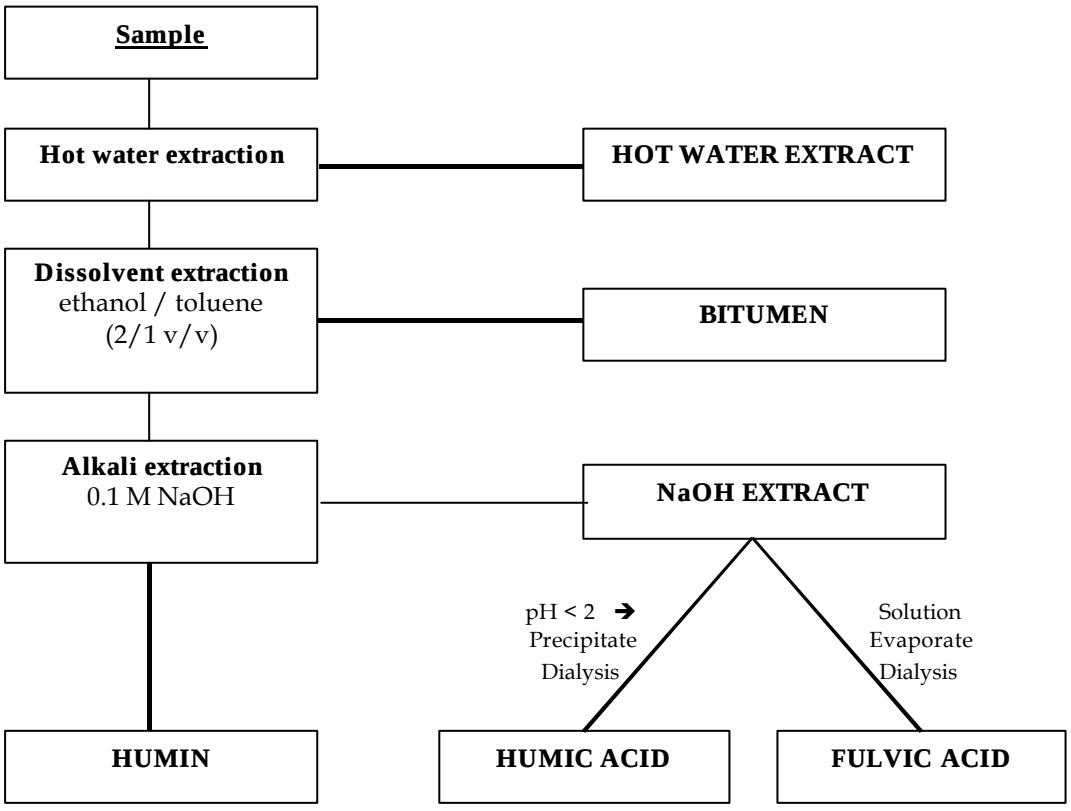


FIGURE 2 Extraction of humic substances (Hänninen et al. 1995).

Humic fractions are determined operationally; they are labelled according to their solubility in base and acid solutions (Hayes et al. 1989). The base-soluble acid-insoluble fraction is called humic acid (HA), and the base- and acid-soluble

fraction is called fulvic acid (FA). The fraction insoluble in both base and acid solvents is humin.

LPB samples are in the initial stage of humification and unused LPB can be taken as non-humic. The humic fraction of LPB is designated as residual fibre, while the extractioned fractions of unused LPB are named as follows: the base-soluble acid-insoluble fraction is a non-humic humic acid analogue (NHNAA), the acid- and base-soluble fraction is a non-humic fulvic acid analogue (NHFAA), and the insoluble fraction is the residual fibre (IV).

Elemental analysis and solubility experiment (I, III, IV, VI)

Elemental analyses (nitrogen, carbon and hydrogen) of cellulose and lignin and of compost, waste liquid packaging board (LPB), peat and coal and their humic fractions were carried out with a Fison Instruments EA 1110 Analyzer (I, III, V, VI). LPB samples stored for six months were analysed with a Fison Instruments EA 1108 CHN Analyzer (VI). Samples were air-dried and homogenised by grinding them through a 0.5 mm sieve. Results were expressed as weight per cent organic matter basis.

Solubilities in distilled water (neutral pH value) and diethyl ether were determined for humic acids of compost matured three years, milled peat, and brown coal and for hydrolysis residues of these humic acids. The amount of sample was 50 mg, and this was mixed with 50 ml of distilled water or diethyl ether. The mixture was left on a mechanical shaker (Heidolph Polymax 2040, revolution 50 rpm/min) for one hour and then vacuum-filtered on a tared Whatman GF/A glass fibre filter (approximate pore size 1.6 μm). The filter was dried in an oven at 60°C for 16 hours and weighed. The amounts of humic acids and hydrolysis residues passing through the Whatman GF/A filter after mixing with distilled water or diethyl ether are presented as percentage of the original sample amount (VI).

Alkaline hydrolysis of humic acids (V, VI)

Humic acid samples were degraded by alkaline hydrolysis at elevated temperature and pressure. This degradation method was chosen because it preserves aliphatic structures.

Alkaline hydrolysis was done to cellulose and lignin, and to HAs extracted from catering waste compost (age 1 week, 1.5 and 3 years), cellulose compost (age 1.8 years), peat (age 2000 and 60,000 years) and coal (age 1,000,000 and 100,000,000 years).

Humic acids (250 mg) were autoclaved in 2 M NaOH solution in nitrogen atmosphere at 170 °C for two hours (Hänninen & Niemelä 1991). After hydrolysis, internal standards (meso-erythrol for hydrophilic phase and 2-hydroxy-3-methoxybenzaldehyde for hydrophobic phase) were added to the solution. Na^+ ions in the solution were exchanged to H^+ ions in Dowex 50 ion-exchange resin, and pH was adjusted to 1.5. Precipitate, labelled here as

hydrolysis residue, formed during 16 hours standing. It had similar solubility to the original HA sample and was removed by centrifugation. The supernatant (a water solution) was extracted with diethyl ether (3 × 50 ml). After the extractions, both hydrophilic (water) and hydrophobic (diethyl ether) phases were analysed.

Model compounds for the hydrophilic and hydrophobic phases, with their response factors, are listed in Table 4. Hydrophilic standards (100 μg) were prepared in distilled water. pH of the hydrophilic standard mixture was adjusted to 1.5 and the mixture was kept at room temperature overnight to allow the lactones to develop. The mixture was then evaporated to dryness under vacuum; the residue was diluted to 1 ml of pyridine and silylated with 250 μl N,O-bis(trimethylsilyl)trifluoroacetamide with 1% (by volume) trimethylchloro-silane (BSTFA). Hydrophobic standards (15 μg) were prepared in dried ethyl ether. The hydrophobic standard mixture was evaporated to dryness under nitrogen, diluted in 0.5 ml pyridine and silylated with 350 μl of BSTFA.

TABLE 4 List of model compounds used for alkaline hydrolysis, with their response factors.

Hydrophilic phase	R	Hydrophobic phase	R
lactic acid	1.4	phenol	4.1
α-hydroxyisobutyric acid	1.3	p-cresol (2-methylphenol)	1.7
glycolic acid	1.1	guaiacol (2-methoxyphenol)	3.4
2-hydroxybutyric acid	1.5	catechol (1,2-dihydroxybenzene)	0.7
levulinic acid	3.2	benzeneacetic acid	0.8
oxalic acid	1.5	4-hydroxybenzalehyde	0.9
malonic acid	1.3	resorcinol (1,3-dihydroxybenzene)	0.5
glycerol	1.1	4-hydroxyacetophenone	0.8
succinic acid	1.1	vanillin (4-hydroxy-3-methoxy-benzaldehyde)	0.8
methylsuccinic acid	1.2	3-hydroxybenzoic acid	0.5
fumaric acid	1.0	3,4-dihydroxy-benzaldehyde + acetovanillone	0.8
glutaric acid	1.4	4-hydroxybenzoic acid	0.2
malic acid	1.0	4-hydroxyphenylacetic acid	0.4
		phthalic acid (1,2-benzenedicarboxylic acid)	0.5
		vanillic acid (4-hydroxy -3-methoxybenzoic acid)	0.5
		3,4-dihydroxyben-zoic + 3,5-dihydroxybenzoic acids	0.4

The hydrophilic phase from alkaline hydrolysis was concentrated to 50 ml under vacuum and an aliquot of 5 ml was taken for chromatographic analysis. This was evaporated to dryness under vacuum and silylated as the hydrophilic standard mixture. The hydrophobic phase from alkaline hydrolysis was dried with Na_2SO_4 and concentrated to 25 ml, and an aliquot of 5 ml was taken for further analysis. The solution was evaporated to dryness with N_2 gas and then treated with BSTFA as the hydrophobic standard mixture.

Degradation products were analysed by the internal standard method with an HP 6890 gas chromatograph equipped with an HP 5973 mass detector. The stationary phase on the column was HP-5MS 5% phenyl methyl siloxane, its length was 30 m, nominal diameter 250 μm, and film thickness of the stationary phase 0.25 μm. The MS detector was run in scan mode (mass range *m/z* 50–550). 3,4-Dihydroxybenzaldehyde and acetovanillin and also 3,4-dihydroxybenzoic and 3,5-dihydroxybenzoic acids had similar retention times and these compounds were quantified together as 3,4-dihydroxybenzaldehyde + acetovanillin and 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid.

Unknown peaks from both phases were identified through a mass spectral library (Wiley 275) search. Peaks whose quality factor exceeded 90 in compost HAs and 80 in peat and coal HAs were accepted for further study. Amino acids were identified in the hydrophilic phase.

Carbohydrate analysis (IV)

Quantitative monosaccharide analysis was carried out for bark, windrow compost samples (composted 1, 23 and 36 weeks) and waste LPB board stored for 6 and 18 months, and for unused LPB and badly deteriorated LPB and their humic fractions (HWE, HA, FA and humin or residual fibre fraction).

Samples (12–25 mg) were hydrolysed according to Cheshire (1979). After hydrolysis the solution was filtered and diluted to 100 ml with distilled water. A subsample of 20 ml was taken for monosaccharide (L-arabinose, D-ribose, D-xylose, L-fucose, D-mannose, D-fructose, D-galactose and D-glucose) analysis and internal standard (myo-inositol) was added. SO_4^{2-} ions were removed by Amberlite IRA-68 ion-exchange resin treatment (Ristolainen 1999). The solution was evaporated to dryness under vacuum. The residue was diluted with 2 ml of pyridine and silylated with 500 μl of BSTFA.

