

DEVELOPMENT OF MICROBIAL COMMUNITY IN THE COURSE OF COMPOSTING OF GARDEN WASTE

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Abstract

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Composting represents one of the technologies of processing of biodegradable municipal waste. Samples collected from composting plants were analyzed chemically, physically and microbiologically. The pH of bio waste samples increased from 6.5 to 8.6. The total carbon to nitrogen ratio in samples of bio waste decreased, in the course of composting, from 40:1 up to the value of 25:1 while the total nitrogen to phosphorus ratio decreased from 10:1 up to 8:1. Indicator groups of microorganisms were monitored in compost samples. Representatives of *Enterobacter* genus, namely *E. cloacae* and *E. aerogenes* were identified in the samples on the basis of biochemical tests. The bacterial groups needed for efficient composting, i.e. order *Bacillales* and *Actinomycetales*, were present in appreciable amounts.

compost, composting, bio waste, aerobic microorganisms

Composting represents the most common manner of aerobic processing of organic waste. It is a biotechnological process based on the ability of microorganisms and invertebrates to transform organic substances featuring the nature of waste material into utilisable products (Giglotti *et al.*, 2005). Compost is, historically, one of the common natural fertilizers used for growing of plants as well as improvement of the quality of soil. In addition to preparation of a high-quality fertilizer, composting also leads to decreasing of the amount of feed waste material as well as decreasing of the amount and activity of pathogenic microorganisms, parasites and weed seeds in the composted material. The quality of compost and course of biochemical processes are largely affected by the composition of feed material, and therefore composting is, to a certain extent, rather a unique process. Some parameters prove to be generalized in the literature – for example the specific temperature increase up to 70 °C in the course of 14 days from the time of stowing. In general, the temperature is a critical parameter determining quality of the composting process (Finstein *et al.*, 1986). In the first stage the microflora decomposes simple organic substances

forming a part of the composted material. The aforementioned decomposition is accompanied by releasing of energy which results in a temperature increase. Microbial activity reaches its peak, which we may observe not only considering the increased amount of cultivable microorganisms but also their increased enzymatic activity (Raut *et al.*, 2008). In this thermophilic stage Raut *et al.* determined the highest level of activity of hydrolases, phosphatases, cellulases as well as proteases. It is followed by decomposition of less decomposable organic substances, which is accompanied by decreases of the temperature of composted materials to 20 °C – 30 °C between 3rd and 7th week from the time of stowing. Products of decomposition of the mesophilic stage are used for synthesis of humic substances in the cooling stage. Termination of the composting process is accompanied by a decrease of the temperature of compost to the ambient temperature as the energy is utilized for synthetic procedures.

However, composting may also prove to be rather problematic, mainly with respect to the composition of feed material. A low level of pH of feed material inhibits the process due to the limited

bacterial activity, which is connected with creation of unpleasant odours (Sundberg and Jönsson, 2007). In this case lactic and acetic acid bacteria (Partanen *et al.*, 2010) and yeasts (Hultman *et al.*, 2010) prove to prevail. Low pH values are encountered mainly in case of food residues (pH approx. 4.9) (Eklind *et al.*, 1997, Adhikari *et al.*, 2008). Therefore, it is more favourable to mix them with fresh garden waste and waste originating from maintenance of municipal vegetation (Sundberg *et al.*, 2004).

Composting is a highly complex microbial process; however, printed materials present only very little information about the composition of microbial associations in compost. It is common to perform cultivation determinations of major groups of bacteria, and the total amount of bacteria, actinomycetes and fungi are often evaluated as well. Nevertheless, no standardized methods of determination of the microbial composition of compost prove to exist (Finstein *et al.*, 1986). Microbial diversity of bio waste is rather significant; it decreases during the composting process in the course of which specific groups of microorganisms gradually prevail (Ryckeboer *et al.*, 2003). Pathogenic microorganisms of *Salmonella*, *Yersinia* genera, opportunistic pathogens such as *Clostridia* sp. or microorganisms pathogenic for plants are often detected in bio waste. Most vegetable and animal pathogens are destroyed by high temperatures in the course of the thermophilic stage of composting. Spore-forming microorganisms prove to be more resistant to high temperatures.

The objective of our paper was to present specifications of the composition of bio waste in various stages of the composting process. We focused on those groups of microorganisms that play a major part as regards soil fertility and evaluation of soil quality. Furthermore, we determined the amount of enterobacteria in the composted material and performed their identification. Concurrently, we monitored the temperature, humidity, value of carbon nitrogen ratio (C/N), pH, total phosphorus (P) and biochemical oxygen demand (BOD) in the samples.

