ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Influence of Bacterial Inoculation on the Composting Process of Organic Wastes.

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ABSTRACT

Inoculation of composting mixture consisting of garden, food, paper and textile wastes (68:20:11:1) by *Bacillus pumilus* KF-4 characterized by high cellulase activity was conducted. For inoculation, 10, 15 and 20% (w/w) of cultural medium with bacteria in their active growth phase were used. Compost without inoculation was used as a control. Effects of inoculation on temperature profile, organic matter and dissolved organic carbon were analyzed. Stability of composts was estimated on the basis of microbial respiration, and maturity on the basis of composts' phytotoxicity. It was found, that inoculation increases composts' organic matter decomposition and that inoculated composts became to be not phytotoxic 30 days earlier than the control one. The optimal dose of inoculation was shown to be 15%.

Keywords: Compost, bacterial inoculation, phytotoxicity, dissolved organic carbon, organic matter decomposition

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INTRODUCTION

Recently a lot of municipal solid wastes are disposed on the landfills causing pollution of underground and surface water, soil, destruction of close situated biocenoses, emission of greenhouse gases etc [1]. Besides, landfilling of organic wastes leads to exclusion of biogenic elements from their cycles. Alternatively, organic wastes can be treated, e.g. by means of composting method [2–7]. The composts obtained may be used as soil fertilizers or soil substitutes [8,9].

Composting is an exothermic process of biological oxidation, where organic substrate is decomposed by microbes in conditions of elevated temperature and humidity, and a resulting product of composting is highly humified product similar to soil [2,5,6,10]. Quality of compost is determined by its characteristics such as stability and maturity. Stability of compost is connected with stability of organic compounds in it, able to be decomposed rapidly. Maturity of compost is connected with its suitability for stimulation of plant growth and humification [3,11–13]. Stability of compost is connected with its microbial activity, therefore it can be estimated using intensity of microbial respiration, or by means of chemical analysis of organic matters' transformation [3,10–12,14]. Maturity of compost may be estimated using several parameters such as phytotoxicity, humification index, humic to fulvic acid content ratio, or organic carbon to total nitrogen content ratio [3,11,14,15]. Mature compost is characterized by absence of phytotoxic compounds such as short chain fatty acids, phenols, alkaloids, aldehydes, ketones, amino acids and ammonia, which are formed in the process of degradation of initial organic compounds [3,4,12].

Biologic decomposition of organic compounds is driven by different microbial groups habiting composted wastes [2], and naturally this process is time consuming. One of the ways to speed up the process of maturation and stabilization of the compost is inoculation of compost mixtures by microbial isolates. In literature, data concerning introduction of bacterial and fungal strains into composts for intensification of composting process are presented, and results obtained are quite controversial [4–7]. These controversial results may be explained by wide variation of chemical properties of initial wastes used for composting. Domination of specific chemicals determines microbes which may be efficient as inoculates for composting intensification. Therefore, some authors suppose that microbes selected from the composts may be the most efficient inoculates for speeding up the process of composting [10,16]. Anyway, inoculating microbes should possess enzymatic activity which enables decomposition of organic biopolymers present in the waste treated. In the process of composting decomposition occurs due to microbial excretion of exoenzymes [17]. In the composting mixture many of recalcitrant compounds such as lignin and hemicellulose are present, however rate of compost maturation mainly depends on decomposition of cellulose [18,19]. From the one hand, fungi are the most active destructors of cellulose, from the other hand, in unfavorable conditions they can produce phytotoxins which decrease fertilizing properties of composts [20]. Therefore usage of bacterial inoculates may be more preferable.

The objective of this study was estimation of efficiency of bacterial cellulolytic isolate for intensification of composting process of mixture consisting of organic fraction of municipal solid waste and fallen leaves.

MATERIALS AND METHODS

For composting, we artificially prepared the mixture of green garden waste (fallen leaves), food waste, paper waste and textile (mass ratio 68:20:11:1). Wastes were selected on the Waste sorting station in the city of Kazan (Russia). 12 independent replicates of compost mixtures (50 kg each) were prepared in the plastic containers. The initial waste mixture had the following characteristics: organic matter content (OM) 87.2±6.4%, total organic carbon content– 46.2±4.1%, total nitrogen content– 1.2±0.2%, C:N ratio – 40.2. The compost mixtures were inoculated by *Bacillus pumilus* KF-4 strain in amounts of 10, 15 and 20% (w/w). Compost mixture without inoculation was used as a control. As a result, 4 compost variants were used in this experiment - Bac10, Bac15, Bac20 and C, three independent replicates of the compost mixtures were used for each variant. For inoculation, cultural medium with bacteria was used.