Monosaccharides were quantified by internal standard method with a Perkin-Elmer Autosystem XL gas chromatograph equipped with flame ionisation (FI) detector and NB-54 column. Some peaks of the monosaccharides were overlapping and those monoshaccarides were quantified together. The mass-based response factors between the peak of myo-inositol and the peaks derived from the different monosaccharides were as follows: L-arabinose + D-ribose 1.23, D-xylose + L-fucose 1.53, D-mannose + D-fructose 1.38, D-galactose 2.00 + D-glucose 1.23.

3 RESULTS

3.1 Biodeterioration of cellulose (III, IV)

Waste liquid packaging board (LPB) and recovered fuel (REF) were stored under different conditions for study of their biodeterioration. The main components of LPB and REF are made from cellulose. Storage experiments lasted 18 months for waste LPB and 3 months for REF. Measurements of temperature, moisture and ash content and elemental analysis (N, C, H) were carried out on both sample matrices. In addition, waste LPB board stored for 6 and 18 months was fractionated by humic extractions, and carbohydrate analysis was carried out on board and the extracted fractions.

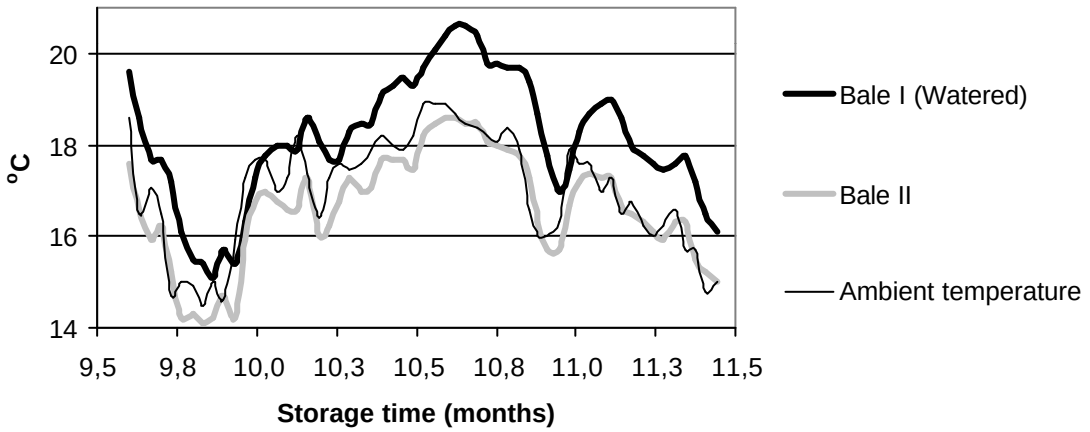


FIGURE 3 Temperature curves of watered and non-watered LPB bales stored inside the shed between 18 Dec. 1995 and 2 Feb. 1996.

Temperatures inside the LPB bales changed in response to the external conditions, with a short time delay (Fig. 3). In general, temperatures were about 2 °C higher in the watered than in the non-watered LPB bale stored inside the

shed. Temperature of the non-watered bale was near the ambient temperature (III).

After storage for 1.3 months, the temperature of the unshielded REF bale rose to 30 °C, while the ambient temperature was 7 °C (Fig. 4 a). According to temperature readings from the REF windrow, biodeterioration was effective in the windrow, and temperatures were at the same level as in composting (Fig. 4 b). The temperature difference was wider between the two REF bales that were stored outdoors than between the LPB bales stored inside the shed. The temperature increase indicates biodeterioration in watered LPB bale and in REF bales and windrow.

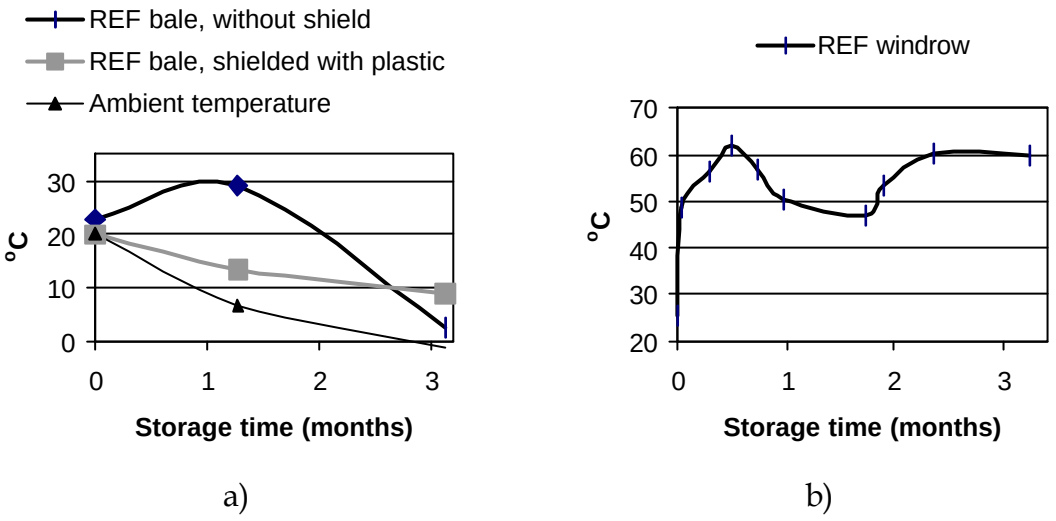


FIGURE 4 Temperatures during storage of three months. a) REF bales stored with and without plastic shield and b) REF windrow.

The non-watered LPB bale inside the storage shed dried during storage of 13 months the moisture content measured inside the bale was only 7%. Humidity measured inside the bale decreased during 11 months from 90% to 60%. Humidity of the other LPB bales was over 95%. After storage of 18 months, moisture content of waste LPB board in different bales varied from 5.1% to 52.6%, and it was 64.7% in the badly deteriorated bale. Concentration of CO_2 was high in those LPB bales with high moisture content and near background concentration in the non-watered bale inside the shed, which had lowest moisture content (Table 5). Owing to the aluminium film, the average ash content of the LPB material was relatively high: 10.6% (III).

Yields of hot water extracts (HWE) were slightly higher in waste LPB board (bales 1 to 4) than in the unused LPB, but amounts of bitumens, humic acids (HA) and fulvic acids (FA) were nearly on the same level in waste LPB board (bales 1 to 4) as in unused LPB (Table 6). The yields of the HWE, bitumen, HA and FA extracts was highest for the badly deteriorated old bale. Extracts from the badly biodeteriorated old bale were darker in external appearance than extracts from the other bales (III).

TABLE 5 Concentrations of CO_2 (%) inside the waste LPB bales (III).

Storage time (months)	7.5	9.5	11	17
Bale 1	2.46	2.47	3.21	3.52
Bale 2	0.52	0.23	0.23	0.25
Bale 3	1.38	0.28	0.18	5.99
Bale 4	2.19	0.46	0.36	2.98
Old bale*	0.75	1.36	0.56	0.47
Background	-	0.19	0.15	0.21

Bale 1 = watered, inside the shed; Bale 2 = non-watered, inside the shed; Bale 3 = in the open air; Bale 4 = in the open air, shielded with plastic film; * badly deteriorated bale

TABLE 6 Variation in the amounts of humic extracts from waste LPB board stored under different conditions (bales 1 to 4); badly deteriorated LPB (old bale) and unused LPB (% OM).

	Hot water extract		Bitumen		Humic acid		Fulvic acid
Storage time (months)	6	18	6	18	6	18	6
Bales 1 to 4	1.8 – 3.7	2.1 – 3.5	1.1 – 1.3	1.0 – 1.6	0.3 – 0.6	0.2 – 0.6	0.1 – 0.2
Old LBP bale*	4.0	2.5	2.3	1.3	2.2	0.5	0.6
Unused LPB	1.5		1.1		0.2		0.2

Bale 1 = watered, inside the shed; Bale 2 = non-watered, inside the shed; Bale 3 = in the open air; Bale 4 = in the open air, shielded with plastic film; * badly deteriorated bale

TABLE 7 Variation in the results of elemental analysis (N, C, H) caaried out on waste LPB stored under different conditions (bales 1 to 4), on badly deteriorated (old bale) LPB and on unused LPB. Board and HWE, HA, FA fractions were analysed.

	Nitrogen		Carbon		Hydrogen	
Storage time (month)	6	18	6	18	6	18
BOARD, w% OM						
Bales 1 to 4	< 0.1	0.2	48.5 – 51.7	57.8 – 61.6	6.9 – 7.8	8.3 – 9.2
Old LBP bale*	1.2	0.4	70.6	56.7	10.9	8.4
Unused LPB	0.2		52.1		7.6	7.0 – 8.6
HOT WATER EXTRACT, w% OM						
Bales 1 to 4	0.9 – 1.5	1.0 – 1.8	45.2 – 46.1	44.2 – 47.8	6.7 – 7.8	
Old LBP bale*	4.5	4.6	45.5	46.7	6.7	7.3
Unused LPB	1.3		41.5		6.6	
HUMIC ACID, w% freeze-dried matter						
Bales 1 to 4	0.8 – 0.9	0.8 – 1.3	44.6 – 46.5	46.3 – 51.1	5.7 – 6.0	5.4 -5.8
Old LBP bale*	4.1	3.4	46.6	49.2	5.8	6.2
Unused LPB	0.7		44.8		6.7	
FULVIC ACID, w% freeze-dried matter						
Bales 1 to 4	0.3 – 1.3	0.6 – 0.9	37.0 – 45.1	30.8 – 36.8	5.1 – 6.3	4.0 – 4.4
Old LBP bale*	3.8	2.3	43.1	37.8	5.6	4.9
Unused LPB	1.0		47.2		7.6	

Bale 1 = watered, inside the shed; Bale 2 = non-watered, inside the shed; Bale 3 = in the open air; Bale 4 = in the open air, shielded with plastic film; * badly deteriorated bale

TABLE 8 Variation in the results of carbohydrate analysis (xylose & fucose, mannose & fructose, galactose, glucose) of waste LPB stored under different conditions (bales 1 to 4), of badly deteriorated (old bale) LPB and unused LPB. Board, HWE, HA, and residual fibre fractions were analysed, % OM.

