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Changes in Cell Number and the ATP Content during the Composting Process

K. Czaczyk, K. Trojanowska, B. Stachowiak, H. Dubisz

Department of Biotechnology and Food Microbiology, University of Agriculture,
Mazowiecka 48, 60-623 Poznań, Poland

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Abstract

The ATP level and the number of mesophilic bacteria, thermophilic bacteria, moulds and yeasts were examined in different composts prepared from lupine *(Lupinus angustifolius* cv Mirela). During the first weeks of composting, the mesophiles increased slowly and the thermophile phase follows. After eight weeks of composting the number of thermophiles decreased rapidly and development of mesophilic bacteria and fungi was observed.

From five ATP extraction methods, the best results were obtained from Celsis-Lumac extracting mixtures and it was chosen for further experiments. The ATP level increased at the beginning of composting and decreased after 8 weeks of the process. After this time, ATP content began to rise to the achieved maximum during the 12^{th} week of composting. There is no correlation between the number of microorganisms in compost and the ATP level.

Keywords: composting process, bioluminescence, ATP

Introduction

Knowledge of the biological and biochemical principles of composting is very important in the refinement of compost engineering. During the composting process, microorganisms convert organic matter into degradation products, CO_2, heat and new cell material. Microbial biomass, activity and cell numbers are fundamental parameters which can be used to characterize the course of composting. Microbial biomass accounts for about 1-3% of the compost organic carbon, but this small fraction controls the turnover of the large amount of organic matter present in this material [9] The relationship between biomass, microbial activity and cell numbers is comparatively constant only under stated conditions. The activity of microorganisms within composts depends upon environmental determinants such as pH, temperature, nutrient and oxygen availability [18].

Plate counts are often used for microbiological enumeration in compost [11]. This very simply but time- and labour-consuming method measures only a fraction of total microbial population. An alternative approach is the use of chemical properties to estimate microbial biomass and metabolic activity. Some authors have used methods based on the measurement of phospholipids or nucleic acid (DNA or RNA) contents or ATP (adenosine triphosphate) levels [7, 10, 19].

Determination of the ATP level is a useful analytical method for estimating total microbial biomass [5]. It is based on the assumption that all living organisms contain ATP in protoplasm. The bioluminescence method based on luciferin-luciferase system of the firefly *(Photinus pyralis)* has been modified into can easy, rapid and sensitive determination. Many experiments connected with composting have used this method. Suberkropp et al. [14] measured ATP in leaf litter, Garcia et al. [3] showed that ATP concentration could be used as an index of microbial biomass and activity of the composting process. Marambe et al. [8] used ATP as a measure of microbial biomass in animal-waste compost.

The aim of this work was estimation of correlation between ATP content and the number of mesophiles,

termophiles, moulds and yeasts during the composting process and posibilities to use ATP bioluminescence technique to control composting.

Materials and Methods

Compost was prepared with *Lupinus angustifolius* cv Mirela and beech bark (in 1:1 weight ratio) and an additional 5% dry matter (DM) extract from seeds of *L. angustifolius* cv Mirela. Three compost processes were studied in the these experiments:

a) Control (a compost without inoculated *Bacillus co agulans),*

b) Compost inoculated with *B. coagulans* (strain No. 6) at the outset of the composting process

c) Compost inoculated with *Bacillus coagulans* (strain No. 6) during composting process (every four weeks).

Each compost bath was placed in 5-1 plastic perforated containers. The composting process was conducted for 24 weeks in an air conditioner chamber at 30°C and 70% humidity.

Inoculation of compost using *Bacillus coagulans* no. 6 strain is connected with fungistatic activity of these strains against plant pathogens [17].

The microbiological analysis of compost by Koch's plate method required determination of the total number of mesophiles (nutrient broth, 37°C, 24h), the total number of thermophiles (nutrient broth, 55°C, 24h) and the total number of moulds and yeasts (Czapek-Doxa medium, 30°C, 72h). All samples were tested in 4 repetitions.

