Improving municipal solid waste compost process by cycle time reduction through inoculation of *Aspergillus niger*

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Abstract

A lack of understanding about the effect of microorganism inoculation on compost production and relatively expensive downstream processing are the main obstacles towards an economic compost production. Our work tries to fill this gap. For this, influence of inoculation on the composting of organic fraction of municipal solid waste (OFMSW) to produce compost with higher agronomic value was evaluated. Three similar aerated bioreactors (A, B and C) with the same size and shape in laboratory scale designed. Reactor A was inoculated with the *Aspergillus niger* IBRC-M 30095, reactor B was inoculated with old compost and reactor C was used as a control. During the composting process temperature, moisture, pH, and electrical conductivity (EC) were evaluated. Also, the ratio of carbon to nitrogen (C/N) and germination index (GI) were measured in during process to evaluate compost maturity. The results of this study showed that the C/Ns decreased to about 63.37%, 59.6% and 46% for bioreactors B, A and control, respectively. Also maximum GI and temperature reached to about 138% and 59 °C in reactor B. Furthermore, our results showed that inoculation with this microorganism reduces process time to 18 days that is better than the results of other researchers and thus results in cost savings. However, we think, *Aspergillus niger* is appropriate candidate for compost production as a model.

Keywords Compost · Inoculation · *Aspergillus Niger* · Bioreactor · Municipal solid waste

Introduction

Nowadays, municipal waste is a problem in terms of quality of life and environmental risks. One useful method for solving the problem of organic waste is composting [6]. In composting, organic material in a biological process by microbial communities are transformed to simpler substances [5]. The

- Municipal waste compost process must be improved by reducing time.
- Inoculation effect on the composting of municipal organic waste was investigated.
- Our results showed *Aspergillus niger* reduces process time to 18 days.
- A significant cost reduction was observed by reducing process time in our work.
- *Aspergillus niger* is proposed as a successful model for compost production.

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microorganisms usually are found in initial waste, which can grow and operate during composting [13]. In the beginning of the composting process, the concentration of organic matter is high but microbial population is low, therefore, use of various inoculations can increase microorganism concentration [16, 17, 19]. Several studies have been carried out in this case, for example Wei et al. [22] showed that microbial inoculation was effective for reduction of process time. Also, Awasthi et al. [3] investigated influence of fungal consortium on composting of organic fraction of municipal solid waste. According to their research, inoculation of fungal consortium significantly improved compost humification. Ke et al. [14] evaluated the effect of *Thermotolerant lipolytic actinomyces* on composting. They showed that this inoculation can reduce compost maturity time. In other work, the effect of *Phanerochaete chrysosporium* inoculation on composting of agricultural waste was studied by Varma et al. [19] and found that this inoculation is effective on final compost quality. Nair and Okamitsu [17] studied the effect of *lignocellulolytic* inoculants (*EM bacteria* and *Trichoderma spp.*) on household waste composting. They showed that there is not any significant difference between inoculated composting and composting

without inoculant. Also, Hart et al. [10] studied the effect of *cellulolytic* fungi for composting of wheat straw and observed that the use of this microorganism can enhance product stability and speed of the process.

However, according to contradictory results in articles it seems there is a lack of inoculation effect on the composting process. Therefore, our work tries to fill this gap.

To achieve the above objective, inoculation effect of *Aspergillus niger* IBRC-M 30095 on composting as a model for pilot plant scale bioreactor process was investigated. Also, as companies always looking for ways to reduce their operating costs, another aim of this research is reduction of process time which leads to less required total investment.

Materials and methods

Microorganism

Aspergillus niger is a fungal microbe of great industrial importance. This mold is used extensively in the production of citric acid and in the production of several enzymes such as amylases, pectinases, and proteases. Therefore, in this study, *Aspergillus niger* IBRC-M 30095 was obtained from Iranian biological resource centre (IBRC).

Methodology

In this work, all waste was collected from Amol in northern Iran in the winter of 2014/15. First, we tried to separate plastics, metals and textiles from waste. Composition of solid waste has been shown in Table 1. Crushing of wastes caused homogenization and more accurate sampling. Crushed wastes mixed well and were loaded into

bioreactors. For crushing of waste, we use the crushing machine (khazar Electric, KHD 18.19). The distance between the blades of shredder was set to the size of the output waste is 30 to 50 mm.