	D-xylose + L-fucose		D-mannose + D-fructose		D-galactose		D-glucose	
Storage time (month)	6	18	6	18	6	18	6	18
BOARD								
Bales 1 to 4	8.1 – 9.5	4.3 – 8.2	4.6 – 5.9	3.2 – 4.0	0.7 – 1.2	0.6 – 1.0	53.0 –58.2	24.8 – 50.2
Old bale*	1.6	6.8	2.3	4.0	1.0	1.0	14.2	47.9
Unused LPB	12.7		3.7		0.4		61.3	
HOT WATER EXTRACT								
Bales 1 to 4	3.8 – 5.0	3.1 – 4.2	0.6 – 0.1	0.7 – 2.1	2.3 – 0.9	1.4 – 3.4	4.1 – 7.1	3.8 – 7.7
Old bale*	2.0	3.2	1.1	1.6	2.1	1.8	5.3	5.0
Unused LPB	4.6		0.4		0.4		32.1	
HUMIC ACID								
Bales 1 to 4	7.2 – 9.1	1.1 – 2.0	2.9 – 3.8	1.0 – 1.4	3.3 – 4.8	0.8 – 2.6	22.1 – 32.2	3.0 – 20.2
Old bale*	1.8	2.2	2.3	1.5	1.6	1.5	7.9	8.6
Unused LPB#	17.0		1.9		4.4		33.5	
RESIDUAL FIBRE								
Bales 1 to 4	8.0 – 8.8	4.9 – 8.5	4.7 – 5.1	4.2 – 5.5	0.4 – 0.6	0.5 – 0.6	55.7 – 59.2	44.0 – 55.8
Old bale*	1.6	8.5	2.1	5.0	0.5	0.3	15.9	61.4
Unused LPB	13.3		4.1		0.3		63.6	

Bale 1 = watered, inside the shed; Bale 2 = non-watered, inside the shed; Bale 3 = in the open air; Bale 4 = in the open air, shielded with plastic film; * badly deteriorated bale; #non-humic humic acid analogue

Nitrogen concentrations were higher in HWE, HA and FA fractions than in waste LPB board (Table 7) and highest in the badly deteriorated old bale, in all extraction fractions (board, HWE, HA, FA). Concentration of carbon was higher in the board than in humic fractions. The high concentration in the board is partly due to the polyethylene coating of LPB. Both the nitrogen and the carbon concentrations increased in the HA fraction during storage (III). Concentration of nitrogen also increased in the REF windrow and in the unshielded REF bale during storage for three months. The increase was from 1.1 to 1.4 w% OM and from 0.9 to 1.1 w% OM in windrow and bale, respectively (Kallunki et al. 2002).

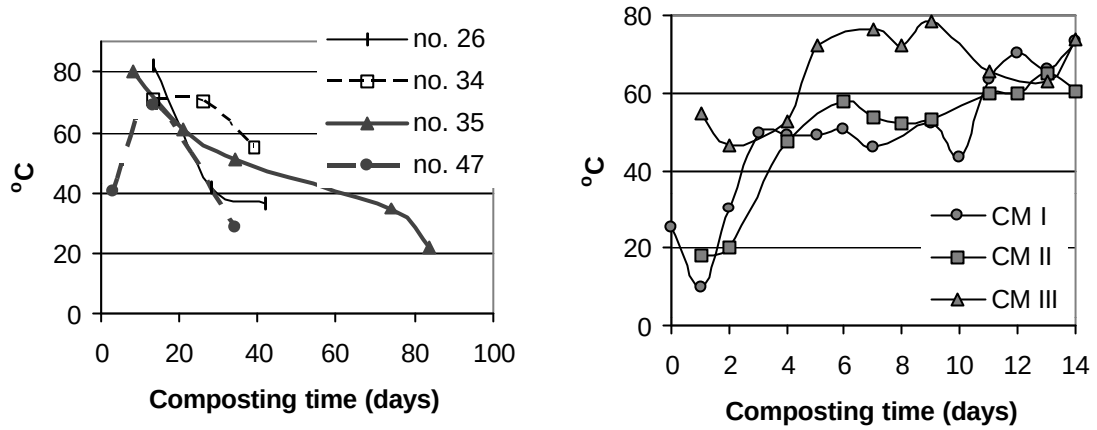
Table 8 shows the results of the carbohydrate analysis of waste LPB board and humic extracts after storage of 6 and 18 months. Concentrations of xylose + fucose, mannose + fructose, and glucose were higher in board and residual fibre fractions owing to the high content of pulp fibres, but concentration of galactose was higher in the HWE and HA fractions. In general, concentrations of xylose + fucose and glucose were higher in unused LPB than in waste LPBs in all sample fractions (IV).

3.2 Composting of catering waste and cellulose (I, II, IV, VI)

Temperature and gas measurements (I, II, VI)

On average, temperatures of 55 to 65 °C were reached within two weeks in windrow composting of catering waste. In winter, because the catering waste and bulking agent were frozen, the delay time before composting began was three to five weeks. After this, the increase in the average composting temperature was dramatic. The temperature of low windrows (height from 1.0 to 1.5 m) rose to 85 °C within a few days of start-up, but temperatures decreased to below 50 °C within 30 to 40 days in spring and summer 1998 (Fig. 5 a, I).

The pilot-scale drum composting experiment was carried out in winter (outdoor temperature varying from -25 to +10 °C); however, temperatures increased during five days to over 50 °C at the feeding end of the drum. Temperatures were at all times relatively even, from 50 to 80 °C in the middle part and at the output end of the drum. The addition of ash in CM II and III did not appear to accelerate the start of the temperature rise, but CM III (containing 20% ash) showed a higher temperature of 80 °C in the middle section of the drum. One reason for this might be that the ash in CM III increased the heat capacity (Fig. 5 b, II).



a) b)

FIGURE 5 Composting temperatures during a) windrow composting (windrow no. 26 was built up on 20 Apr. 1998, no. 34 on 30 April 1998, no. 35 on 25 May 1998 and no. 47 on 4 Sept. 1998) and b) drum composting with ash as composting additive (CM I containing 0% ash, CM II 10% ash and CM III 20% ash) (I, II).

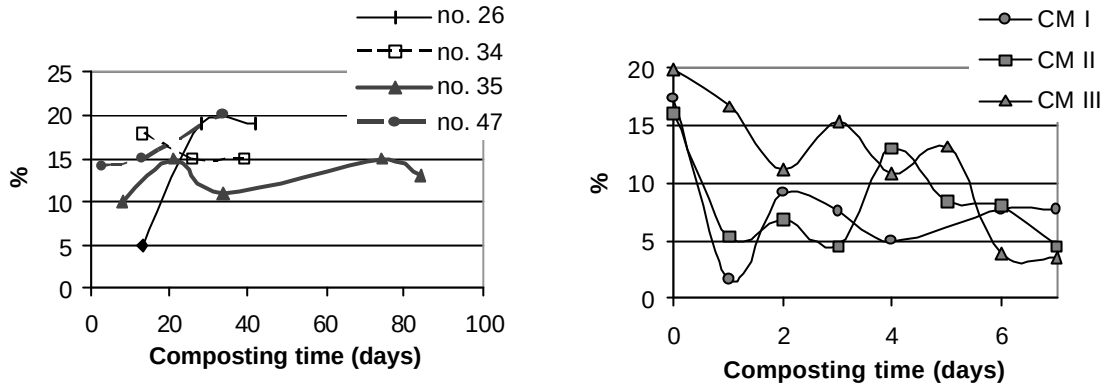
For maturation compost masses were combined to larger curing piles after composting in lower windrows. Temperatures continued to be high, about 60 to 70 °C, for three to six months, until they decreased to 40 to 50 °C (I). After the drum composting phase, temperatures decreased relatively quickly in curing heaps during the drum composting experiment. This may be a consequence of the small size of the heaps (9 m^3), so the loss of the heat was greater than in larger compost windrows (II). The temperature of the cellulose compost rose to 55°C during composting, but regular addition of urea or turning of the compost was required to maintain temperatures above 40 °C. The composting process was ceased during winter owing to the low ambient temperatures (VI).

Oxygen concentrations of small windrows (height 1.0 to 1.5 m) were relatively high, indicating sufficient aeration (Fig. 6 a). Adequate aeration of the windrows was guaranteed when the initial height of the windrow was less than 1.5 m and the blending ratio for catering waste and bulking agent was one tonne of catering waste to one cubic metre of wood chips (I).

Large and compact windrows made during the early phase of the project emitted odours when the windrows were turned. Temperatures were below 50 °C inside. To improve the aeration, more bulking agent was used, and this improved the process, which worked actively, without odour problems, during winter and spring 1997. Subsequently, in view of the high cost of bark, the ratio of catering waste and bulking agent was reduced back to 1/1 (w/v) and the bulking agent was changed to waste-wood chips. At the same time a crusher for catering waste was brought on line. The height of the windrows was also lowered from 2.0 m to 1.5 m. After these changes the composting process worked well, mainly without odour problems (I).

Oxygen concentration during the ash composting experiment varied from 3.5 to 19.8% in the middle section of the drum (Fig. 6 b). Highest values were

measured at the beginning of the composting and lowest values after seven days. CM III had the highest oxygen concentrations, except at the end of the measuring period. Addition of ash improved the availability of oxygen. The accelerated oxygen consumption also indicated accelerated composting (II). Concentration of carbon dioxide is inversely proportional to oxygen concentration.



a) b)

FIGURE 6 Oxygen concentrations during a) windrow composting (windrow no. 26 was built up on 20 Apr. 1998, no. 34 on 30 April 1998, no. 35 on 25 May 1998 and no. 47 on 4 Sept. 1998) and b) drum composting with composting additive (CM I containing 0% ash, CM II 10% ash and CM III 20% ash) (I, II).

Methane concentrations varied from 0 to 0.4%, being lowest in CM III and highest in CM II during drum composting phase. The concentration of hydrogen sulphide varied from 0 to 2.3 ppm. The concentration was at the same level, from 0 to 1 ppm, in CM II and CM III (II).