MATERIAL AND METHODS

Samples of analyzed material were collected in the composting plant located near the city of Slavkov u Brna, which is in the South Moravian Region of the Czech Republic. The composting plant was erected and equipped in 2009, mainly on the basis of subsidies granted by the EU (Cohesion Fund), State Environmental Fund of the Czech Republic and Environmental Fund of the South Moravian Region. The composting plant processes, on average, 10.000 – 15.000 kilograms of waste per week. The waste originates mainly from maintenance of municipal vegetation and comprises also bio waste transported from residents of Slavkov. Sample No. 1 was collected from fresh bio waste, sample No. 2 was from waste composted in banded piles for the time

period of 14 days, sample No. 3 was composted for 63 days and the last sample No. 4 represented fresh compost (75 days), more specifically its sub sieve fraction prepared for distribution as a top quality fertilizer suitable for gardening or soil reclamation purposes.

All the aforementioned samples were collected and stored in sterile plastic containers in accordance with ČSN ISO 10381-6 Standards and immediately transported to a laboratory where they were instantly processed. The results specified herein represent average values based on ten repetitions for each sample.

Methods of Determination of Microorganisms

The amounts of microorganisms present in samples of composted materials were determined using the cultivation dilution method in accordance with ČSN EN ISO 6887-1:1999. 20 g of a sample was added to 180 ml of sterile distilled water and shaken for 20 minutes in a shaker (Heidolph Promax 1020, 130 rpm). The sample was subsequently diluted using a decimal series. Suitable dilutions were inoculated in Petri dishes and the adequate cultivation agar medium was poured over them. In order to determine spore-forming bacteria, the diluted sample was incubated in a water bath for 10 minutes at the temperature of 80 °C. Meat-peptone agar No. 1 (Merck, CZE) was used for the purpose of determination of the heterotrophic plate counts as well as the amount of spore-forming bacteria. Czapek-Dox agar (Himedia, CZE) was used for cultivation of micromycetes, Actinomyces agar (Himedia, CZE) was used for cultivation of actinomycetes, Ashby agar (Himedia, CZE) was used for determination of fixators of atmospheric nitrogen, and Endo agar (Merck, CZE) was used for cultivation of enterobacteria. All the samples were cultivated in a thermostat at the temperature of 25 °C for 72 hours, only the amount of enterobacteria was determined after cultivation at 37 °C for 24 hours.

The amount of microorganisms was recalculated considering the determined dry matter of the sample as expressed as the amount of colony forming units in 1 g of dry weight (CFU/g d.w.).

Enterotest 24 (Erba Lachema, CZE), a biochemical identification kit, was used for identification of enterobacteria. Results were evaluated using the TNW identification software (Erba Lachema, CZE).

Physical and Chemical Analyses

All the samples underwent an analysis of dry matter (ČSN EN 14346:2007) as well as ash-free dry mass (ČSN EN 15169:2007). The total solid content and ash-free dry mass in the samples were determined using an electric muffle furnace LMH 07/12 which is designed to measure incineration processes, drying, degradation, re-heating, thermal treatments, etc. Analytical laboratory balances Radwag AS 220/X, for precise weighing, readability to 0.0001 g. A well-mixed sample (10 g) is evaporated in a weighed dish and dried to a constant weight in an

electric muffle furnace at $(105 \pm 2)^\circ\text{C}$. The increase in weight over that of the empty dish represents the total solids TS [%]. After the total solid assessment the dish with the sample is put back to the electric muffle furnace at $(550 \pm 5)^\circ\text{C}$. The increase in weight over that of the dish after the total solid assessment represents the ash-free dry mass [%].

For the analysis of pH, (10 ± 0.05) g of compost samples were mixed with 50 ml of de-ionised water. The mixture was stirred for 10 minutes and pH of the fluid phase was subsequently measured at the temperature of $(20 \pm 2)^\circ\text{C}$ using WTW 340i pH-meter (Germany).

Total amounts of nitrogen and phosphorus were determined spectrophotometrically in the steep liquor prepared during determination of pH using HACH LANGE DR 2800 (Germany) spectrophotometer. The total carbon content was determined using TOC Sievers 800 (USA) analyzer. Determination of BOD was performed using OxiTop Control, WTW (Germany) pressure-gauge instrument. The temperature of compost was measured using OMEGA HH506A (USA) thermometer and its humidity using TESTO 635 (Germany) humidity measuring instrument.

