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Changes in L-Glutaminase and L-Asparaginase Activities During Vermicomposting of Organic Solid Wastes

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ABSTRACT

Background and Objective: The vermicomposting of solid wastes has attracted much attention during the last decade because animal manure, crop residues and other solid wastes are increasingly cumulating and generating some environmental problems. In this study, L-glutaminase (E.C. 3.5.1.2) and L-asparaginase (E.C. 3.5.1.1) activities were considered as possible tools for the characterization of cow manure (CM) and wheat residue (WR) biodegradation during 60 days of the vermicomposting process. The objectives of this study were to investigate the effects of vermicomposting process on the activity of the amidohydrolases which are effectively involved in N mineralization and subsequent N supply to the plants and to find a bio-indicator reflecting the maturity of the vermicomposts. **Materials and Methods:** Organic carbon (OC), total nitrogen (TN), L-glutaminase and L-asparaginase activities were determined on seven occasions with 10-day intervals (T0, T10, T20, T30, T40, T50 and T60). **Results:** The both solid wastes, general decreasing trends in C/N ratio were observed. In contrast, an increasing trend was monitored in the N concentration of the products. Two distinct phases were monitored in L-glutaminase and L-asparaginase activities. During the first phase which last for 30 to 40 days, L-glutaminase was increased in CM and WR by 5.3 and 2.9 fold, respectively. The values for L-asparaginase were 2.3 and 2.5 fold. During the second phase, the enzyme activities decreased slightly or remained unaffected. **Conclusion:** The enzyme activities were shown to be responding bio-indicators to vermicomposting and thus they can be used as bio-indicators of vermicompost maturity.

KEYWORDS

Biodegradation, amidohydrolases, N mineralization, plant available N, enzyme activities, maturity index

INTRODUCTION

Eisenia fetida has been widely used for conversion of organic wastes to vermicomposts[1]. The earthworms enhance the rate of organic matter biodegradation and enzyme activities[2]. The L-glutaminase (E.C. 3.5.1.2) and L-asparaginase (E.C. 3.5.1.1) are among amidohydrolases contributing to N mineralization in soils, solid wastes, forest litter and plant residues and hence, play an important role in supplying N to the growing plants and soil-dwelling microorganisms[3]. The amidohydrolases are responding bioindicators reflecting the soil organic matter status[4]. The response of urease activity to the process of vermicompost production from animal manure and plant residues has been investigated. It has been shown that urease activity was negatively correlated with the vermicomposting time. Urease activity has eventually reached

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a threshold level, that was considered as maturity[5]. Other amidohydrolase responses to vermicomposting have not been investigated. So, it is hypothesized that vermicomposting of solid wastes would change the availability of energy, C and N for microbial communities and therefore would influence the enzyme activities. The objective of this study was to investigate the response of L-glutaminase and L-asparaginase activities to the vermicomposting process during vermicomposting of cow manure (CM) and wheat residues (WR).

MATERIALS AND METHODS

The study took place at Isfahan University of Technology, Isfahan, Iran from August to December, 2021. The CM and WR were collected from Lavark Research Station, Isfahan University of Technology, Isfahan, Iran. Before the experiment, the solid wastes were milled and passed through a sieve (2 mm in diameter). Twenty earthworms (*Eisenia fetida*) were added per kg of the initial substrates including CM and WR. The moisture was adjusted at 70% of water holding capacity and the temperature was adjusted at 25°C during a 60 day incubation. The products were sampled every 10 days. The sampling time T0 represents the start of the process before introducing the earthworms to the initial substrates. The T10, T20, T30, T40, T50 and T60 represent the consecutive sampling times corresponding to 10, 20, 30, 40, 50 and 60 days of incubation, respectively. Organic carbon (OC) and total N (TN) were determined by wet digestion and Kjeldahl digestion, respectively[4]. The activities of L-glutaminase and L-asparaginase were assayed by a toluene and buffer-based technique[3].

RESULTS AND DISCUSSION

Vermicomposting decreased the OC content by 31.1 and 13.1% in the CM and WR treatments, respectively (Table 1). In the CM treatment, TN was increased by 36% until T50 whereas, a decrease was monitored in the last sampling time (T60). Instead, a consistent increase in TN was observed in WR, so the TN values increased by 69% (Table 1). The C:N ratio was decreased in the CM and WR by 44 and 73 %, respectively. The initial activity of the L-glutaminase was 573 mg NH_4^+-N kg h^{-1} in the CM treatment and was increased until T50 by 5.3 fold followed by a slight decrease to 2742 mg NH_4^+-N h^{-1} at T60. The behavior of L-glutaminase activity in the WR was somewhat different. Even though it was gradually increased by 2.9 fold until T30, a decrease happened between T30 and T40, so that the final activity of L-glutaminase was not different from that of initial WR at T0 (Table 1). The L-asparaginase activity in the CM was increased until T40 by 2.3 fold and remained unaffected to the T60. The L-asparaginase activity of the WR was increased by 2.5 fold until T30 and then decreased gradually to T60 (Table 1).

The overall decreasing trend in the OC contents can be attributed to the release of CO_2 during vermicomposting[5]. Digestion of organic substrates within earthworm guts enhances the rate of CO_2 evolution[6]. It is reported that TN was increased at the end of vermicomposting period in different feed mixtures, because of mineralization of organic matter[7].