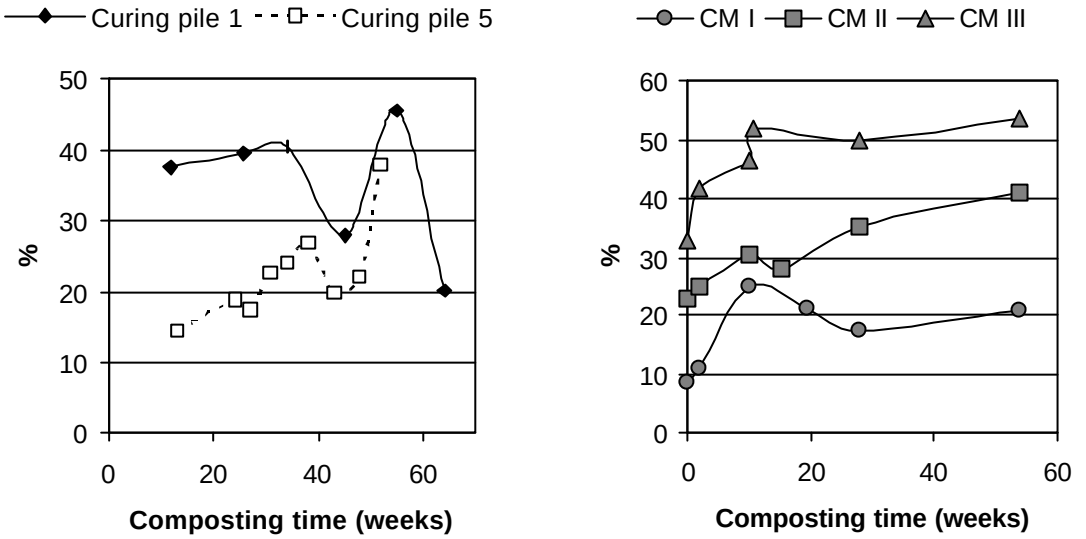
Moisture content and pH (I, II)

Moisture content of large windrows exceeded 70%. These windrows became compacted, which caused anaerobic zones to form inside the windrows, and some odour problems arose in the composting field. Proper moisture conditions were achieved when an adequate amount of bulking agent was used and the height of the windrows was reduced (I).

pH values were higher in the smaller windrows than in larger ones. An increase of pH from 4 to 6 persisted for four months in large windrows, while an increase of pH from 4 to 7 persisted for only 1.5 months in small windrows (I). Addition of ash raised pH values during the drum composting. At the output of the drum the pH of CM III was 2.8 units higher and that of CM II was 0.7 units higher than the pH of CM I. The pH value of CM I increased during the curing phase. Finally, there were no marked differences in the pH values between various CMs in the curing heaps after maturation of eight weeks (II).

Carbon mineralisation (I, II)

Mineralisation of organic matter in windrow composting is a relatively slow process. Doubling of the ash content took from six to nine months (Fig. 7 a, I). Mineralisation seemed to be faster in the drum composting experiment where ash was used as composting additive. Increase in ash content of the compost or degradation of the organic matter was considerably faster in CM III (containing 20% ash) and CM II (containing 10% ash) than in CM I (without ash addition). Measured in absolute percentage units, the ash increased by 12% in CM I, 18% in CM II, and 20% in CM III during composting of one year.



a) b)

FIGURE 7 a) Ash content of two curing piles and b) ash content during drum composting, where ash was used as composting additive (CM I 0% ash, CM II 10 % ash, CM III 20% ash).

Application of catering waste compost (I, II)

The guideline value for the conductivity of mature compost is below 4 mS/cm (Anon., 1992). The average value of the conductivity was 4.4 mS/cm in small windrows and 2.6 mS/cm in large windrows (I). The conductivity varied in the ash composting experiment as follows: CM I from 0.43 to 3 mS/cm, CM II from 0.61 to 4.11 mS/cm, and CM III from 1.32 to 2.95 mS/cm. The lowest values in compost mixes were measured in the samples from composts matured one year (II). A general disadvantage of compost as fertiliser if the conductivity is too high is weakening of the water intake of plants through too high salt concentrations. Conductivities can be reduced by blending compost with mineral soil.

Commercial soil amendments (such as catering waste compost) for use in Finland must not contain more than specified concentrations of arsenic (As) and

heavy metals (Hg, Cd, As, Ni, Pb, Cu and Zn). Concentrations of heavy metals in windrow composting were considerably below the concentration limits (I). Addition of ash increased the heavy metal concentrations of CM II and CM III, but the concentrations were not so high as to restrict the use of either CM. Measured against guideline values in an EC working document for Biological Treatment of Biowaste, the concentration of cadmium was too high (II, Anon. 2001).

Water-soluble calcium and magnesium concentrations decreased during windrow composting. The concentration of NO_3-nitrogen was low in all windrow compost samples except the sample from a curing pile cured for 25 weeks. NH_4-nitrogen varied from <10 to 1510 mg/g of dry matter. Low nitrate levels in conjunction with high ammonium concentrations indicate instability and potentially high microbial activity (I, Leege et al. 1998). After composting for four months, total nitrogen content was lower in CM II and CM III, which contained ash, than in CM I without ash. Differences between total nitrogen content in different compost mixes diminished during maturation. When the pH and temperature of the compost increased, the evaporation of ammonia increased. Total concentrations of phosphorus were higher in CM II and III, but the phosphorus was in sparingly soluble form (II).

According to Saharinen (1998), city refuse compost is mature when the cation exchange capacity (CEC) is over 60–67 meq/100g. $ECEC_{om}$ values increased during composting (Fig. 8). The increase was 10 and 15 cmol+/kg during composting of nine weeks in typical windrows. These values indicate that the composting had begun rapidly. The values for curing pile increased from 49 to 83 cmol+/kg OM indicating that the maturation had proceeded well.

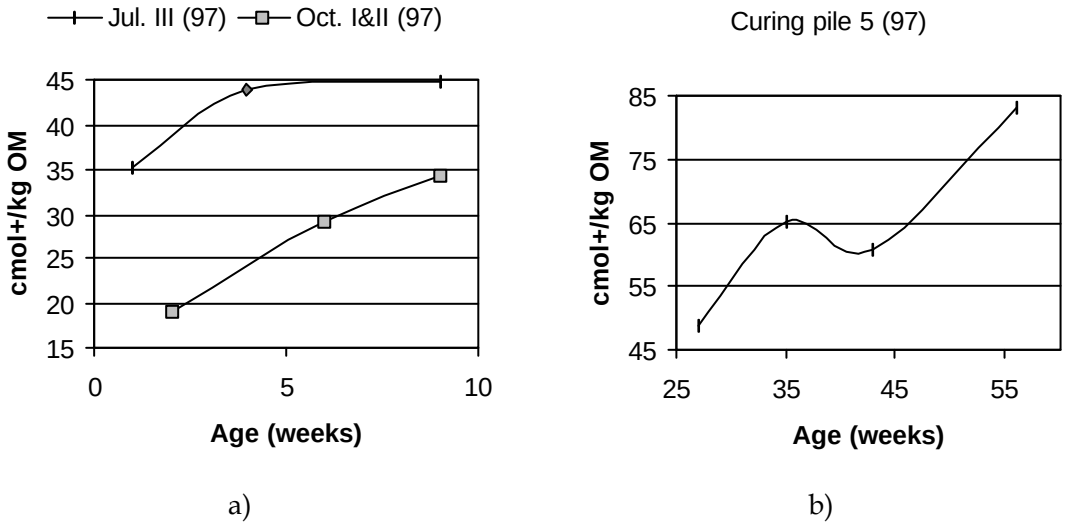


FIGURE 8 $ECEC_{om}$ data from a) two windrows and b) a curing pile from the windrow composting experiment.

As measured by pathogenic microbes (*Salmonella*), windrow composts were at all times hygienic. *Salmonella* was found in only two of 36 samples. This result was as expected since food in Finland rarely contains *Salmonella*. The number of

indicator microbes of pathogenicity (*faecal chain coccus* and *thermophilic faecal coliform bacteria*) was high throughout the first year of composting even though the temperature of the large windrows exceeded 60°C for a very long time. In smaller windrows made since summer 1997, the number of these microbes decreased during the process, and the windrows were sanitised fairly quickly. The number of indicator microbes decreased as expected when the catering waste was crushed with a screener-crusher bucket that was not used elsewhere in the landfill (I).

3.3 Humification in the early stages (I, II, IV, VI)

Extraction of humic substances (I, II, VI)

The amounts of hot water extracts (HWE), bitumen, humic acids and humin extracted from compost samples are shown in Table 9. The yield of HWE remained almost the same, while bitumen decreased slightly during windrow composting of 1.5 years (I). Yields of HWE and bitumen decreased sharply in CMs during the first year of maturation in the drum composting experiment where ash were used as composting additive (II). Yields of bitumen were lower in a catering waste compost that had matured for three years than in the same compost matured for seven years (VI).

Yields of HA increased during windrow composting but the increase was quite slow; the amounts of HA were noticeably increased after composting of nine months (I). In catering waste compost, where ash was used as composting additive, yields of HA clearly increased during maturation. Evidently the addition of ash boosted the humification process. The increase of HA was most rapid in CM III (containing 20% ash). After composting of six months the differences levelled off, but after 12 months CMs II and III contained clearly higher amounts of humic acids than CM I. Yields of HA stayed on the same level after composting of one year (II). The yields of HA increased in cellulose compost as well, being 1.3% OM in composts matured 1.8 years and 8.2% OM in 3.8 years (VI).

TABLE 9 Yields of hot water extract (HWE), bitumen, humic acid (HA) and humin extracted from different composts.

	Age/ weeks	HWE % OM	Bitumen % OM	HA % OM	Humin % dm
WINDROW COMPOST					
Bark	-	0.5	3.7	8.7	69.0
March II (97)	1	3.6	6.1	9.6	60.4
Curing pile 3	23	3.8	4.3	10.6	77.2
Curing pile 1	37	3.5	2.5	18.3	68.4
Cruring piles 1 & 2	77	5.2	4.9	20.7	86.0
DRUM COMPOST (ash as an additive)					
CM I (0% ash)	0	1.6	8.0	5.6	68.6
CM I (0% ash)	2	19.7	13.7	4.1	95.9
CM I (0% ash)	10	12.1	2.3	11.1	105.8
CM I (0% ash)	28	1.8	1.8	10.0	7.0
CM I (0% ash)	54	3.8	3.6	10.5	-
CM II (10% ash)	0	20.8	7.0	3.9	65.1
CM II (10% ash)	2	21.9	8.5	3.8	68.0
CM II (10% ash)	10	13.7	2.3	8.0	73.0
CM II (10% ash)	28	2.0	2.0	11.9	73.0
CM II (10% ash)	54	6.1	3.1	8.8	-
CM III (20% ash)	0	19.8	8.3	3.7	69.8
CM III (20% ash)	2	11.5	2.8	5.0	117.7
CM III (20% ash)	10	12.1	3.1	11.9	86.8
CM III (20% ash)	28	1.6	1.6	13.7	79.0
CM III (20% ash)	54	7.0	4.0	13.6	-
DRUM COMPOST (catering waste)					
matured 3 years		-	7.2	24.8	63.1
matured 7 years		5.0	9.9	26.4	-
CELLULOSE COMPOST					
matured 1.8 years		13.1	2.5	1.3	65.8
matured 3.8 years		10.9	5.7	8.2	51.9

- not measured

Alkaline hydrolysis of humic acids in the early stages (VI)

Effectiveness of the alkaline hydrolysis, as well as the overall resistance of the humic acids extracted from composts, can be estimated on the basis of the amount of hydrolysis residue. Cellulose, one of the references, was totally dissolved under conditions of alkaline hydrolysis, whereas lignin, the other reference, was more or less resistant to hydrolysis (the amount of hydrolysis residue was 39%). The amount of hydrolysis residue was low in cellulose compost HA (10%) and it was on the similar in humic acids extracted from catering waste composts (23%).