RESULTS

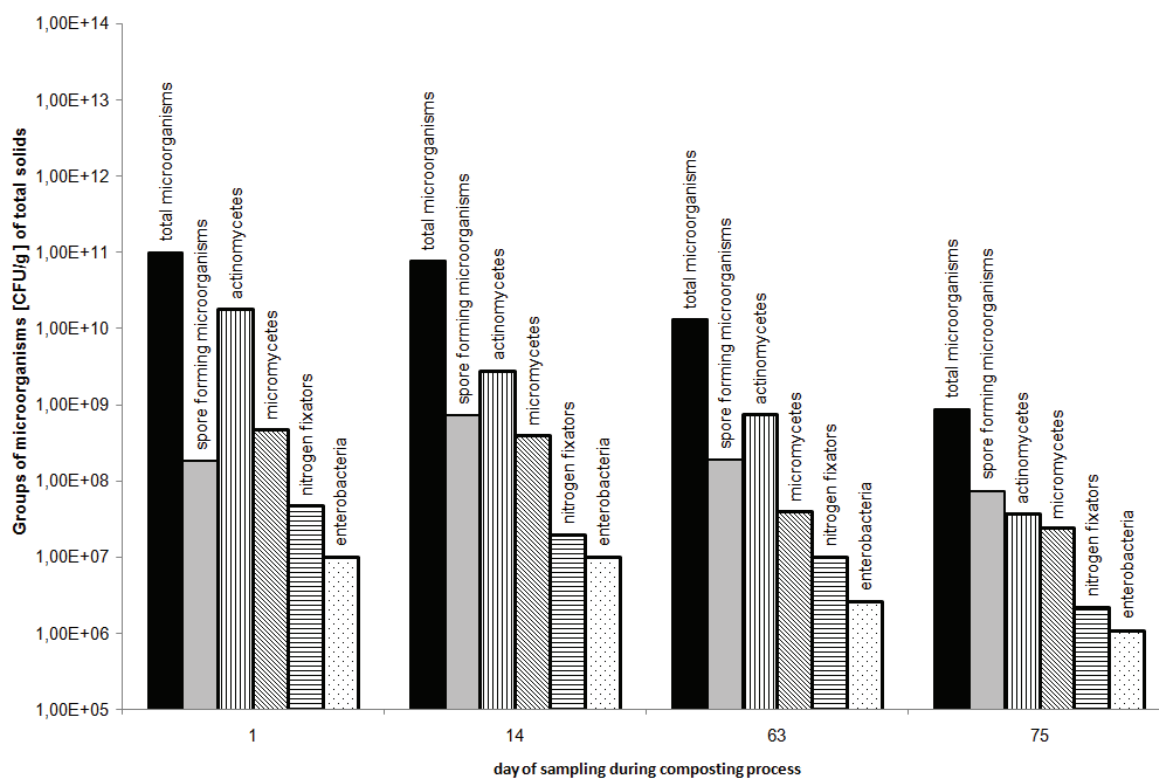
Representation of several indicator groups of microorganisms was determined in samples collected from freshly composted materials as well as after 14 days, 63 days and 75 days of composting.

It pertained to determination of the heterotrophic plate counts, amount of spore-forming bacteria, actinomycetes, retainers of atmospheric nitrogen, micromycetes and enterobacteria (Fig. 1).

