ORIGINAL RESEARCH

Accelerated bioconversion of cow dung into concentrated organic fertilizer using microbial composition

Gulnaz F. Rafikova^{1*}, Tatyana Yu. Korshunova¹, Elena V. Kuzina¹, Oleg N. Loginov¹

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Abstract

Purpose The purpose of this research was to evaluate the effectiveness of compositions based on the strains of microorganisms and intended for cow dung processing.

Method Cow dung in an amount of 6 kg was placed into fermentation containers. To process the waste, we used microbial compositions. Sampling was performed on the 1st, 5th, 12th, 19th and 29th days. During the experiment, an analysis of microbiological, physicochemical and phytotoxic parameters was carried out.

Results The number of micromycetes in the compostable mixture decreased by half compared to the control sample on the 5th day. When treated with microbial compositions at a dose of 25 ml/kg, no *Salmonella* bacteria was detected in the compostable mixture on the 29th day. In the variants of the experiment with the introduction of microbial compositions, the temperature increased to 45-51°C in a month after the experiment, the humidity decreased to 69%, and the pH of the compostable mixture was set at a neutral level. It was shown that the amount of total nitrogen increased by 7.1-38% when treated with microbial compositions. After 29 days in almost all experimental samples with the introduction of a liquid bacterial culture, the rate of germination and seedling emergence exceeded the growth rate of the control sample.

Conclusion The possibility is shown to use the given compositions with bacteria of various functional groups as a basis of biological products for the accelerated processing of organic waste, such as cow dung.

Keywords Microbial compositions, Bioconversion, Cow dung, Organic fertilizer

Introduction

A greater demand for meat and milk and the associated increase in their production stimulate active development of animal husbandry (Post 2012). Recently, more and more research has been done in the field of technologies for the production of meat substitutes (Kumar et al. 2017) and meat produced by animal cell culture (Specht 2020). However, despite this fact, traditional animal husbandry will continue to occupy a leading position in providing the human population with food for a long time to come. The intensification of cattle industry leads to accumulation of a large amount of such organic waste as cattlemanure (Zhong et al. 2017). There are about 330 million heads of cattle in the world for meat production and 234 million heads of cows for milk production (FAOSTAT 2016). Moreover, one cow, depending on its weight, age, etc., produces, according to various estimates, 936 to 2226.5 kg of dung per year (Noorollahi et al. 2015). About 3 billion tons of cow dung is formed annually in China (Xie et al. 2017), 335 million tons in the USA (USDA-ARS 2005), and about 400 million tons in Russia, and 170 million tons of cow dung is suitable for producing biogas and biofertilizers (Namsaraev et al. 2018).

Today, industrial animal waste in the form of manure represents a big environmental problem. If the manure storage system is poorly organized, chemical air pollution can occur with toxic gases (ammonia, hydrogen sulfide, mercaptan, etc.) as well as pollution of soil, surface and ground waters with urea, phenols,

[🖂] Gulnaz F. Rafikova

rgf07@mail.ru

¹ Ufa Institute of Biology, Ufa Federal Research Center of Russian Academy of Sciences, Ufa 450054, Russian Federation

etc. In addition, pathogenic microorganisms causing human and animal diseases may be present in animal waste (Nie et al. 2015; Wang et al. 2017). In this regard, issues related to the need of its management are of particular relevance.

On the other hand, it is well known that manure is a source of nutrients (nitrogen, phosphorus, potassium) necessary for plant growth and development of plants (Bai et al. 2016). The amount of nutrients in organic waste is largely determined by the conditions of feeding and keeping animals. Manure obtained in large industrial-type complexes when feeding animals with a considerable amount of concentrated feed is characterized by an increased content of nutrients necessary for plants.

Currently, there are many ways to utilize organic animal waste: burning, using biogas, synthesis of gas and feed, vermicomposting, passive composting, biofermentation, etc. (Wani and Rao 2013; Xiao et al. 2018; Esteves et al. 2019; Bai et al. 2020).

Among these methods, biofermentation using active strains of microorganisms is the simplest, environmentally friendly and economically feasible technology for processing organic waste in large volumes.

The purpose of this work is to evaluate the effectiveness of compositions based on the strains of microorganisms and intended for cow dung processing.