Use of model compounds and search of an MS spectral library allowed the identification of 23 carboxylic acids and one alcohol in the hydrophilic phase of degradation products of compost HA, cellulose and lignin. Five of these carboxylic acids were amino acids. Eighteen aromatic and 14 aliphatic compounds were identified in the hydrophobic phase of alkaline hydrolysis. No aromatic hydrophobic compounds were detected in cellulose, whereas 12 aromatic compounds were quantified and two identified in lignin.

Eight aliphatic compounds were the same in the hydrophilic phase of cellulose and lignin. Three major compounds were the same in the degradation products of lignin and compost HA. These were lactic, glycolic and oxalic acids. Yields of malic acid were higher in degradation products of compost HAs than in cellulose or lignin.

Total yields of quantified compounds were 31.2% OM, and 2.8% OM in the hydrophilic phase of cellulose and lignin, respectively (Table 10). Total yields were higher in the hydrophilic phase of compost HA than in the corresponding phase of lignin. Yields of lactic and glycolic acids were higher in cellulose HA than in lignin or compost HA. Yields of these acids were similar in HA extracted from catering waste composts. The highest oxalic acid yields were measured in lignin and in compost HA.

Twelve carboxylic acids, including five amino acids, were identified but not quantified, through search of an MS spectral library, in the hydrophilic phase of compost HA and cellulose or lignin. Hydroxycarboxylic acids, of which the most abundant compounds were 3-hydroxypropanoic and 2-hydroxypentanedioic acid, were identified in degradation products of compost HA. Amino acids were identified in the hydrophilic phase of compost HA: alanine, glycine, valine, leucine and proline.

Guiacol and phenol were major compounds in the hydrophobic phase. The combination 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid was clearly detected in compost HA but was only a minor constituent in lignin. No hydrophobic compounds were quantified in cellulose. The total yield of quantified compounds in the hydrophobic phase of lignin was 4.2% OM (Table 11). At the same time, total yields from compost HA varied from 0.2 to 2.1% OM. Concentrations of 3-hydroxybenzoic acid and of 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid were higher in compost HA than in lignin. HA extracted from lignin-free cellulose compost produced aromatic degradation products in an amount of 0.2% OM.

On the basis of an MS library search, seven aromatic and 20 aliphatic compounds were identified, but not quantified, in the hydrophobic phase. Benzoic and homovanillic acids were found in lignin and in compost HAs, except in cellulose compost HA. 1,4-Benzenedicarboxylic acid was identified in cellulose compost HA. 2-Hydroxybutyric, 2-hydroxy-2-methylbutyric, 2-hydroxypentonic, and methylmaleic acids were identified in the cellulose sample. Methylmaleic acid was identified in all compost HAs. 2,5-Cyclohexanediene-1,4-dione was identified in lignin and in 3-year-old compost HA. Also, many compounds quantified in the hydrophilic phase were identified in the hydrophobic phase (VI).

TABLE 10 Yields of four major compounds and total yields of quantified compounds in the hydrophilic phase of alkaline hydrolysis, % OM (mean ± s).

	Cellulose	Lignin	March II (97) HA (after 1 week)	Curing pile 1&2 HA (after 1.5 years)	Drum composted catering waste (after 3 years)	Cellulose compost HA (after 1.8 years)
n	2	3	2	3	3	3
lactic acid	16.7 ± 0.9	0.9 ± 0.1	2.6 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	3.1 ± 1.0
glycolic acid	2.6 ± 0.1	0.7 ± 0.1	1.9 ± 0.1	1.4 ± 0.4	1.9 ± 0.1	1.8 ± 0.6
oxalic acid	0.2 ± 0.1	1.2 ± 0.5	3.3 ± 1.3	1.6 ± 0.3	2.1 ± 0.3	1.1 ± 0.9
malic acid	0.2 ± 0.1	< 0.1	0.7 ± 0.6	0.8 ± 0.1	0.9 ± 0.1	1.3 ± 0.5
Total yield	31.2 ± 2.5	2.8 ± 0.5	10.2 ± 1.2	7.1 ± 0.8	8.5 ± 0.5	9.0 ± 3.3

s = standard deviation; n = number of replicates; total yield = lactic, α-hydroxyisobutyric, glycolic, 2-hydroxybutyric, levulinic, oxalic, malonic, succinic, methylsuccinic, fumaric, glutaric and malic acids and glycerol

TABLE 11 Yields of four major compounds and total yields of quantified compounds in the hydrophobic phase of alkaline hydrolysis, % OM (mean ± s).

	Cellulose	Lignin	March II (97) HA (after 1 week)	Drum composted catering waste (after 3 years)	Cellulose compost HA (after 1.8 years)
n	3	2	2	3	3
phenol	nd	0.2 ± 0.06	0.3 ± 0.05	0.4 ± 0.12	0.1 ± 0.05
guaiacol	nd	2.6 ± 0.5	1.0 ± 0.18	0.4 ± 0.11	< 0.1
3-hydroxybenzoic acid	nd	nd	<0.1	0.1 ± 0.04	< 0.1
3,4-dihydroxy-benzoic acid + 3,5-dihydroxybenzoic acid	nd	< 0.1	0.1 ± 0. 1	0.3 ± 0.12	< 0.1
Total yield	nd	4.2 ± 0.7	2.1 ± 0.2	1.9 ± 0.4	0.2 ± 0.03

s = standard deviation; n = number of replicates; nd = not detected; Total yield = phenol, p-cresol, guaiacol, catechol, 4-hydroxybenzaldehyde, resorcinol, 4hydroxyacetophenone, vanillin, 3-hydroxybenzoic acid, 3,4-dihydroxybenzaldehyde + acetovanillone, and 4-hydroxybenzoic, 4 hydroxyphenylacetic, phthalic, vanillic acids, and 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid

Elemental analysis of nitrogen, carbon and hydrogen (I, VI)

The atomic ratio of C/N decreased from 48 to 23 during windrow composting of 18 months indicating maturation of the compost (Table 12). Nitrogen and carbon contents increased slightly during composting of 8.5 months, while the hydrogen content stayed on the same level. Nitrogen content was higher and carbon content slightly lower in the HA fraction than in unfractionated compost samples. Also, the atomic ratio of C/N was lower in the humic acid fraction (I).

Cellulose had lower carbon and higher hydrogen contents than lignin: the atomic ratio of H/C was 1.8 in cellulose and 1.0 in lignin. Nitrogen was not detected in either cellulose or lignin. Nitrogen was enriched in the HA fraction of cellulose compost during composting. However, the addition of urea during composting may have caused increased microbial growth in the compost mass and these microbial remains may then bonded to HA, or urea may have chemically bonded to HA possibly through Maillard reaction. The nitrogen content was 7.8 w% dm in HA from cellulose compost matured 3.8 years and 2.6 w% dm in hydrolysis residue of HA from cellulose compost matured 1.8 years (VI).

TABLE 12 Elemental analysis (N, C, H) of compost samples and their humic acid factions. C/N and H/C are presented as atomic ratio.

	Age/ weeks	N	C	H	C/N	H/C
Cellulose		nd	43.3	6.5		1.79
Lignin		nd	47.8	3.8		0.95
COMPOSTS						
March II (97), w% OM	1	1.3	53.6	6.5	48	1.5
Curing pile 3, w% OM	23	2.1	55.5	6.6	31	1.4
Curing pile 1,w% OM	37	2.7	55.5	6.5	24	1.4
Curing pile 1 & 2, w% OM	77	2.5	49.5	5.9	23.1	1.4
HUMIC ACIDS						
March II (97) HA, w% OM	1	2.7	51.7	5.4	22	1.2
Curing pile 3 HA, w% OM	23	3.6	52.4	5.5	17	1.3
Curing pile 1 HA, w% OM	37	4.0	52.3	5.2	15	1.2
Drum compost, HA, matured 3 years, w% dm		3.8	50.0	4.8	15	1.2
Cellulose compost, HA matured 3.8 years, w% dm		7.8	43	4.6	6.5	1.3
HYDROLYSIS RESIDUES OF ALKALINE HYDROLYSIS						
Lignin, w% dm		> 0.1	56.6	4.3	-	0.89
March II (97) HA, w% dm	1	0.4	46.5	3.7	146.2	0.94
Drum compost, HA matured 3 years, w% dm		1.6	53.2	3.9	37.7	0.87
Cellulose compost HA, matured 1.8 years, w% dm		2.6	46.8	4.5	20.8	1.14

nd = not detected

Atomic ratio of H/C was higher in catering waste compost HA than in the hydrolysis residues. A lower ratio of H/C indicates more carbon–carbon double bond character in hydrolysis residues. The ratio of H/C was similar in the

hydrolysis residue of the HA of compost matured 3 years and the hydrolysis residue of lignin.

Carbohydrate analysis of composts and their humic fractions (IV)

Decrease in the total concentration of carbohydrates during composting was a clear trend in the compost samples and in hot-water, humic acid, fulvic acid and humin fractions (Table 13). A main cause of this was the decreasing concentration of glucose during composting. Concentrations of xylose + fucose, and also mannose + fructose, were similar during composting in unfractionated, HA and FA fractions. Concentrations of quantified monosaccharides were highest in the compost sample and the humin fraction. Concentrations of the separate monosaccharides were similar in the humic acid fraction, varying from 2 to 14 mg/g OM, except for glucose, the concentration of which decreased from 71 to 28 mg/g OM during composting of eight months. The concentrations of monosaccharides were slightly higher in the FA than the HA fraction.