The compost variants were wetted till 55%, and after mixing, incubated for 124 days. Every three days, compost mixtures were properly mixed to provide aeration. Moisture content was maintained on the level of 55%. In the process of composting, temperature control was conducted. Compost samples were taken on 1, 14, 21, 36, 43, 50, 57, 71, 83, 106 and 125 days.

OM content was measured using dry combustion method by 550^{0}C. DOC was according to ISO 14240-2, 1997 [21], respiration activity (RA) – according to [22], and phytotoxicity – according to [23] with oat plant (*Avena sativa L.*). For phytotoxicity experiments, haplic greyzem soil sampled in the forest area close to Kazan (Russia) was used. Shoot height and plants' biomass grown in this soil were used as control parameters in phytotoxicity experiments.

The sampling and the chemical and biological analyses were conducted in triplicate. The results are expressed on an air-dry soil basis. The data from the experiments were processed using statistics package and of Origin 8.5 (OriginLab, Northampton, USA). The means were compared using Fisher's Protected Least Significant Difference at α = 0.05. The values in the figures and tables were expressed as the means ± S.E. of the corresponding replicates.

RESULTS AND DISCUSSION

Temperature profiles of all 4 mixtures were similar, and typical for composting process: after 4 days, increase of temperature in all 4 variant up to 48-55^{0}C was observed. Further, temperature was higher than 55^{0}C in all the mixtures during 16-19 days. After 23rd day, we observed monotonous decrease of temperature in all 4 variants to 26-27^{0}C on the 124th day.

Components of composted wastes consist of organic compounds, and about half of them are decomposed in the process of composting to CO_2 which is released, another half is transformed to more stable compounds. Therefore, decrease of OM content can be used as an index of compost stabilization [24]. As presented on Fig. 1A, initial content of OM in composts was about 88-90%.

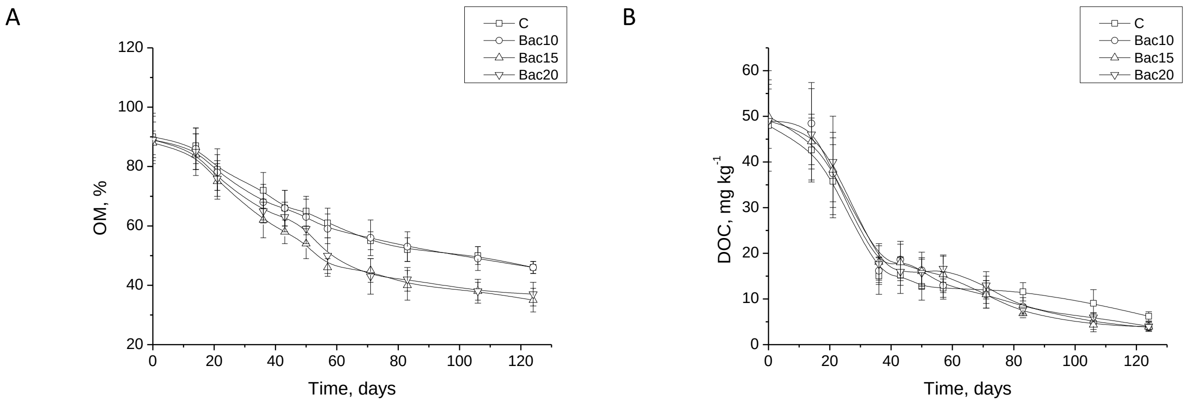

Figure 1: Dynamics of contents of OM (A) and DOC (B) in the process of composting