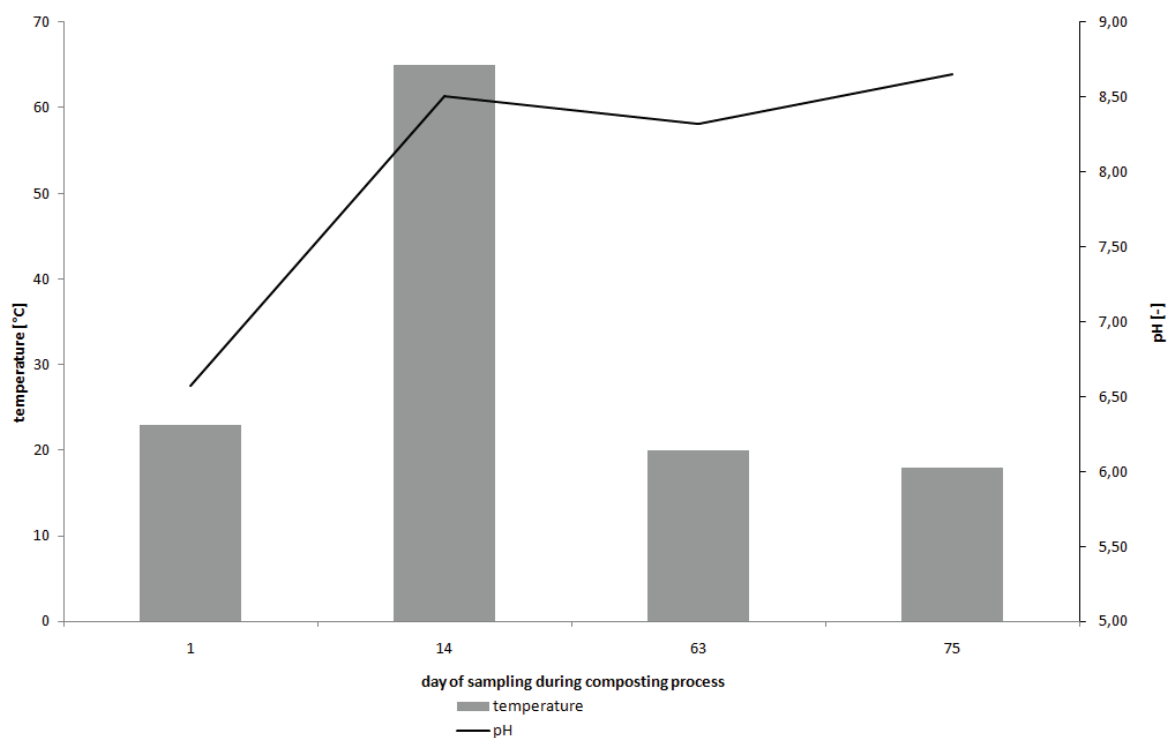
Using biochemical identification tests we managed to identify representatives of *Enterobacter* sp., namely *E. cloacae* and *E. aerogenes*, in all the tested samples. In most cases the highest amounts of microorganisms were determined in fresh materials; only spore-forming bacteria reached the maximum value after 14 days of composting. In the course of 75 days the heterotrophic plate counts decreased by three orders of magnitude, up to the value of $8.8 \cdot 10^8$ CFU/g d.w. The rapid decrease of the amount of actinomycetes determined during the first 14 days continued in the course of the whole process and reached the value of three orders of magnitude. The amounts of micromycetes and fixators of atmospheric nitrogen were rather stable in the course of the composting process; nevertheless, a decrease of the amounts by one order of magnitude was observed even in these groups. A surprisingly high amount, which reached up to $1 \cdot 10^7$ CFU/g d.w. (Fig. 1), was encountered in case of enterobacteria.

The temperature of composted material as well as pH value of its water steep liquor was measured throughout the whole composting process (Fig. 2). An almost neutral pH determined at the beginning of composting was substituted by its rapid increase up to the value of 8.65.