Materials and methods

Lab-composting process

The objects of investigation were the samples of cow dung formed during litterless livestock housing. The experiment was carried out under model conditions. Material in an amount of 6 kg was placed into fermentation containers (height 0.40 m, diameter 0.20 m, effective volume 10.00 L) covered with heatinsulating material. In total, 15 fermentation containers were prepared for the experiment and randomly divided into 5 groups: one control and 4 experimental groups. Compositions containing bacterial strains of various functional groups were used for waste processing. Microorganisms included into the compositions did not have antagonism towards each other. Therefore, these bacterial strains can be used to create microbial compositions. In order to do this, bacterial strains were cultured on the optimal growth medium and then mixed in equal proportions. The titer of each bacterial strain included into the microbial composition was 10⁸-10⁹ CFU/mL. Microbial compositions in the form of a mixture of liquid culture were introduced into the processed waste in an amount of 5 ml/kg and 25 ml/kg (0.5% and 2.5%). The structure of microbial compositions is given in Table 1. A waste sample without introducing a microbial composition served as the control. To optimize the air regime of the processed waste, manual mixing was performed once every 3 days. To determine the temperature in the fermentation containers, it was measured at several depths. Samples were taken at 5 points averaged by quartering. Sampling was carried out on the 1st, 5th, 12th, 19th and 29th days after starting the experiment. The samples were stored at 4°C for physicochemical and microbiological analyzes.

Microbiological parameters analysis

Isolation and quantification of microorganisms in the processed waste was conducted according to the generally accepted method by plating the suspension on Petri dishes with nutrient medium as described in (Gerhardt 1981). The total number of microorganisms

Table 1 Bacterial strains in microbial compositions used in the bioconversion process

Composition 1 (C 1)	Composition 2 (C 2)
Bacillus cereus BMCH-IB-B-4	B. cereus BMCH-IB-B-4
B. methylotrophicus BMCH-IB-ONF-K1	B. subtilis BMCH-IB-B-5
B. subtilis BMCH-IB-ONF-14	B. thuringiensis var. kurstaki BMCH-IB-B-6
Cellulomonas persica BMCH-IB-C-35	Cellulomonas persica BMCH-IB-C-35
Lactobacillus pentosus BMCH-IB-M-8	Lactobacillus pentosus BMCH-IB-M-8
L. plantarum BMCH-IB-M-3	L. plantarum BMCH-IB-M-3
Penibacillus ehimensis IB 739	Penibacillus ehimensis IB 739

was determined in a Petri dish with nutrient agar (peptone 10 g (w/v), yeast extract 5 g (w/v), NaCl 5 g (w/v), agar-agar 15 g (w/v)), *E. coli* bacteria on Endo agar (Merck, Germany); *Salmonella* on bismuth sulfate agar (Merck, Germany); micromycetes on acidified Czapek-Dox medium (NaNO₃ 2 g (w/v), KH₂PO₄ 0.7 (w/v), K₂HPO₄ 0.3 g (w/v), KCl 0.5 g (w/v), MgSO₄·7H₂O 0.5 g (w/v), FeSO₄ 0.01 g (w/v), sucrose 30 g (w/v), agar-agar 15 g (w/v) (pH 4.5)).

All the media were prepared as per standard procedure and sterilized in an autoclave at 121°C and pressure of 15 lb for 20 min. After sterilization, the medium was dispersed into Petri dishes under a sterile atmosphere in the laminar air flow, and after solidification, these dishes were used to isolate microorganisms. Dilution plating was used to calculate the microbial population (Ariffin et al. 2008). One gram of a compost sample was added to 9 mL of sterile distilled water in a test tube in order to get 10 mL⁻¹ serial dilution. The spread plate technique was used to isolate microorganisms by spreading 0.1 mL of the desired dilution onto the surface of solid agar medium. Microorganisms were grown in a thermostat at 28°C. The incubation period varied for different microorganisms: 24 h for bacteria, 72 h for fungi. The colonies grown in each plate were counted using a colony counter as CFU. The total colony forming units were calculated with consideration given to the dilution factor (Chander et al. 2018).

The *Salmonella* cultures in bismuth sulfite agar (BSA) form brown, grey or black colonies, sometimes with a metallic sheen. BSA plates are incubated for 24 h at 35°C. As a rule, the surrounding medium is initially brown, but may turn black with a longer incubation period creating the so-called halo effect (Abdelkhalek et al. 2016).