TABLE 13 Concentrations of monosaccharides in compost and their hot water extract, humic acid, fulvic acid and humin or residual fibre fraction, mg/g OM.

	Age/ month	Ara + Rib	Xyl + Fuc	Man + Fru	Gal	Glc	Sum
UNFRACTIONATED							
Bark		16	32	32	32	223	335
March II (97)	0.2	13	30	46	37	238	363
Curing pile 3	5.3	17	31	39	37	206	329
Curing pile 1	8.5	9	33	29	24	199	295
HOT WATER EXTRACT							
March II (97)	0.2	21	15	45	79	319	477
Curing pile 3	5.3	22	22	42	82	54	222
Curing pile 1	8.5	6	9	19	24	30	88
HUMIC ACIDS							
Bark		7	2	5	7	50	71
March II (97)	0.2	7	3	8	13	71	101
Curing pile 3	5.3	5	6	9	14	25	59
Curing pile 1	8.5	4	5	6	9	28	52
FULVIC ACIDS							
Bark		4	9	21	28	55	118
March II (97)	0.2	5	13	46	53	69	186
Curing pile 3	5.3	4	14	16	29	28	91
Curing pile 1	8.5	2	10	13	20	30	74
HUMIN							
Bark		20	50	50	39	331	490
March II (97)	0.2	13	37	69	43	269	431
Curing pile 3	5.3	18	43	43	32	269	405
Curing pile 1	8.5	9	37	30	24	223	323

Sum = Ara+Rib+Xyl+Fuc+Man+Fru+Gal+Glc

3.4 Intermediate and final stages of humification (V, VI)

Extraction of humic substances (V)

The amounts of hot water extracts, bitumen, humic acids and humin extracted from peat and coal samples are shown in Table 14. The amounts of HWE and bitumen decreased in the order milled peat, interglacial peat, brown coal and black coal (V).

In terms of quantity the most important fraction in peat samples was the HA fraction; the amount of HA was 51.3% OM in milled peat and 56.7% OM in IGP. The amount of HA was lower at the brown coal stage and lower still at the black coal stage (V).

TABLE 14 Amounts of hot water extract (HWE), bitumen, humic acid (HA) and humin extracted from peat and coal.

	HWE % OM	Bitumen % OM	HA % OM	Humin % dm
Milled peat	6.7	11.8	51.3	26.0
IGP	2.5	12.8	56.7	16.0
Brown coal	1.5	7.5	25.0	–
Black coal	–	1.2	4.8	–

- not measured

Alkaline hydrolysis of peat and coal humic acids

The effectiveness of the alkaline hydrolysis was estimated on the basis of the amount of hydrolysis residue. Cellulose dissolved totally during alkaline hydrolysis, while lignin, the other reference, was relatively resistant to hydrolysis; the amount of hydrolysis residue was 39%. The amount of hydrolysis residue was low in milled peat HA (15%) and higher in interglacial peat HA (23%). Brown and black coal HA fractions were the most resistant towards alkaline hydrolysis; the amounts of hydrolysis residue were 41% and 35%, respectively.

With use of model compounds and the search engine of an MS spectral library, 41 carboxylic acids (seven of which were amino acids and six benzenedicarboxylic acids) and two alcohols were identified in the hydrophilic phase obtained in alkaline hydrolysis of peat and coal HAs and in cellulose and lignin. Thirteen aliphatic compounds out of 16 were the same in lignin and cellulose. Lactic, glycolic and oxalic acids were major compounds in the hydrophilic phase.

The total yield of quantified compounds was 31.2% OM in cellulose and 2.8% OM in lignin (Table 15). Yields of lactic, glycolic and malic acids were higher in cellulose than in lignin.

The total yields of hydrophilic compounds were higher in milled peat HA (13.9% OM) than in IGP HA (4.5% OM), brown coal HA (3.3% OM) or black coal HA (1.4% OM) (Table 15). The highest yield of oxalic acid was in milled

peat HA. Yields of lactic, glycolic, fumaric and malic acids decreased with the sample age in milled peat HA, IGP HA, brown coal HA and black coal HA.
Twenty-nine carboxylic acids (of which seven were amino acids and six benzenecarboxylic acids) and one aromatic alcohol were identified, but not quantified. Compounds identified in more than two samples are listed in Table 16. 3-Hydroxypropanoic acid, five-carbon saccharinic acid and 3-hydroxybuturic acid were identified in five samples out of six, and glysine, 2-hydroxyhexadioic acid and six-carbon saccharinic acid were identified in four samples out of six. Amino acids were identified in the hydrophilic phase obtained in alkaline hydrolysis of peat and coal HA. The most common amino acids were glycine, leucine, valine and alanine. In addition, urea, praline, hexanedioic, 3-deoxypentitol-2-carboxylic, 4-hydroxyphenl acetic and homovanillic acids were identified in two separate samples, and six aliphatic carboxylic acids and two aromatic compounds were identified in a single sample.

TABLE 16 Compounds identified by mass spectral library search in more than two samples in the hydrophilic phase (X = identified, nd = not detected).

	Lignin	Cellu-lose	Milled peat HA	IGP HA	Brown coal HA	Black coal HA
ALIPHATIC COMPOUNDS						
2-hydroxy-2-propenoic acid	nd	nd	X	X	nd	X
alanine	nd	nd	nd	X	X	X
3-hydroxypropionic acid	X	X	X	X	X	nd
3-hydroxybutyric acid	X	nd	X	X	X	X
valine	nd	nd	X	X	X	nd
leucine	nd	nd	X	X	X	nd
glysine	nd	nd	X	X	X	X
2-hydroxy-1,5-pentadioic acid	X	X	nd	X	nd	nd
2-hydroxy-1,7-hexadioic acid	X	X	X	nd	X	nd
saccharinic acid C(5)	X	X	X	X	X	nd
1,2,3-propanetricarboxylic acid	nd	nd	X	X	X	nd
saccharinic acid C(6)	X	X	X	nd	X	nd
AROMATIC COMPOUNDS						
phthalic acid	nd	nd	X	X	X	X
vanillyllactic acid	X	nd	nd	X	X	nd

With the use of model compounds and the search engine of an MS spectral library, 38 aromatic and 47 aliphatic compounds were identified in the hydrophobic phase obtained in alkaline hydrolysis. Of these compounds, three aromatic and 11 aliphatic compounds were also identified in the hydrophilic phase.

TABLE 15 Yields of four major compounds and total yields of quantified compounds in the hydrophilic phase obtained in alkaline hydrolysis, % OM (mean ± s).

	Cellulose	Lignin	Milled peat HA	IGP HA	Brown coal HA	Black coal HA
n	2	3	2	3	3	1
lactic acid	16.7 ± 0.9	0.9 ± 0.1	4.2 ± 0.4	1.4 ± 0.1	0.6 ± 0.1	0.6
glycolic acid	2.6 ± 0.1	0.7 ± 0.1	2.8 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.2
oxalic acid	0.2 ± 0.1	1.2 ± 0.5	3.7 ± 0.4	1.1 ± 0.5	1.3 ± 0.3	0.3
malic acid	0.2 ± 0.1	< 0.1	1.0 ± 0.1	0.3 ± 0.1	0.1 ± 0.01	nd
Total yield	31.2 ± 2.5	2.8 ± 0.5	13.9 ± 0.4	4.5 ± 0.3	3.3 ± 0.5	1.4

n = number of replicates; Total yield = lactic, α-hydroxyisobutyric, glycolic, 2-hydroxybutyric, levulinic, oxalic, malonic, succinic, methylsuccinic, fumaric, glutaric and malic acids and glycerol

TABLE 17 Yields of four major compounds and total yields of quantified compounds in the hydrophobic phase obtained in alkaline hydrolysis, % OM (mean ± s).

	Cellulose	Lignin	Milled peat HA	IGP HA	Brown coal HA	Black coal HA
n	3	2	2	1	3	1
phenol	nd	0.2 ± 0.06	0.5 ± 0.29	1.6	0.3 ± 0.05	< 0.1
guaiacol	nd	2.6 ± 0.5	0.3 ± 0.20	1.1	0.8 ± 0.13	< 0.1
3-hydroxybenzoic acid	nd	nd	0.1 ± 0.03	0.2	0.2 ± 0.01	0.1
3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid	nd	< 0.1	0.4 ± 0.09	0.3	0.2 ± 0.01	< 0.1
Total yield	nd	4.2 ± 0.7	2.7 ± 0.34	4.3	2.7 ± 0.2	0.2

n = number of replicates; nd = not detected; Total yield = phenol, p-cresol, guaiacol, catechol, 4-hydroxybenzalehyde, resorcinol, 4-hydroxyacetophenone, vanillin, 3-hydroxybenzoic acid, 3,4-dihydroxybenzaldehyde + acetovanillone, and 4-hydroxybenzoic, 4-hydroxyphenylacetic, phthalic, vanillin, 3,4-dihydroxybenzoic and 3,4-dihydroxybenzoic acids

Major compounds in the hydrophobic phase were guiacol, phenol and 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid. The total yield of quantified aromatic compounds was 4.2% OM in lignin, and the amount varied from 2.6 to 4.3% OM in peat HAs and from 0.2 to 2.7% OM in coal HAs (Table 17). No aromatic hydrophobic compounds could be quantified in cellulose. The yields were noticeably higher in IGP, brown coal and milled peat HA than in black coal HA.

Yields of 4-hydroxyacetophene and 4-hydroxybenzoic and vanillic acids and 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid decreased in peat and coal HA with the age of the sample; values were highest in the youngest milled peat HA and lowest in the oldest black coal HA. 3-Hydroxybenzoic acid was not detected in lignin, and yields were similar in milled peat, IGP peat and brown coal HA.

TABLE 18 Compounds identified by mass spectral library search in more than two samples in the hydrophobic phase (X = identified, nd = not detected).