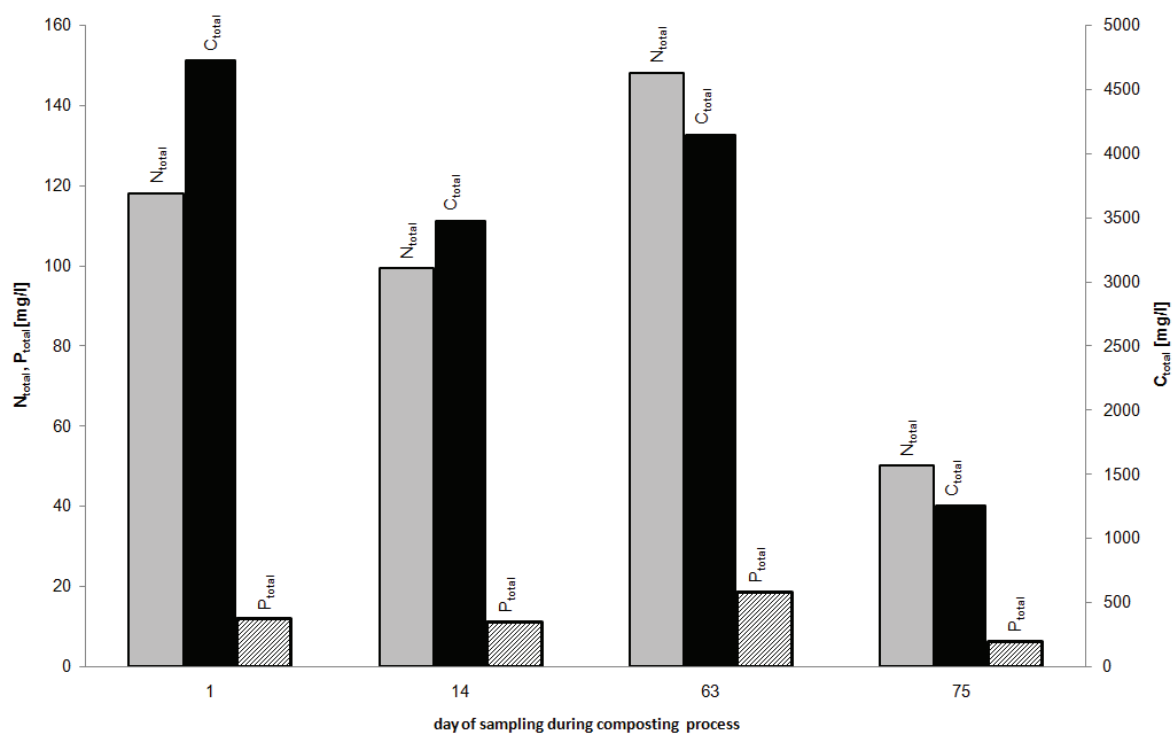
The total carbon to nitrogen ratio in samples decreased, in the course of composting, from 40:1



1: Presence of indicator groups of microorganisms in the course of composting



2: Average values of pH and temperature in the course of composting

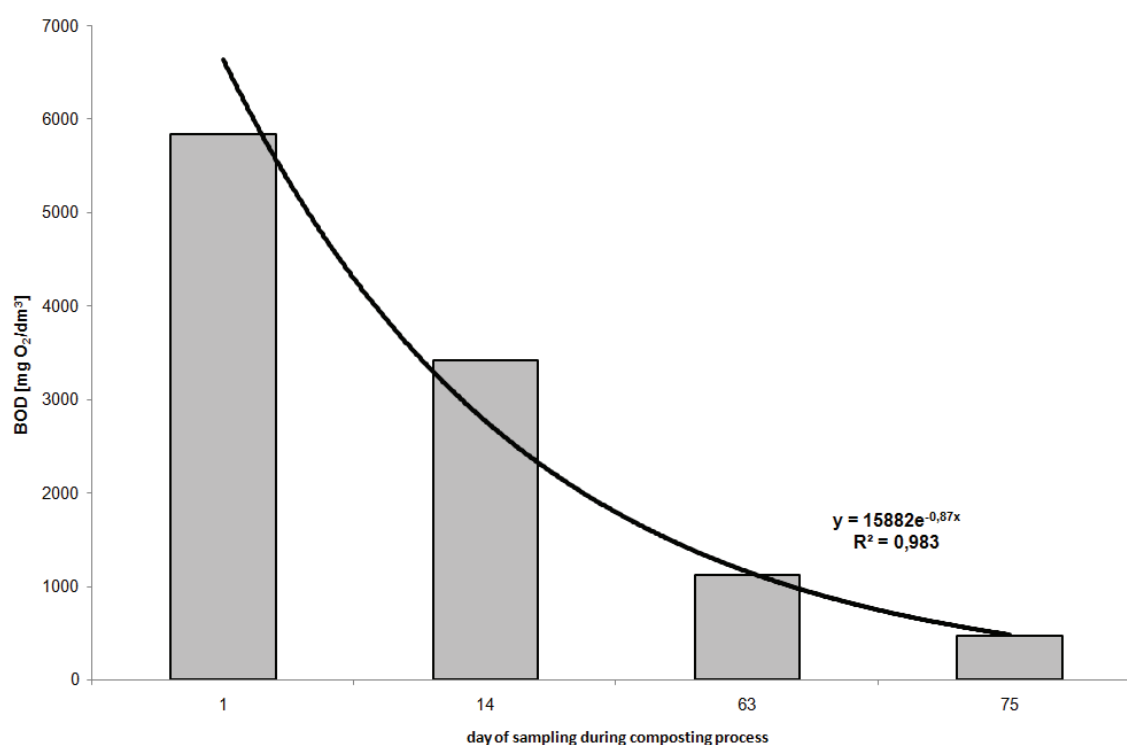


3: Values of total carbon, nitrogen and phosphorus in the course of composting

up to the value of 25:1 while the total nitrogen to phosphorus ratio decreased from 10:1 up to 8:1 (Fig. 3).