Composting bioreactor

To study of the effect of inoculation on composting, three similar aerated bioreactors with the same size and shape was designed in laboratory scale. A schematic diagram of the bioreactors is shown in Fig. 1. The volume of each bioreactor was 40 L. The walls of the bioreactors were insulated using Styrofoam. The air provided by an air compressor and distributed through a perforated PVC pipe. An equal mass feed of homogeneous waste (20 kg) was entered in each of the bioreactors. In this work retention time was 25 days. Air flow rate was adjusted at 2 L/min.kg of raw wet waste. Leachate collected during the process was returned to the bioreactor, because moisture content should not be changed in during process. Digital thermometer (TK-110) was used to measure temperature. Raw materials before entering the bioreactor were homogenous. For stirring the material in the bioreactors, a stirrer (Honeywell® Zone Valve Motor) was placed inside each bioreactor. In this study, stirrer speed was 20 rpm and samples for test (20 g wet weight) were collected daily.

Temperature, moisture content (MC), electrical conductivity (EC), pH, organic matter (OM), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), C/N and germination index (GI) were evaluated to determine the stability of the compost and compost maturity.

The germination index is a maturity test based on seed germination and initial plant growth using a liquid extract from the compost. Organic matter is an important chemical

Table 1 Comparison of the results obtained for reduction of C/N ratio with comparable studies

Reference	Feedstock	Changes of C/N (%)	Initial C/N	Initial moisture content (%)	Final C/N	Time (day)	Inoculation type	Composting system
Our work	Organic municipal solid Waste	57.7 59.6 63.4	42.59	75	19.68 17.19 15.6	18	Non inoculated <i>Aspergillus Niger</i> Mature compost	Small scale bioreactor
Awasthi et al. [3]	Organic municipal solidwaste	52.6	36.12	60	17.12	35	Thermophilic fungal consortium	Windrow
Varma et al. [19]	Agricultural waste	52	~ 25	80–85	~12	20	<i>Phanerochaete chrysosporium</i>	Rotary drum composter
Wei et al. [22]	Municipal solid waste	23.5 27	20.1	60	15.4 14.6	35	Complex microorganisms ligno-cellulolytic (<i>Trichoderma</i> and <i>White-rot fungi</i>)	windrow
Yeoh et al. [25]	Palm Oil Mill Organic Waste	52.1	38.26	65–70	18.3	63	Complex microorganisms	laboratory scale
Zhang et al. [26]	Agricultural wastes	46.6	30	55	16	42	<i>Phanerochaete chrysosporium</i>	laboratory scale

Fig. 1 Schematic diagram of experimental reactors: Bioreactor A was inoculated with the *Aspergillus Niger* IBRC-M 30095, bioreactor B was inoculated with old compost and bioreactor C used as a control without inoculation; (1) composting tank; (2) air compressor; (3) gas flow meter; (4) air regulator; (5) thermal probe; (6) exhausted gas; (7) mixer; (8) effluent; (9) moisture content probe; (10) sampling; (11) electric motor; (12) pump

parameter to check the stability and maturity of compost prepared by municipal solid waste. In this research work, the toxicity test of compost was evaluated by using GI and maturity of compost was also evaluated by using TOC and OM.

Fig. 2 Temperature evolution during the composting test for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control reactor

Changes in TOC and OM also represent the speed of the process. Meanwhile, GI and C/N measurements represent an indicator for completing the compost production process.

Microbial inoculum preparation

Potato dextrose broth (PDB) was used for cultivation. This solution was autoclaved at 121 °C for 20 min and after cooling, a loop of *Aspergillus niger* IBRC-M 30095, grown on a potato dextrose agar plate (PDA), was added to 100 ml PDB medium in 250 ml Erlenmeyer flask. The flask was then incubated at 150 rpm and 37 °C in an incubator shaker (Jal-Tajhiz Shaking Incubator) for 48 h. A 1.5 L volume of this inoculum was prepared and was used to inoculate to bioreactor A. For preparation of old compost inoculant, a compost that a week had been passed from its maturity phase was used and then its moisture content adjusted at 60%. Also 1.5 L of this inoculant sprinkled on reactor B. Reactor C was used as control system (Without inoculation).

Analytical procedures

The samples were collected from 6 different points of the bioreactor, mixed and sieved through a 5 mm mesh screen. The quantity of dry matter and moisture content was measured by drying of samples at 105 °C for 12 h in an electric furnace (Azar furnace). The amount of ashes and organic matter were determined by burning in furnace according to ASTM D 2974–87 [2]. In this method, ash content of compost sample is determined by igniting the oven-dried sample from the moisture content determination in a muffle furnace at 440 °C. The substance remaining after ignition is the ash. The ash content is expressed as a percentage of the mass of

Fig. 3 Moisture changes during the composting process for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

the oven-dried sample. Organic matter is determined by subtracting percent ash content from 100.