The *E. coli* culture on Endo agar forms mediumsized flat red-coloured colonies, sometimes with a dark metallic sheen. The results were recorded after 24 h of inoculation at 37° C (Angelika et al. 2020).

Physicochemical parameters analysis

Samples spread out thinly were dried at 105°C for 3 or 4 h to determine the moisture content according to the Russian standard (State Standard GOST 26713). The temperature was measured with a digital thermometer. The total nitrogen was analyzed using the Kjeldahl method, P_2O_5 with spectrophotometry. The pH was measured with stirring 5.0 g sample in 50 mL of distilled water via a pH meter. Total carbohydrates in air-dried samples were determined using double hydrolysis with H_2SO_4 (4 M and 0.5 M), as reported by Cheshire and Mundie (1966); carbohydrate contents were measured by anthrone colorimetry (Brink et al. 1960).

Phytotoxicity parameters analysis

The phytotoxicity of compost extracts was evaluated by the seed germination technique (Zucconi et al. 1981). A water extract of each compost sample was prepared by shaking the samples with distilled water at 1:10 w/v ratio for 1 hour, and then filtered (Zucconi et al. 1981). Sterile radish seeds (*Raphanus sativum* L.) were used. Five ml of water compost extract was applied to filter paper in a Petri dish and 10 seeds were then placed on the filter paper. All experiments were run in triplicate. The Petri dishes were incubated for 96 hours at room temperature (22°C). The seed germination and root length of the plants in distilled water were measured and used as the control.

The relative seed germination (RSG), relative root growth (RRG) and germination index (GI, the product of relative seed germination and relative root elongation) were calculated as follows (Zucconi et al. 1981):

RSG (%) = (number of seeds germinated in compost extract/ number of seeds germinated in control) ×100 RRG (%) = (mean root length in compost extract/ mean root length in control) ×100 GI (%) = (RSG × RRG)/100

Statistic analysis

Data in Table 2 and Figs are given as the average of three repetitions \pm SE calculated in all treatments using MS Excel. Significance was evaluated using the T-test.

Results and discussion

Microbial composition

To assess the effect of microorganisms on the change in the quantitative indicators of organic waste, bacteria of various physiological groups were selected from the collection of microorganisms of the Institute of Biology (Ufa Federal Research Center, Russian Academy of Sciences) and Closed Joint Stock Company Research

Production Enterprise "BioMedChem". To and create compositions, microorganisms were selected according to their ability to produce a certain kind of metabolites (hydrolytic enzymes, antibiotic substances, organic acids, etc.) that might favorably influence the composting of the waste. Representatives of four genera of bacteria were included into the composition (Table 1). The basis of both compositions was cellulosolytic bacterium Cellulomonas persica BMCH-IB-C-35 with its ability to decompose cellulose and starch to easily accessible substrates; lactic acid bacteria Lactobacillus pentosus BMCH-IB-M-8 and L. plantarum BMCH-IB-M-3 that synthesize bacteriocins inhibiting the growth of pathogenic microbiota and produce organic acids to preserve nutrients in a compostable mixture; Penibacillus ehimensis IB 739 bacterial strain possessing a complex of enzymes with glucanase, chitinolytic and proteolytic activity, as well as antagonistic phytopathogenic microorganisms (Aktuganov et al. 2003; Aktuganov et al. 2007). In addition, the content of the microbial compositions was represented by various types of bacteria of the genus Bacillus, which are capable of synthesizing proteolytic enzymes and substances with antibiotic activity. The difference in microbial compositions was determined by different species of the bacilli.

Microbiological analysis

At the initial stage of composting (on the 5th day) the total number of microorganisms reached its maximum

when introducing microbial compositions, which was apparently due to external addition of microbial consortium culture dominated by bacterial isolates). Similar results were obtained by Chander et al. (2018) when introducing microbial compositions for composting cow dung and straw.

When analyzing the total number of microorganisms, it was noted that on the 29th day in the control sample (without treatment) the number of microorganisms increased by 4 times. On the contrary, when processing the organic waste with microbial compositions, this indicator somewhat decreased (Table 2), thus complying with the data obtained by Chander et al. (2018). The decrease probably occurred due to the fact that the bacteria of the compositions suppressed individual microorganisms in cow dung and also due the accelerated exhaustion of the most preferable substrate for nutrition of microorganisms.