Regardless of the solid waste type, both enzyme activities revealed an early increase. Earthworms directly as a consumer or indirectly by stimulation of microbial activity enhance the rate of mineralization, which results in a more rapid conversion of the initial material into humus-like substances[8]. Although there are no previous reports found regarding the response of L-glutaminase and L-asparaginase to vermicomposting process in the literature, greater activities of cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase are reported Edwards and Arancon[8].

Following the early increase in the activity of L-asparaginase and L-glutaminase (Table 1) the enzyme activities showed a tendency to be stabilize presumably due to the decrease in available organic substrates during the final stages of the vermicomposting. Current study findings corroborated the results suggesting a stabilization period at the end of vermicomposting process for other enzyme activities[9]. Two phases were observed during the vermicomposting process (Table 1). The first phase (hydrolytic phase) occurs

Table 1: Variation of some chemical and biochemical characteristics of the organic materials

Time (d)	OC (g kg^{-1})	TN (g kg^{-1})	C:N	LGL (mg NH_4^+ kg h^{-1})	LAS (mg NH_4^+ kg h^{-1})
CM					
T0	177.4 (7.3)^{ad}	11.3 (0.36)^{d}	15.5^{a}	573 (23.0)^{f}	851 (19.7)^{d}
T10	227.8 (2.4)^{b}	15.1 (1.07)^{bc}	15.1^{a}	1970 (8.7)^{e}	1172 (39.8)^{c}
T20	237.9 (7.5)^{a}	15.0 (0.15)^{bc}	15.8^{a}	2589 (29.5)^{c}	1388 (12.4)^{b}
T30	186.6 (6.7)^{d}	14.5 (0.10)^{c}	12.9^{b}	2458 (6.6)^{d}	1425 (48.3)^{b}
T40	209.9 (3.4)^{c}	16.4 (0.19)^{ab}	12.8^{b}	2975 (18.3)^{a}	1924 (20.5)^{a}
T50	154.2 (2.4)^{e}	17.6 (1.50)^{ab}	8.7^{c}	3031 (9.0)^{a}	1771 (34.1)^{a}
T60	122.2 (3.6)^{f}	14.1 (1.50)^{c}	8.7^{c}	2742 (9.8)^{b}	1856 (37.2)^{a}
WR					
T0	372.7 (4.5)^{b}	6.3 (0.25)^{f}	59.5^{a}	918 (36.1)^{cd}	154 (3.5)^{ef}
T10	399.9 (13.7)^{a}	8.5 (0.27)^{de}	47.2^{b}	1088 (42.6)^{c}	198 (3.9)^{cd}
T20	352.7 (12.4)^{bc}	8.4 (1.07)^{e}	42.3^{bc}	1837 (26.1)^{b}	245 (6.8)^{b}
T30	404.5 (15.8)^{a}	10.3 (0.34)^{d}	39.2^{c}	2630 (54.6)^{a}	388 (6.8)^{a}
T40	334.8 (13.0)^{cd}	15.8 (0.51)^{b}	21.2^{e}	686 (15.1)^{e}	211 (3.9)^{c}
T50	356.3 (15.5)^{bc}	12.8 (0.26)^{c}	27.7^{d}	1054 (26.9)^{c}	177 (3.9)^{de}
T60	323.7 (6.2)^{d}	20.1 (2.40)^{a}	16.2^{e}	811 (49.3)^{de}	129 (3.9)^{f}

[a]Values in parenthesis are standard errors, CM: Cow manure, WR: Wheat residue, OC: Organic C, TN: Total N, LGL: L-glutaminase activity, LAS: L-asparaginase activity and Data within each column with the same letter are not significantly different ($p<0.05$)

as a stimulation of metabolic activity probably because of the degradation of readily available organic substrates by the microorganisms. The second phase is characterized by a reduction of enzyme activities, due to biodegradation of readily decomposable organic constituents. The second phase has been considered as the stabilization phase[9].

It is clearly shown that the amidohydrolase activities can be considered as maturity indices for the vermicomposts production. It is noteworthy that the maturity index could only be used when the early sage of the decomposition has been passed. Therefore, there is a need to evaluate the duration of the early degradation phase for various initial materials and other earthworm species.

CONCLUSION

The L-glutaminase and L-asparaginase were responding to the progress of the vermicomposting process. A two-phased behavior was observed indicating that regardless of the solid waste type, the enzyme activities increased at different rates and then stabilized for the rest of the process. Our findings support the hypothesis suggesting that the amidohydrolases can be used as maturity indices of solid waste composts.

SIGNIFICANCE STATEMENT

Processing the solid wastes and transforming them into a value-added soil organic amendment may provide the growers with a qualified organic fertilizer supplying macro- as well as micronutrients. The duration of the vermicomposting process reaching a mature organic amendment is always a major question. Different maturity assessment techniques have been developed nevertheless, a biological indicator is still required to reflect the stage of the biodegradation process. It is hypothesized that the amidohydrolases may indicate the maturity of the vermicomposts. This study aims to find a reliable bio-indicator to show the maturity of vermicomposts. The findings revealed that the amidohydrolases show a two-phase process, by which the maturity would be achieved at the end of the first phase.

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