The temperature in the centre of waste in bioreactor was measured daily. The amount of TKN and TOC were measured according to American Public Health Association kjeldahl and modified Walkley–Black method [1]. In this research work, the moisture content of the feedstock and the ratio of carbon to nitrogen (C/N) in bioreactors were measured about 75% and 42, respectively. In this study to measure GI, the seeds of Cress (*Lepidium sativum*) were used. GI was used for evaluation of compost negative effects on plant growth. The number of germinated seeds was counted and root length was measured as described by Institute of Standards and Industrial Research of Iran ISIRI 13320 [11]. All analyses for each sample were performed in triplicate.

Results and discussion

Monitoring of temperature, pH, EC and moisture content during the compost production

Figure 2 shows the temperature changes of bioreactors during composting. As seen in Fig. 2, due to presence of more biodegradable materials at begin of the composting process, a rapid rise in temperature was observed in 6 days in all bioreactors. A maximum of 59 °C was observed in reactor B and 49 °C and 45.5 °C in bioreactors C and A, respectively. According to [19], organic matter degradation during composting is directly indicated by the temperature patterns of the compost, and it can be correlated to the rate at which the degradation is being carried out. As a result, it seems production of compost can be

Fig. 4 The pH profile during composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

Fig. 5 The electrical conductivity profile during composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

carried out in the bioreactor B more quickly. We also think because of the presence of various microorganisms in the bioreactor B, temperature of reactor B is higher than the temperature of the other two reactors. In other side, it seems the indigenous microorganisms cannot compete with the fungi in bioreactor A. These results are compatible with the results of Varma et al. [19] and Nair and Okamitsu [17]. Varma et al. [19] studied the carbon decomposition by inoculating *Phanerochaete chrysosporium* during drum composting of agricultural waste. They reported that and this might be due to higher indigenous microbial populations in this trial. Also, Nair and Okamitsu [17] studied the effect of inoculation *Trichoderma* on composting of putrescible kitchen wastes. Their results showed that the maximum temperature in the inoculated reactor was less than the inoculated reactor. Figure 3 shows the changes of moisture content during the composting period in these

bioreactors. In the early days, it seems high speed aerobic processes lead to increase water production. Our result showed that moisture content rose in the first 3 days, indicating more biological water was produced than water vaporized. Increase in water content at the beginning of composting was also reported in a previous study [20, 24]. In this experiment, total moisture losses were found to be 10%, 9% and 18% in bioreactors C, A and B.

Changes of pH versus time are shown in Fig. 4. At the beginning of process, initial pH of all bioreactors was measured 6. As seen in Fig. 4, the pH of all the bioreactors, except bioreactor B, decreased in early stage of composting. Due to the fermentative metabolism it seems the large amount of organic acid is produced [3, 15, 25]. Of course, due to the higher process speed in reactor B, this reduction in the pH has not been observed. Increasing of pH was attributed to

Fig. 6 Total organic carbon profile during composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

Fig. 7 Total nitrogen profile during the composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

the production of ammonia along with degradation of raw materials such as proteins. Decomposition of organic acids, also played as another factor in growth of pH [8, 21]. In the end, although pH of the bioreactors was close to neutral range but total change in pH of control bioreactor is lower than inoculated bioreactors. Therefore, these relatively low changes in pH showed the slow rate of decomposition.

To investigate of compost consumption as fertilizer, the electrical conductivity as an important factor was measured. In fact, EC indicates total salt content in compost. The high EC value could cause a negative effect on plant [23]. For our work, EC profile is shown in Fig. 5. As seen in Fig. 5, at the end of 18th days, the EC increased to about 2.99, 3.3 and 2.91 mS/cm for bioreactor B, A and control, respectively. We think the release of mineral salts and ammonium ions through decomposition of organic matter could be considered as the cause of this increase [13].

Monitoring of TOC, TKN and C/N ratio during compost production

Changes of TOC and TKN are shown in Figs. 6 and 7. As seen in Fig. 6, maximum reduction of TOC was observed in reactor B. TOC reduction during composting was probably due to destruction of more sensitive organic material by bacteria [18]. As seen in Fig. 6, TOC reduced from 38.3% to 36.1% during the 18 days for reactor A. Therefore according to our work 6% of TOC was assumed by the *Aspergillus niger* IBRC-M 30095. In addition, TKN in all bioreactors increased during composting and no significant difference was found between bioreactors A and B. According to Dias et al. (2010), the increase of TKN might be caused by the weight loss of the compost material as a result of carbon dioxide evolution and water loss. Change of C/N versus time is also shown in Fig. 8. Generally, C/N ratio of 15–20 can be used as an indicator of

Fig. 8 C/N ratio changes during composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