On the basis of literature data five different methods of ATP extraction were performed:

a) Shaking with 10% trichloroacetic acid (TCA) was used. A lg sample of compost was added to a test tube containing 4 ml of 10% (w/v) TCA/4 mM EDTA and the tube contents mixed for 5 min using a Vortex mixer. The mixture was then centrifuged (1000g) and the super natant was used for ATP determination [15].

b) Shaking with 20% TCA was used. The further pro cedure was the same as described above [15].

c) The boiling Tris-buffer was used. A lg sample of compost was dropped into a test tube containing 8 ml of boiling Tris-buffer (0.1 M, pH 7.75) and the mixture boiled for a further 60s. The tube was cooled and after centrifuging (1000g) the supernatant was used to ATP determining [12].

d) The H_2SO_4 extraction method was used for deter mining ATP. A lg compost sample was mixed with 4 ml 0.3 M H_2SO_4 for 5 min using Vortex mixer. The sample was neutralized with 1 M NaOH, centrifuged (1000g) and the supernatant was used for ATP determination [6].

e) Extraction method based on the Celsis-Lumac ex tracting mixture. A lg compost sample was mixed with 4 ml distilled water using Vortex mixer. The sample was centrifuged (1000g) and nucleotide release reagent (NRM) in proportional volume (1:2) was added to the determination of ATP (Instruction of Biocounter M 1500 Celsis-Lumac) [16].

ATP analysis was performed with Celsis-Lumac Luminometer Biocounter M 1500. All Calculations of ATP concentration were based on internal calibration using ATP standard (Sigma). A 100 µl sample of the ATP containing extract was pipetted into a reaction tube and 100 µl Lumit QM (Lumit QM and Lumit QM diluent - mixture luciferin-luciferase) was added, and then the light output from the reaction mixture was measured in the luminometer. The ATP concentration of the extract sample was calculated by comparing the light output of the sample with that of ATP standard. All samples were tested in 8 repetitions.

The dry matter (DM) of compost samples was determined after drying at 60°C for 2 h and at 105°C to constant weight.

Results

Microbiological Analysis

The changes in microbial population in the different composts during the process are presented in Figures 1 to 3.

The initial number of mesophilic bacteria (Figure 1) in control compost was 2×10^7 CFU (Colony Forming Units)/g and 4×10^9 CFU/g in inoculated composts. The number of mesophilic microorganisms during the process of composting varied. During the first weeks of composting the number of these microorganisms remained stable (10^9-10^{10} CFU/g of compost). The lowest level of mesophiles in compost was recorded in the 12^{th} week of composting for the control and compost inoculated in the beginning. From that moment on the number of mesophiles began to increase and remained at the level 10^{12} and 10^{14} CFU/g for control and compost inoculated at the beginning, respectively. In the case of compost inoculated of *Bacillus coagulans* during composting process the number of mesophilic bacteria increased continuously to the 20^{th} week of process to the level 2×10^{15} CFU/g.

The number of thermophiles (Figure 2) at the beginning of the process was on the level 4.2×10^4 CFU/g for control and 1.1×10^6 CFU/g for inoculated composts. The highest number of this kind of microorganism was observed in the 4^{th} week for the compost inoculated on the beginning (at the level of 2.6×10^{10} CFU/g) and in 8^{th} week for the other composts (at the level of 10^{10}-10^{11} CFU/g). In the next weeks of composting the number of thermophiles decreased and did not vary to the end of the process.

The number of moulds and yeasts (Figure 3) in the examined composts during the first four weeks increased from the level of 10^4 to 10^5-10^7 CFU/g of compost. The highest number of fungi was observed in the 16^{th} week of composting process for all examined composts (10^8 CFU/g). At the end of the ripening process the number of moulds and yeasts was on the level 10^7-10^8 CFU/g of compost.