After 14 days, OM content decreased, and a maximum rate of decrease was observed during the first 2 months of composting and corresponded to the time of temperature decrease. After 60 days, decrease rate slowed down, and at the end of composting OM content was measured to be 46% for both C and Bac10 variants, and significantly lower (35% and 37%) for Bac15 and Bac20 variants, correspondingly. Thus, inoculation of compost mixtures by bacterial preparation in amounts of 15% and 20% intensified decomposition of OM. Most likely, this effect is explained by the fact that the microbes inoculated possess cellulolytic activity. Cellulose was a main component of wastes treated, and cellulolytic enzymes produced by microbes intensified its decomposition. Our results are in line with those obtained by other authors [5]. DOC changes are suggested as index of OM decomposition efficiency and compost stability [25–27], some authors connect DOC changes with phytoxicity [24]. In the initial mixtures, DOC content was estimate to be 48-50 mg kg^{-1} (Fig. 1B). This content is typical for composts obtained from municipal, wooden, green wastes and sewage sludge [26,28]. In the process of composting decrease of DOC content was observed, with maximal decrease rate in the first three weeks of the process. In the literature, different data concerning maximal rate of decrease of DOC content are published: from 5-7 days [28], to 2-4 weeks [25], and even 2-3 months [26]. After

4 months of composting, DOC content ranged between 3.8-6.2 mg g^{-1}, and these values characterize the composts obtained as stable and mature ones [11,24,25]. Taking into account more significant decrease of OM content in Bac15 and Bac20 variants as compared with the other 2 ones, it can be expected that DOC content is higher in these variants, since DOC is a product of decomposition of organic biopolymers presented in OM. However, in our study DOC content did not differ significantly between the variants – it was estimated to be 87, 92, 92 and 92% for C, Bac10, Bac15 and Bac20, correspondingly. As suggested by [6], this can be connected with the fact that introduction of bacterial inoculate may not only intensify decomposition of biopolymers but also intensify utilization of soluble carbonic products of this decomposition.

Respiration based on CO_2 release measurement is often used for estimation of microbial activity of composts, and of composts' stability [10,24,25,27]. In the beginning of composting, RA levels ranged from 6.1 to 6.4 mg CO_2-C g^{-1} 24 h^{-1} (Fig. 2). These results are supported by data published by other authors [13,26,29].