Fig. 9 Germination index changes during the composting test for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

compost maturity [12]. In our work, Changes of C/N were measured until 25 days, however, after 18 days, no significant changes were observed in the C/N (data not shown). Therefore we think C/N of 18 days can be used as an indicator of end point of process. Most change in ratio of carbon to nitrogen was observed in the bioreactor that inoculated with old compost. This result is compatible with our observation that the rate of decomposition in bioreactor B is more than other bioreactors. Table 1 shows a comparison between the reductions of carbon to nitrogen ratio in several studies. Awasthi et al. [3] evaluated the inoculation effect on the process by windrow composting for 35 days and showed that the carbon to nitrogen ratio of organic municipal waste was changed from about 36 to about 17. Yeoh et al. [25] also studied the effects of inoculation on composting of palm oil organic waste in laboratory scale

reactor. According to their research, carbon to nitrogen ratio reduced from 38.26 to 18.31 during the 63 days. In our work, the C/N ratios were 19.68, 17.19, 15.6 during 18 days in bioreactors control, A and B respectively. Therefore, according to our method, compost can be produced with highest speed in reduction of C/N. However, we think inoculation in bioreactors, especially ones that inoculated with the old compost, led to a significant increase in process speed. In other hand, it is noted that the reduction of particle size can improve the process [9]. As it has been said, it is necessary to achieve a carbon to nitrogen ratio of less than 20 to produce high quality compost. Accordingly, reactors A and B arrived at this value after the 10th day, but the control reactor on the 18th day. Therefore it seems that the use of *Aspergillus niger* as an inoculant can have a significant effect on reducing the process time. However, cost

Fig. 10 Determination of degradation rate constant (k) for composting for bioreactors A, B and C, respectively inoculated with *Aspergillus niger*, old compost and control

reduction is the process used by companies to reduce their costs and increase their profits. We think that due to the reduction of time to less than 18 days in the compost production, our technique can reduce costs for companies and researchers.

Seed germination test

Germination index (GI) accurately reflects the maturity and quality of the compost.

According to California compost quality council standard [7], GI > 90% shows that process has been completed and subsequently the final compost has been matured. In this research, GI changes during the composting test shown in Fig. 9.

As shown in Fig. 9, GI index has been reached above 90%, therefore we think our produced compost has been matured. The GI values decreased in the early stages of composting. We think this can be due to production of fatty acids and ammonia. After 3 days, on the contrary, it seems the decomposition of these acids leads to a decrease in GI values. Also maximum GI value observed in bioreactor B. This may be because the amount of phytotoxicity in this compost is lower than those observed in bioreactors A and control. However, according to the results of this study, GI values in bioreactors A and B were better than the values for control bioreactor. In other words, inoculation with *Aspergillus niger* IBRC-M 30095 can be successfully chosen as a good model for the optimum compost production. These results are also compatible with the result of [3].

Process kinetics

To find out the speed of the process in each bioreactor, determination of process kinetic can be useful. Review of the literature showed that first order is the most common form of description of the composting process [4]. Therefore, a first-order equation was adopted in this study to model the composting process.

We assume that the degradation of organic matter obeys from a first-order kinetics as below:

$$\frac{dM}{dt} = -kM \quad (1)$$

where M is the quantity of organic matter at any time in kg, k is the reaction rate constant and t is the time in days. Solving eq. (1) we find:

$$-\ln \frac{M}{M_0} = kt \quad (2)$$

Figure 10 shows a comparison between obtained values of reaction rates for these three bioreactors. As seen in Fig. 10, maximum degradation rate was observed in bioreactor B and minimum degradation rate was observed in bioreactor A. It seems *Aspergillus niger* IBRC-M 30095 wins in competition

with other microorganisms and it cannot act alone on all organic materials, therefore less organic matter decomposition was observed in the reactor A. These results imply that inoculation with *Aspergillus niger* IBRC-M 30095 has an impressive effect on degradation speed, but inoculation with compost showed a greater effect on increasing the speed. This result is compatible with result of [13].

However, we suggest *Aspergillus niger* IBRC-M 30095 as an efficient model for production of compost in bioreactors. Advantages would be a reduction of process time.

In fact, the most important result of our research is the reduction of the process time, which could be very attractive to compost companies. This is a very economically important issue. However, the results may help the possibility of close cooperation between engineering and basic research groups. Finally, it has to be mentioned that optimization of the amount of inoculation and therefore achievement of higher yields of *Aspergillus niger* IBRC-M 30095 remains as some gaps for future studies.

Conclusion

In this research work, the effect of *Aspergillus niger* IBRC-M 30095 inoculation on the compost production process from the organic fraction of municipal solid waste was investigated. According to this research, the use of inoculation can have a positive effect on composting processes. A significant effect on C/N ratio and GI was observed using this method. However our results showed that inoculation of *Aspergillus niger* IBRC-M 30095 can increase product quality and reduce the time of process. Also, as companies always looking for ways to reduce their operating costs, therefore it seems reduction of process time leads to less required total investment. This study was performed on a laboratory scale and the results can be used to improve composting on a larger scale.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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