	Lignin	Cellu-lose	Milled peat HA	IGP HA	Brown coal HA	Black coal HA
AROMATIC COMPOUNDS						
benzoic acid	X	X	X	X	X	nd
1-methyl-3,5-dihydroxybenzene	nd	nd	X	X	X	X
2-hydroxybenzoic acid	nd	nd	nd	X	X	X
2-hydroxyphenylacetic acid	X	nd	X	X	X	X
p-hydroxyphenylpropionate	nd	nd	X	X	X	nd
4-hydroxyphenylpropionic acid	X	nd	X	X	nd	nd
homovanillic acid	nd	nd	X	X	X	nd
1,4-benzenedicarboxylic acid	nd	nd	X	X	nd	X
ALIPHATIC COMPOUNDS						
hexanoic acid	nd	nd	X	X	X	X
2-methyl-2-hydroxybutyric acid	nd	X	X	X	X	nd
heptanoic acid	X	nd	X	nd	nd	X
2-hydroxypentanoic acid	X	X	X	X	nd	nd
octanoic acid	X	nd	X	nd	X	X
nonanoic acid	nd	nd	nd	X	X	X
methylmaleic acid	X	X	X	X	X	X
2-hydroxypentanedioic acid	X	X	nd	X	X	X
2-methylglutaric acid	nd	nd	X	X	X	nd
alkane C(>13)	X	nd	nd	X	X	X
octanedioic acid	nd	nd	nd	X	X	X
alkane C(>13)	nd	nd	nd	X	X	X
nonanedioic acid	X	nd	X	X	nd	X
hexadecanoic acid	X	X	nd	X	nd	nd
stearic acid	X	X	X	X	X	X

Fifty carboxylic acids, nine alcohols, six long chain alkanes, one ester, one aldehyde and one ketone were identified, through a mass spectral library search, in the hydrophobic phase. Compounds identified in more than two samples are listed in Table 18. Ortho- and meta-cresol and vanillyllacetic, 2-hydroxy-4-methylpentanoic, thiophene-2-carboxylic, 2-oxo-4-methylpentanoic, 2,3-dimethylsuccinic, decanoic and heptanedioic acids and two alkanes C(>13) were identified in two samples out of six. Further, 11 aromatic and 14 aliphatic compounds were identified in a single sample.

Elemental analysis (V)

Nitrogen and hydrogen concentrations were higher in peat and its HA fraction than in coals and their HA fractions (Table 19). Carbon content was highest in unfractionated black coal, 78.8 w% OM, and noticeably lower in the HA fraction, 54.7 w% OM. Cabron contents were, nevertheless, higher in coal HAs than in peat HAs. Nitrogen contents were relatively low in the hydrolysis residue of IGP and coal HAs; they varied from 1.0 to 0.3 w% dm. Carbon content varied in hydrolysis residues from 46.8 to 62.0 w% dm.

The atomic ratio of H/C was greater than 1.0 in milled peat, IGP peat and brown coal and in peat HAs. The H/C ratio was below 1.0 in black coal and in coal HAs. Atomic ratio C/N was higher and H/C was lower in hydrolysis residues than in the original HA, except in the hydrolysis residue of brown coal HA.

TABLE 19 Results of the elemental analysis (N, C, H) of peat and coal and their humic acid factions, and of the hydrolysis residues obtained in alkaline hydrolysis. C/N and H/C are presented as atomic ratio (V, VI).

	Unit	N	C	H	C/N	H/C
UNFRACTIONATED						
Cellulose	w% dm	nd	43.4	6.5		1.8
Lignin	w% dm	nd	60.1	4.8		1.0
Milled peat	w% OM	1.8	60.1	7.7	390	1.5
IGP	w% OM	1.6	67.3	8.9	500	1.6
Brown coal	w% OM	0.7	63.6	5.7	110	1.1
Black coal	w% OM	0.9	78.8	4.5	100	0.7
HUMIC ACIDS						
Milled peat HA	w% OM	2.3	49.7	5.3	25	1.3
IGP HA	w% OM	2.2	50.7	5.2	27	1.2
Brown coal HA	w% OM	0.9	58.8	3.4	78	0.7
Black coal HA	w% OM	1.0	54.7	3.2	63	0.7
HYDROLYSIS RESIDUE						
Lignin	w% dm	nd	56.6	4.3		0.9
IGP HA	w% dm	1.0	62.0	5.4	74	1.0
Brown coal HA	w% dm	0.3	61.5	4.8	208	0.9
Black coal HA	w% dm	0.9	60.7	2.9	82	0.6

nd = not detected

Solubility experiment

Over 90% of a sample of milled peat HA, the hydrolysis residue of HA of compost matured three years and the hydrolysis residue of milled peat HA dissolved in distilled water (Table 20). The colour of these water solutions was dark brown. In contrast, only 2 to 14% of HAs of compost and brown coal and the hydrolysis residue of brown coal HA dissolved in water. Only minor quantities of HA and almost none of the hydrolysis residues dissolved in diethyl ether. All water solutions were coloured, even the hydrolysis residue of brown coal, but the ether solutions were transparent (VI).

TABLE 20 Results of the solubility experiment. Dissolved matter as a percentage of the amounts of the original sample, mean ± s (VI).

	Distilled water		Diethyl ether	
	Dissolved, %	Colour	Dissolved, %	Colour
Compost HA, 3 years	12 ± 1.1	light brown	7.5 ± 2.6	transparent
Hydrolysis residue	97	dark brown	-	transparent
Milled peat, HA	93 ± 2.6	dark brown	2.2 ± 1.1	transparent
Hydrolysis residue	96 ± 0.2	dark brown	0.8	transparent
Brown coal, HA	4.0 ± 0.8	straw-coloured	1.0 ± 1.0	transparent
Hydrolysis residue	2.2	straw-coloured	0.4	transparent

s = standard deviation; - = not measured

4 DISCUSSION

4.1 Water-solubility of humic substances

The high hydrophilicity of humic and fulvic acids is evident from their solubility in natural waters (Aiken 1985). In view of this, it was deemed relevant to study the hydrophobic and hydrophilic solubilities of humic acids.

Our solubility experiments carried out in distilled water (neutral pH value) and organic solvent showed that the typical brown colour of HA appeared only in water solutions. The extent of the dissolution in water varied, peat HA being most soluble. HA from the compost matured three years alsodissolved in water to some extent. The alkaline hydrolysis residues of compost and peat HA were water-soluble as well. The experiment indicates that HAs are of hydrophilic nature and emphasises the importance of analysing the hydrophilic phase of HA degradation products.

4.2 Humification by biodeterioration

Humic acids extracted from badly deteriorated LPB bale showed that the cellulose material had started to transform into humic substances. The amount of humic acids extracted from a sample of this bale was five times as great as the amounts extracted from the younger and less deteriorated LPB.

The most important factor affecting the biodeterioration during storage is moisture content, especially if the ambient temperature is suitable for growth of microbes. Production of CO_2 inside the bales indicated active aerobic microbes. Organic waste residues within board fibres serve as a starting point for the growth of microbes, which penetrate inside the board and trigger biodeterioration Factors such as dirty packages and long and open storage of bales may enhance the biodeterioration and humification.

The relatively high nitrogen content in the badly deteriorated liquid packaging board was an indicator of microbial enrichment. As microbes die, their nitrogen becomes available to living microbes, and microbial utilisation of the cellulose in board can be expected to intensify with time.

4.3 Humification by composting

We have assumed that the humic substances produced by composting represent humification in its early stages. On this account, composting was employed as a synthetic process for the production of HAs for structural study.

During composting of cellulose, wood and lignin were deliberately excluded through the use of polypropylene bottle caps as bulking agent. Cellulose compost produced HAs, indicating that humification and formation HAs can occur without a lignin contribution. These HAs further produced aromatic degradation products at 0.2% of OM after alkaline hydrolysis. This indicates that aromatic compounds developing during the early stages of humification are not dependent on the presence of lignin. In the case of cellulose compost they originate from microbes.

Both windrow and drum composting processes worked smoothly during our experiments. Normal composting temperatures of 55 °C to 65 °C were reached within two weeks in the large-scale windrow composting experiment, except in winter when the starting material was frozen and they were reached within 4 to 5 weeks. Occasionally, temperatures rose as high as 80 °C in low windrows. The composting process worked well in low windrows and during most of that time the odour level in the composting field was satisfactory and nearby residents experienced no odour problems. Ash content increased during composting, but slowly. The amount of humic acids doubled during composting of 77 weeks and a notable increase in HA concentrations occurred after 37 weeks. Measurement of cation exchange capacity indicated that compost made in low windrows matured well.

Ash enhanced the rate of mineralisation of compost and the formation of humic acids, indicating that the ash may have some abiotic effects on the formation of humic substances. The acidification phase of the compost is shorter and milder when the composting is done with ash. According to Fang et al. (1999), coal fly ash can be used for the replacement of lime in compost materials to prevent a quick drop of pH during composting. Results of the pilot-scale drum composting experiment demonstrate the feasibility of using ash as an additive in composting of catering waste.

The significance of the study of the hydrophilic phase obtained in alkaline hydrolysis is that it provides additional organic matter for analysis. The yields of aliphatic compounds in the hydrophilic phase were clearly higher than the yields of aromatic compounds in the hydrophobic phase obtained in alkaline hydrolysis of compost HA.

4.4 Humification in peat and coal formation

Peat is a geologically young and biologically active pedological environment that will undergo considerable further diagenetic processes in the future before becoming coal. It is crucial that this be remembered in comparing peat with coal (Hawke et al. 1999). Although marked similarities among the degradation products of the HAs were found in this work.

Amounts of humic acids decreased in order IGP, brown coal and black coal. Evidently diagenetic processes in coal formation result in decrease of humic acid. On the basis of the results obtained, we may state that the diagenesis of the black coal is much more advanced than that of the brown coal.

In the degradation study, more aliphatic than aromatic compounds were identified in the hydrolysis products of the alkaline hydrolysis of peat and coal HAs. The number of aliphatic compounds was 72, whereas the number of aromatic compounds was 42. Also, the yields were higher in the hydrophilic than in the hydrophobic phase. Major degradation products were oxalic, lactic and glycolic acids in the hydrophilic phase and phenol, quaiacol and 3,4-dihydroxybenzoic acid + 3,5-dihydroxybenzoic acid in the hydrophobic phase. Degradation products of black coal HA clearly differed from those of peat and brown coal HAs; black coal HA had only low content of phenolic compounds and more benzenecarboxylic compounds.