Comparison of ATP Extraction Methods

In preliminary experiments the five different ATP extraction methods described above were tested. Because the extraction method based on the Celsis-Lumac extracting mixtures was found to be the most effective it was used as a reference method (extraction capacity

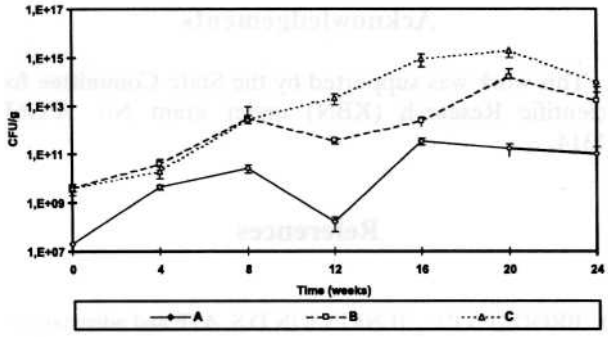

Fig. 1. Changes in mesophilic bacteria numbers during composting process.
(A - control compost; B - compost inoculated of *Bacillus coagulans* no 6 on the beginning of composting process; C - compost inoculated of *Bacillus coagulans* no 6 every four weeks).

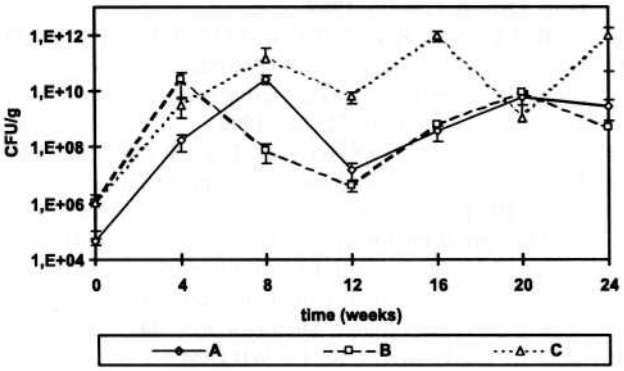

Fig. 2. Changes in thermophilic bacteria numbers during composting process.
(A - control compost; B - compost inoculated of *Bacillus coagulans* no 6 on the beginning of composting process; C - compost inoculated of *Bacillus coagulans* no 6 every four weeks).

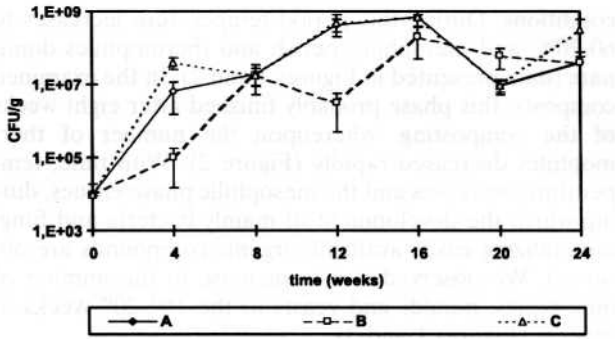

Fig. 3. Changes in fungi numbers during composting process.
(A - control compost; B - compost inoculated of *Bacillus coagulans* no 6 on the beginning of composting process; C - compost inoculated of *Bacillus coagulans* no 6 every four weeks).

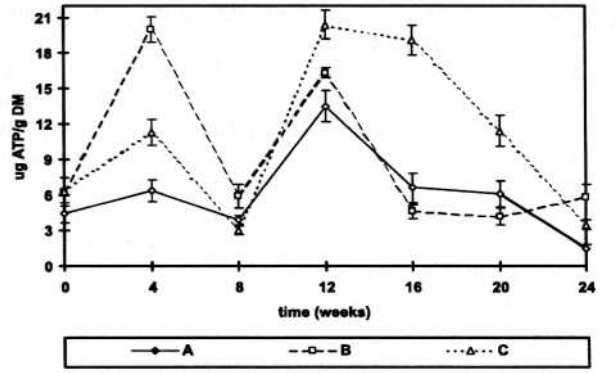

Fig. 4. ATP levels during composting process. (A - control compost; B - compost inoculated of *Bacillus coagulans* no 6 on the beginning of composting process; C - compost inoculated of *Bacillus coagulans* no 6 every four weeks).