The evaluation of the number of microscopic fungi showed that in the control sample this indicator did not change over time; however, when microbial compositions were introduced, the number of micromycetes in the compostable mixture decreased by half compared to the control sample on the 5th day (Table 2). This is perhaps associated with the bacterial antagonistic activity in microbial compositions against phytopathogenic micromycetes. In particular, this can be explained by the antifungal activity of the strain *P. ehimensis* IB 739 because of both the synthesis of enzymes destructing the fungal cell wall - β -1,3-gluconase, chitonase and chitosanase (Aktuganov

Table 2 The number of different groups of microorganisms in bioconversion of organic waste

Treatments	Time (day)	Total number 10º CFU/g	Micromycetes 10 ⁶ CFU/g	Coliform bacteria 10 ⁶ CFU/g	Salmonella 10 ⁶ CFU/g
Without treatment	5	0.5±0.02ª	10.0±0.45ª	$10.0{\pm}0.48^{a}$	100.0±4.30ª
	29	2.0 ± 0.09^{b}	11.2 ± 0.50^{b}	$50.0{\pm}2.30^{b}$	20.0 ± 0.94^{b}
Dose of inoculants	- 5 mL/kg				
Composition 1	5	2.0 ± 0.08^{b}	0.1±0.005°	9.0±0.41°	7.0±0.30°
	29	1.5±0.06°	0.1±0.004°	$3.0{\pm}0.12^{d}$	$3.0{\pm}0.12^{d}$
Composition 2	5	$2.0{\pm}0.09^{\text{b}}$	$1.0{\pm}0.05^{d}$	12.0±0.53°	26.0±1.10 ^e
	29	0.5±0.01ª	$0.1 \pm 0.004^{\circ}$	$7.0{\pm}0.30^{\rm f}$	$2.0{\pm}0.09^{\rm f}$
Dose of inoculants	- 25 mL/kg				
Composition 1	5	3.5 ± 0.16^{d}	0.1±0.004°	$11.0{\pm}0.61^{ae}$	$2.3{\pm}0.08^{\text{g}}$
	29	0.7±0.03°	0,1±0.004°	3.0 ± 0.13^d	not found
Composition 2	5	$4.3{\pm}0.23^{\rm f}$	$0.1 \pm 0.005^{\circ}$	12.8±0.60°	$1.0{\pm}0.05^{\rm h}$
	29	$0.2{\pm}0.01^{g}$	$0.1 \pm 0.004^{\circ}$	2.0±0.11g	not found

Note: significantly different means are marked with different letters ($p \le 0.05$, t-test).

et al. 2003; Aktuganov et al. 2007) and the synthesis of antibiotic substances by bacteria of the genus Bacillus that suppress the growth and development of microorganisms, including fungi (Kumar et al. 2011). The change in the number of microscopic fungi did not depend on the dose of the compositions introduced. If it is assumed that the decrease in the number of micromycetes occurred mainly due to a decrease in the number of phytopathogens, then there can be a positive effect of microbial compositions on the phytosanitary quality of the resulting organic fertilizer. This is indirectly indicated by the data on phytotoxicity (see section "Phytotoxicity"). Besides, this can allow the use of the resulting compost as an organic fertilizer with additional antifungal properties, as shown by Al-Dhabi et al. (2019).

When adding microbial compositions at the initial stage (on the 5th day), there was no suppression of coliform bacteria. On the contrary, a slight increase was observed in their number apparently due to the insufficient concentration of antibiotic substances in the compostable mixture.

Later on, under the active development of bacteria in the microbial compositions, the number of antimicrobial metabolites increased in the medium and the treatment of organic waste with microbial compositions had a positive effect on the sanitary and hygienic characteristics of the compostable mixture: the number of bacteria of the *Escherichia coli* group and *Salmonella* was lower compared to the control sample (Table 2). Therefore, when treating with Composition 1 and Composition 2 at a dose of 25 mL/kg, salmonella in

the compostable mixture was not detected on the 29th day.