Hänninen & Niemelä (1992) found the major degradation products in the hydrophilic phase of alkaline hydrolysis of peat HA to be oxalic, lactic and glycolic acids. They also stated that lactic and glycolic acids and carboxylic acids with three and four carbon atoms are typical products of alkaline degradation of several polysaccharide constituents. Tsutsuki & Kuwatsuka (1979) studied soil HA samples by alkaline KOH hydrolysis, but they focused on the hydrophobic phase. 3,4-Dihydroxybenzoic, and p-hydroxybenzoic acids and 1,3,5-trihydroxybenzene were the main aromatic compounds, whereas succinic and glutaric acids were the main aliphatic compounds. Maximum yields of those main aromatic compounds varied from 0.6 to 1.3% (w/w) of products in the studied soil HA samples, and the maximum yield of succinic acid was 1.4% (w/w) of the products. The yields of the main aromatic compounds in soil HAs were only slightly higher than those in our study. Results here emphasise the importance of compounds having aliphatic and olefinic structures in the HAs.

Nitrogen content was higher in peat and peat HA than in coal and coal HA. Likewise, the atomic ratio of H/C was higher in peat fractions. Identification of amino acids in brown coal HA may indicate the presence of microbial activity.

4.5 (Bio)synthesis of humus

At the present moment, the structure and biosynthesis of humus are an open question. Classical lignin theory, and also polyphenol theory, emphasise the aromaticity as the structural entity of humus (Hatcher & Spiker 1988, Hendges 1988, Stevenson 1994). Classical lignin theory is supported by the recalcitrant nature of lignin, but lignin is nevertheless degradable in both aerobic and anaerobic environments (Phelan et al. 1979, Colberg & Young 1982 Young & Frazer 1987 Falcón et al. 1995). Carbohydrate theory, is built around the Maillard reaction, emphasises aliphaticity as the structural unit of humus (Cheshire 1979, Ikan et al. 1986, Cheshire et al. 1992, Stevenson 1994).

Carbohydrates considered to be compounds that rapidly degrade during composting and humification (Stevenson 1994, Stott & Martin 2001). The decrease in total carbohydrate concentration during composting was a clear trend in windrow composts as well as in their hot water extract, humic acid, fulvic acid and humin fractions. On component basis, the main cause of the decrease in carbohydrates was the declining concentration of glucose during composting. Concentrations of arbinose, ribose, xylose, fucose, mannose, fructose and galactose were similar in compost HAs of different age. Galactose, mannose, rhamnose and fucose are primarily of microbial origin, while arabinose and xylose are predominantly plant derived and glucose originates from both plants and microbes (Cheshire 1977). Arabinose may also be derived from synthetic fungal products (Coelho et al. 1988).

Saccharinic acids are indicator compounds of carbohydrate structures (Meller 1960, Hänninen & Niemelä 1992). In this study two saccharinic acids (C-5, and C-6) were identified among the hydrolysis products of brown coal HAs. Actually, polymeric carbohydrates and compounds of carbohydrate origin are found throughout the coalification series (Hänninen et al. unpublished results). These findings imply that carbohydrates, and structures originated from carbohydrates are recalcitrant as well. However, the nature of their recalcitrance is different from that of lignin. Easily degradable carbohydrates are utilised by microbes as an energy source, and the microbes then generate new types of polysaccharides (Murayama 1988, Martens & Frankenberger 1991). Carbohydrates and carbohydrate structural parts are thus recycling during the humification of soil organic matter.

Some polysaccharides are also protected from decomposition in soil through the formation of complexes with Fe, Cu and Zn. Further, polysaccharides may form insoluble salts with HAs in highly organic soils (Cheshire 1977). It is probable that carbohydrates of both plant and microbial origin are to some extent incorporated into the end products of the humification process via this abiotic mechanism, too.

An evolutionary perspective is enlightening in considering the role of lignin in the humification process. It is believed that life has existed on Earth for more than one billion years. There is evidence to suggest that non-vascular plants were forming organic matter in soils already in the Late Ordovician and

Early Silurian. Non-vascular and aquatic vascular plants do not contain lignin, unlike terrestrial vascular plants, which contain lignin in their cell walls. Vascular plants emerged in the Early Devonian (Retallack 1990), but since the Carboniferous vascular plants have dominated the landscape (Campbell 1996). On the time scale of the evolution of photosynthetic organisms, lignification is therefore a relatively recent process. Sophisticated life existed on Earth long before lignin (Barghoorn 1964, Boudet 2000).

We surmise that major pedogenetic factors—weathering, erosion, leaching, vegetation development and decomposition of organic matter (humification)— have remained the same from the beginning. And from this we propose that the humification process is older than lignin. Quite likely black coal formation began during geological eras when there was no lignin in plants (van Krevelen & Schuyer 1956), in which case the aromaticity of black coal could not depend on the contribution of lignin.

5 CONCLUSIONS

In this work was studied humification of organic matter from early stages (compost and biodeteriorated cellulose) through intermediate stages (peat) to the final stage (coal):

1) Idea of using composting as synthetic method of producing HAs was found useful.
2) It was possible to produce HAs by composting pure carbohydrate (cellulose) with lignin free bulking agent.
3) Alkaline degradation of HAs from cellulose, and catering waste composts, peat and coals produced compounds in hydrophobic and hydrophobic phases. In general, mostly compounds in hydrophobic phase are analysed. In this study compounds from both phases were analysed. This allowed more of the original organic matter of HAs to be analysed.
4) The yields of aliphatic compounds in the hydrophilic phase were clearly higher than those of aromatic compounds in the hydrophobic phase.
5) Phenolic degradation products were found only in minor amounts.
6) Two saccharinic acids were identified from HAs of peat and brown coal HAs.
7) These results support the argument that, in degradative studies of HAs, the hydrophilic phases and hydrophilic degradation products should not be neglected.
8) From the evolutionary point of view humification of carbohydrates is an older process than humification of lignin.
9) An already existing humification process did not have to change radically when lignin emerged; we propose that lignin was adopted into the existing humification and degradation system.
10) HAs from lignin-free cellulose compost, produced aromatic degradation products in small amounts.

This being the case, the question of the formation, and structure of HAs needs to be approached from a new perspective:

- Nitrogen was enriched during humification, showing microbial invovelment
- Carbohydrates are recycling by microbes, this gives them recalcitrance. So there is no reason to consider lignin more important than carbohydrates as precursors to the humification process,
- So there is no reason to consider aromaticity more important that aliphaticity in the structural studies of HAs.
- This work suggests that humification theories should be based on a more balanced contribution of all plant and microbial materials.

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YHTEENVETO (Summary in Finnish)

Orgaanisen aineen humustuminen tarkasteltuna ajan funktiona

Humustumisprosessissa kuollut orgaaninen aines stabiloituu humusaineiksi, (humushappo (HH), fulvohappo (FH) ja humiini). Humushapot vaikuttavat maaperän, sedimenttien ja veden kemiallisiin ominaisuuksiin. Humustumiseen vaikuttavat sekä biologiset että abioottiset tekijät, mutta HH:jen (bio)synteesi sekä rakenne on tuntematon. Vuosien varrella on esitetty lukuisia humustumisteorioita; valtaosa niistä perustuu humusaineiden aromaattiseen rakenteeseen ja vain harvat niiden alifaattiseen rakenteeseen.

Tässä työssä humustumista tutkittiin ajan funktiona. Humushappo näytteet ryhmiteltiin humustumisen alku-, keski ja loppuvaiheeseen, näytteinä näissä ryhmissä olivat kompostin HH, turpeen HH ja hiilen HH. Humustumisen alkuvaiheessa olevaa ligniinivapaata HH tuotettiin kompostoimalla valkaistua selluloosaa käyttäen tukiaineena polypropeenista valmistettuja pullonkorkkeja, jotka eivät sisällä ligniiniä. Tuhka, jota käytettiin kompostoinnin lisäaineena, lisäsi kompostimassan mineralisatiota ja HH muodostumista, tulokset viittaavat tuhkan abiottisiin ominaisuuksiin humustumisessa kompostoitumisen aikana.

Humushappojen rakennetutkimuksen painopiste oli eri-ikäisten HH alkalisen hydrolyysin hydrofiilisten ja hydrofobisten jakeiden tutkiminen. Tutkimuksen kohteeksi saadaan suurempi määrä hajotustuotteita analysoitaessa molemmat faasit. Rakennetutkimuksessa keskityttiin myös hiilihydraattien merkitykseen HH rakenteissa.

Humushappojen määrä oli suurin turpeen HH ja vastaavasti pienempi kompostin HH ja hiilen HH. Alkalisen hydrolyysin hajotustuotteiden saannot olivat Kaikissa HH näytteiden ikäryhmissä selvästi suuremmat hydrofiilisessä faasissa. Hydrofiilisen faasin yhdisteet olivat suurimmaksi osaksi alifaattisia, kun taas hydrofobisessa faasissa yhdisteet olivat pääosin aromaattisia, mutta myös alifaattisia yhdisteitä identifioitiin. Humushappojen hajoitustutkimukset osoittivat, että sekä hydrofiilinen ja hydrofobinen faasi ovat olennainen osa HH. Tämän vuoksi HH hajoitustutkimuksissa tulisikin tutkia molemmat faasit.

Humustumisen aikana helposti hajoavat hiilihydraatit hajoavat nopeasti, mutta toisaalta mikrobit myös tuottavat uusia polysakkarideja orgaanisen aineksen hajotuksen aikana. Hiilihydraattiperäisiä yhdisteitä on löydetty HH koko hiiltymissarjan ajalta. Tässä työssä ruskohiilen HH hydrofiilisestä jakeesta identifioitiin sakkariinihapoja, jotka ovat tyypillisiä polysakkaridien alkalisen hydrolyysin hajoamistuotteita. Tulosten perusteella voidaan myös hiilihydraatteja pitää kestävänä yhdisteryhmänä, mutta niiden kestävyys perustuu hiilihydraattien kiertoon orgaanisen aineksen hajotessa toisin kuin ligniinin joka hajoaa hitaasti maaperässä. On mahdollista, että humustumisprosessissa

sekä kasvi- ja mikrobiperäiset hiilihydraatit sitoutuvat jossain määrin humus-happorakenteisiin.

Ligniinin osuutta humustumiseen voidaan tarkastella myös evolutiivisesta näkökulmasta. Ennen putkilokasvien kehittymistä maapallolla oli jo humusta muodostavaa vihreää kasvillisuutta. On mahdollista, ettei humustuminen muuttunut radikaalisti ligniinin kehittymisen myötä, todennäköisemmin ligniin sopeutui olemassa olevaan humustumis- ja hajotusprosesseihin.

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