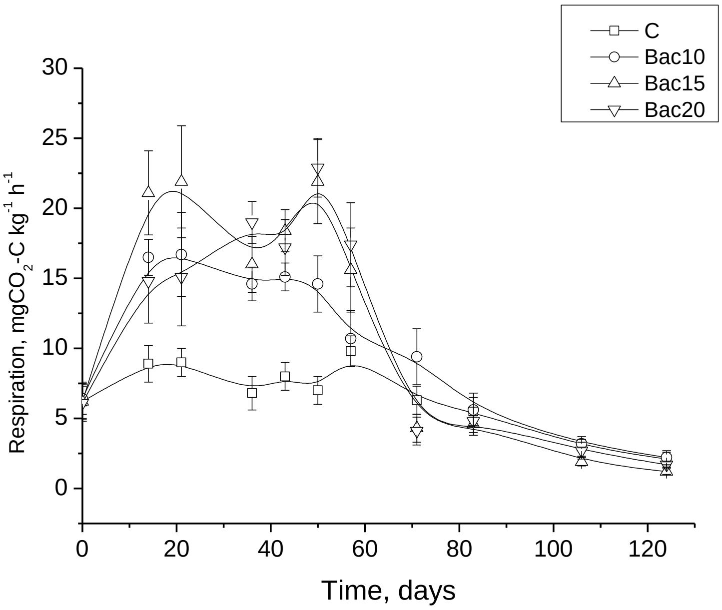

Figure 2: Respiration activity dynamics in the process of composting

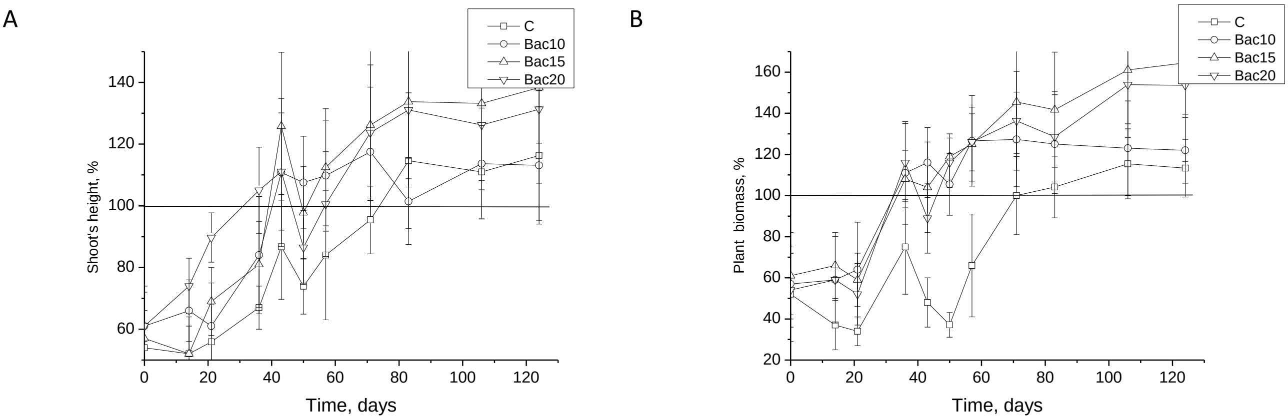

Figure 3: Influence of 4 composts on shoot's height (A) and biomass (B) of *Avena sativa*

During the first 2 weeks of the process, RA rose in all the compost variants. On the 14^{th}-21^{st} day this parameter increased in 1.4-3.6 times. Further weeks were characterized by fluctuations of microbial respiration, and from the day 80 monotonous decrease of this parameter occurred. At the end of composting, RA levels were estimated to be 2.1, 1.9, 1.2 and 1.7 mg CO_2-C g^{-1} 24 h^{-1} for C, Bac10, Bac15 and Bac20 variants, correspondingly. Decrease and stabilization of this parameter at the end of composting suggests that all the biodegradable compounds were decomposed by microbes. According to literature, composts are stable if their

RA ranges between 2.0-5.0 mg CO_2-C g^{-1} 24 h^{-1}, and very stable if it is less than 2 mg CO_2-C g^{-1} 24 h^{-1} [13,24,26]. In our study, Bac15 and Bac20 compost variants became to be stable after 71 days of the process, and on the 124th day they can be classified as very stable. Maturity of composts is one of the main characteristics of composting process. In our study, we measured composts' maturity by means of composts' toxicity towards oat plants (*A. sativa*) (Fig. 3).

Immature composts may be phytotoxic due to presence of phenolic compounds, fatty acids as well as NH_4-N^+ ions, which are formed the process of organic matter transformation. The declination of composts phytotoxicity usually means that these phytotoxic compounds are decomposed and composts maturate [3,13,24,30,31]. In our study, all 4 compost variants were phytotoxic in the first 2 months. Further, phytotoxicity decreased, and at the end of composting in C and Bac10 variants shoot length was slightly higher (11-16%) as compared with non-treated soil. Interestingly, soil treatment by Bac15 and Bac20 variants caused more pronounced stimulating effect on plants: already 71 days old composts caused 26-38% increase of shoot lengths. As for effects on plants' biomass, C compost variant inhibited plant biomass till 71st day, whereas Bac10, Bac15 and Bac20 variants only till 28th day and after 71 days they influenced positively on plant biomass. The values obtained are in line with results presented in literature [5,13,15,24,30,31]. It can be concluded that Bac15 and Bac20 variants influenced more positively on plants. Taking into account economical costs of inoculates preparation, Bac15 variants may be recommended for practical use.

FINDINGS

At the end of composting OM content was measured to be 46% for both C and Bac10 variants, and 35% 37% for Bac15 and Bac20 variants, correspondingly. In the process of composting 87, 92, 92 and 92% decrease of DOC content from the initial 48-50 mg kg^{-1} to was observed for C, Bac10, Bac15 and Bac20, correspondingly. RA rose during the first 2 weeks of the composting, than fluctuated, and from the day 80 monotonous decrease of this parameter occurred. At the end of composting, RA levels were estimated to be 2.1, 1.9, 1.2 and 1.7 mg CO_2-C g^{-1} 24 h^{-1} for C, Bac10, Bac15 and Bac20 variants, correspondingly. At the end of composting, C and Bac10 variants shoot length was slightly higher (11-16%) as compared with non-treated soil, whereas soil treatment by Bac15 and Bac20 variants caused more pronounced stimulating effect on plants. C compost variant inhibited plant biomass till 71st day, whereas Bac10, Bac15 and Bac20 variants only till 28th day.

CONCLUSIONS

Thus, it can be concluded that inoculation of composts by bacterial strain *B. pumilus* KF-4 characterized by high cellulase activity leads to increase of organic matter decomposition rate and therefore to speeding up of compost maturation. Among three inoculation variants studied, 15% variant can be recommended in terms of effectiveness and costs.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University. This work was supported by the subsidy of Russian Ministry of Education and Science No RFMEFI57814X0089.

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