By the means of measuring BOD values in samples we managed to supplement the cultivation

determination of microorganisms by information about their activity expressed in the form of oxygen consumption irrespective of individual cultivation groups (Fig. 4). BOD reached its peak in the raw



4: BOD in the course of composting

feed material while in the course of composting it decreased to the values around 461 mg O₂/dm³.

DISCUSSION

Biodegradable municipal waste as well as waste from gardens and maintenance of municipal vegetation represents material suitable for composting. The resulting high-quality compost may be used for fertilization and soil reclamation purposes. The feed material featured optimal pH values which allowed for a rapid commencement of the composting process. We observed increases of pH values in the course of composting, which corresponded to the conclusions of Raut *et al.*, 2008. The authors, who described problems related to composting of organic material featuring low pH values, isolated from compost a high amount of micromycetes while the presence of bacteria was rather low (Sundberg and Jönsson, 2008). pH of our compost samples was optimal for development of the bacterial population. After the elapse of 75 days of composting, the total carbon to nitrogen ratio decreased to the value of 25:1. The measured value approximates the applicable statutory requirement, which stipulates the value of 30:1 in ripe compost (ČSN 46 5735:1991). The number of spore-forming bacteria increased during 14 days of composting. We presume that these were representatives of *Bacillus* genus, in case of which such an increase during the thermophilic stage is often described (Goyal *et al.*, 2005). Other authors recorded the maximum concentration of spore-forming bacteria after 14 days of composting, which is confirmed by

our results. Bacterial biomass dominated over the biomass of micromycetes throughout the whole period of composting. Amounts of micromycetes as well as bacteria of all indicator groups gradually decreased in the course of the 75 days of composting. The aforementioned cultivation determinations were supported by conclusions resulting from BOD measurements. High temperatures play a major part in case of elimination of pathogenic microorganisms in compost (Haug, 1993). Composted material reached the maximum temperature after 14 days when we measured values of 65 °C. Even though a decrease of the amount of enterobacteria occurred in the samples, but they have not been completely eradicated. In addition to high temperatures, decreases of the amount of pathogens are also affected by other mechanisms such as antagonistic relations between individual groups of microorganisms, production of antibiotics or effects of salts and pH (Haug, 1993). The aforementioned potentially pathogenic bacteria are a common cause of nosocomial infections with weak patients, mainly in their respiratory or urogenital tract. They form a part of the microflora present in the digestive tract of both humans and animals. It most probably entered the composted material through peels of fruit and vegetables from which representatives of this genus are often isolated. Dog excrements from municipal vegetation (lawns) may serve as another source. Zeschmar-Lahl *et al.*, 1994 mention *Enterobacter* sp. amongst common microorganisms present in household waste.

SUMMARY

This article deals with the composting of biodegradable waste collected from inhabitants and green waste collected from maintenance of urban greenery. Basic physical and microbial parameters have been monitored in taken samples. The process of composting was accompanied by physical and chemical changes as well as changes in the amount and composition of microbial communities and associations of higher organisms. Maintaining of the optimal pH, humidity, aeration and a suitable C:N:P ratio, results in occurrence of temperature changes accompanied by a decrease of the heterotrophic plate counts, fixators of atmospheric nitrogen, actinomycetes, micromycetes and potentially hazardous enterobacteria.

Biodegradable waste was processed at the composting plant, which uses technology of belt fill. From these belt fills the samples of material during exact intervals have been taken. In the course of composting the heterotrophic plate counts decreased by three orders of magnitude, up to the value of $8.8 \cdot 10^8$ CFU/g d.w. The rapid decrease of the amount of actinomycetes determined during the first 14 days continued in the course of the whole process and reached the value of three orders of magnitude. The amounts of micromycetes and fixators of atmospheric nitrogen were rather stable in the course of the composting process; nevertheless, a decrease of the amounts by one order of magnitude was observed even in these groups. A surprisingly high amount, which reached up to $1 \cdot 10^7$ CFU/g d.w., was encountered in case of enterobacteria. A total sanitation of the material does not occur. Therefore, it is essential to comply with certain sanitary rules applicable to handling of compost.

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