Temperature

The process of direct biofermentation of manure implies an increase in temperature and the onset of the thermophilic phase, at which the compostable waste can be heated up to 50°C or higher. The thermophilic phase arises due to the presence of a large amount of readily available organic substrate at the beginning of the composting process, which ultimately leads to heat generation as a result of microbial metabolism (Xie et al. 2017). A sharp increase in temperature is known to play an important role in the neutralization of waste from pathogenic organisms, including pathogenic microorganisms and helminths, and also has a detrimental effect on weed seeds that may be present in cattle manure.

The maximum temperature indicator is reached approximately on the 5th day (Fig. 1). In the variants of the experiment with the introduction of microbial compositions, the temperature rose to 45-51°C, whereas without any treatment, the compostable waste was slightly heated. Sivakumar et al. (2007) supposed that temperature higher than 55°C eliminates helminths and pathogens and enables maximum disinfection during the process of composting. In view of the fact that the temperature of the compostable mixture did not exceed 55°C during the termophylic phase, the resultant compost should perhaps be considered as immature.



Fig. 1 Change of the temperature during bioconversion. Initial temperature 28°C

Moisture content

Measurements of the moisture content in the processed cow dung showed that the use of microbial compositions for fermentation led to a more intense loss of moisture compared to the control (Fig. 2). This is probably due to more intense microbiological

processes in the experimental sample associated with the release of heat. One month after the experiment, the moisture content remained at a level not lower than 69% that does not require special actions to moisten the compostable mixture, since a moistening range of 53-58% is considered optimal for active destruction of organic compounds (Norbu et al. 2005).



Fig. 2 Change of moisture during bioconversion. Initial moisture 89.2%

рΗ

The change in pH levels in the processed waste is associated with the conversion of organic compounds. At the initial stages of composting this change is determined by the balance of organic acids, NH_4 -N and CO_2 produced during microbial metabolism (Gavilanes-Terán et al. 2016). It was found that all

samples had a slightly acidic reaction of the medium after the introduction of compositions in the first 2 weeks (Fig. 3). One month after the treatment, the pH of the compostable mixture was set to be neutral. Such a reaction of the medium (close to neutral) is most optimal for active development of microorganisms. In the control sample, the pH level increased sharply and was close to neutral on the 12th day.



Fig. 3 Change of pH during bioconversion

Total nitrogen

One of the most important indicators characterizing the quality of the obtained organic fertilizer is the nitrogen content. Large losses of this element often occur when storing cattle manure. With weak development of microbiological processes, the losses of nitrogen in the form of ammonia are possible, as well as of other water-soluble compounds that can be washed out of manure with sediments and melt water. In addition, there is ammonia evaporating from manure (Fischer et al. 2015). Through an analysis, it was shown that in the control sample the amount of total nitrogen changed insignificantly, whereas the treatment with microbial compositions made this indicator increase by 7.1-38% (Fig. 4). The nitrogen content reached its maximum in the experimental sample with the introduction of Composition 1 in a high dose (25 mL/kg). An increase in the nitrogen content in the processed samples of organic waste could occur both due to both the binding of ammonium ions with organic acids produced by the microorganisms of the compositions and the ability of *P. ehimensis* IB 739 to fix atmospheric nitrogen.



Fig. 4 Change of total nitrogen during bioconversion. Initial percentage 1.4%

As shown in our earlier studies (Minnebaev et al. 2019), this bacterial strain possesses nitrogenase at a level of 0.67 μ g N₂/mL per hour. With insufficient nitrogen in the substrate, diasotrophic microorganisms switch over to fixation of nitrogen from the air. Due to this fact, they have a competitive advantage compared to other groups of microorganisms found in the compostable mixture and actively increase the biomass that enriches the compostable mixture with nitrogen when dying off.

Besides, according to the assumption made by Hayawin et al. (2011), an increase in the nitrogen content can largely be associated with the effect of concentration as a result of losses of organic carbon in the form of carbon dioxide during decomposition of the substrate.

Total carbohydrate

While measuring the carbohydrate content in the compostable mixture, a clear downward trend (Fig.