100%) (Table 1). From the other four methods of extraction of ATP the highest results gave the methods with boiling Tris-buffer (extraction capacity 64%). The extraction methods with 10% TCA and H_2SO_4 showed poor results (extraction capacity 15% and 11%, respectively). The Celsis-Lumac extractant was considered to be the most effective extractant and was therefore selected for further experiments.

Table 1. Comparison of ATP extraction methods.

Extraction method	Extraction capacity [%]
TCA 10%	15 (± 0.43)
TCA 20%	30 (± 0.77)
Boiling Tris-buffer	64 (± 1.14)
H_2SO_4	11 (± 0.50)
Celsis-Lumac Extractant	100 (± 1.90)

(Mean ± SD)

Determination of ATP Levels during Composting Process

The ATP level in examined composts is presented in Figure 4. At the beginning of composting process the ATP level in control compost was 4.5 µg/g DM and in inoculated compost 6.3 µg/g DM of compost. After four weeks of composting process the amount of ATP increased rapidly in all examined composts. In this time the highest level of ATP was observed in inoculated composts (11.3 - 20.0 µg/g DM). After eight weeks of decomposting, the ATP concentration was reduced to 3.9 - 6.0 µg/g DM and in 12th week come back to the high amount (13.5 - 16.3 µg/g DM). After this time, the level of ATP decreased to the end of composting process for all examined samples.

Discussion

Schaub and Leonard [13] reported that naturally occurring aerobic organisms rapidly colonized substrate during composting under suitable moisture and oxygen

conditions. During this period temperature increases to 60-70°C and mesophiles perish and thermophiles dominate (data presented in Figures 1 and 2). In the examined composts, this phase probably finished after eight weeks of the composting, whereupon the number of thermophiles decreased rapidly (Figure 2). With time, temperature decreases and the mesophilic phase ensues, during which the development of mainly bacteria and fungi mineralizing easily available organic compounds are observed. We observed a slow increase in the number of mesophiles, moulds and yeasts to the 16^{th}-20^{th} weeks of process (Figures 1 and 3).

The influence of inoculation composts by *Bacillus coagulans* no. 6 were observed only in the case of the number of mesophilic bacteria (Figure 1).

The aim of this study was the application of ATP determination to the process control of composting, especially to the control of quantity of microflora present in them. There is no correlation between ATP content in lupine composts and the number of mesophiles, thermophiles and the number of moulds and yeasts (coefficients of correlation $R < 0.3$; $n = 56$).

The curve representing the ATP level (Figure 4) showed that the ATP content increased until mesophilic temperatures were exceeded. In the beginning of the thermophilic stage there appeared a step of decrease in ATP content probably caused by thermal inactivation of mesophilic microorganisms. During the thermophilic stage of the process the ATP content began to rise again, which is probably connected with regeneration of ATP concentration due to thermophilic or mesophilic growth (or both).

The results reported by various authors showed the relationships between microbial biomass and microbial ATP in compost and soil [1, 2, 3]. In above-mentioned investigations, the fumigation-incubation or fumigation-extraction methods were used for estimating microbial biomass. Counting different microbial groups by direct and indirect methods is very laborious and tends to be misleading because of the presence inactive forms of microorganisms. Also, the presence of eucariotic microorganisms and remains of organic matter particles, which contain ATP, may falsify the results.

The critical points in the determinations of ATP are the extraction of this substance from biological materials in samples. In our studies, in all testing methods of extraction of ATP, the sample was centrifuged (1000 g). This process removes big organic molecules, but the smallest one, which also contains ATP, may be left over. No influence of inoculation composts by *Bacillus coagulans* no. 6 on the level of ATP was observed in these investigations.

However, the use of ATP measurement as an index of microbial activity is debatable also because many cellular activities are unrelated to the amount of biomass. Although ATP turnover rates are relatively fast, ATP concentration does not always have a direct and constant relation with the living biomass in various environmental situations.

In conclusion, ATP measurement was shown to be an effective method for control phases of composting processing, but there is no correlation between the number of microorganisms (thermophiles, mesophiles, moulds and yeasts) and the ATP level in compost.

Acknowledgements

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