5) was recorded. When processing with the microbial compositions, the carbohydrate content decreased quicker than in the control, and in 20 days it comprised 0.8-1.8% in distinction to 2.4% in the control. This is a classic mechanism associated with the decomposition of organic matter to final products - water and carbon dioxide. Bernal et al. (2009) noted in their research that composting implies partial mineralization of the organic substrate resulting in carbon losses throughout the entire process. The emission of CO₂ is the reason for the loss of carbon (including carbohydrates) in the processed waste. With the introduction of the inoculant, the carbohydrate content decreased quicker than in the control sample, this being explained by a more intensive course of microbiological processes and the need to consume a greater amount of easily accessible carbon substrate by the microorganisms of the compositions (Lu et al. 2018; Chen et al. 2019). In their earlier study, Aira et al. (2007) also reported about the losses of carbon from the substrates as a result of microbial CO₂ respiration.



Fig. 5 Change of total carbohydrate during bioconversion. Initial percentage 5.4%

Total phosphorus

The analysis of the total phosphorus content in the processed waste is given in Fig. 6. It shows that the introduction of Composition 1 in an amount of 5 mL/kg and Composition 2 in all doses resulted in a slight increase in the phosphate content by the end of the experiment compared to the control values. A slight increase in phosphorous during the composting process

might occur by virtue of phosphorous mineralization by bacterial enzymatic activities of the microbial compositions, especially the phosphatase enzyme activity and the synthesis of organic acids by bacteria, thus complying with the data obtained by Kutu et al. (2019). However, it is impossible to draw an unambiguous conclusion about the positive effect of the compositions on the phosphorus content in organic fertilizer.



Fig. 6 Change of total phosphorous during bioconversion. Initial percentage 0.24%

Phytotoxicity

Phytotoxicity is one of the most important criteria for evaluating the suitability of compost for agricultural purposes and to avoid environmental risks before these composts can be recycled back to agricultural land (Cooperband et al. 2003). At the end of the research on the quality of organic fertilizer obtained as a result of manure fermentation, biotesting was carried out. As shown in the research works of some authors, the germination value of test plants for the mature compost should not be less than 80% (Mitelut and Popa 2011; Sangamithirai et al. 2015) testifying to the absence of its toxicity towards plants.

In this study, the use of active strains of microorganisms for processing organic waste improved the phytotoxic properties of the resultant fertilizer. After 29 days, the GI in the samples treated with Composition 1 was 75.5 \pm 3.3 and 85.0 \pm 4.2% (at a dose of 5 and 25 mL/kg, respectively), and in the samples treated with Composition 2, the GI was 80.0 ± 3.7 and 80.0 \pm 3.9% (at a dose of 5 and 25 mL/kg, respectively). All experimental samples, with the exception of the sample with the addition of Composition 2 at a dose of 5 mL/kg, showed seed germination at a level of 80-85%, which exceeded that for the control (75.0 \pm 3.4%). This could be associated with both a decrease in the negative effect of the toxic components of organic waste due to their accelerated decomposition, and the suppression of phytotoxic and phytopathogenic fungi by the microorganisms of the compositions. Besides, the structure of microbial compositions includes bacteria that produce biologically active substances promoting plant growth and development of plants, which could also have a beneficial effect on the germination and emergence rate. In addition, an increase in the content of trace elements necessary for plants in composts can have a positive effect on GI (Romero et al. 2013).

Conclusion

Thus, the result of the experiment showed that the use of the microbial compositions under investigation to treat cow dung led to stimulation of organic matter decomposition and fixation of nitrogen in the resultant organic fertilizer. In addition, when introducing active bacterial strains, there was a noticeable decrease in the number of pathogenic microbiotas and a decrease in the phytotoxicity of the processed waste.

The studied parameters proved that the microbial composition consisting of the bacterial strains *Bacillus cereus* BMCH-IB-B-4, *B. methylotrophicus* BMCH-IB-ONF-K1, *B. subtilis* BMCH-IB-ONF-14, *Cellulomonas persica* BMCH- IB-C-35, *Lactobacillus pentosus* BMCH-IB-M-8, *L. plantarum* BMCH-IB-M-3 and *Penibacillus ehimensis* IB 739 (Composition 1) was the most effective. A dose of 25 mL/kg of this microbial

composition increased the nitrogen content in the compostable mixture by 38%, reduced the development of *E. coli* bacteria by 94%, completely suppressed the development of Salmonella bacteria, and reduced the phytotoxicity of the compost (it was shown by an increase in the seed germination index up to 85%) compared to the untreated samples. The obtained results indicate the possibility of using microbial compositions based on bacteria of various functional groups as a basis of biological products for the accelerated processing of organic waste, such as cow dung.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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