Valorization of agro-industrial waste from palm oil mill into matured fertilizer: Enzymatic profiles during vermicomposting process

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Abstract

Palm oil mill effluent (POME), a polluted agro-industrial waste, is generated in large amount due to crude palm oil production in Malaysia. In this study, biological (microbial population, dehydrogenase and hydrolase activities) parameters were evaluated during the vermicomposting of POME using *Eudrilus eugeniae*. With time, dehydrogenase and hydrolase activities decreased while the microbial population increased due to the biodegradation of POME. The biological parameters showed that the use of earthworms helped increase the biodegradation rates and promoted microbial activities. All the parameters, except dehydrogenase activity, indicated a matured vermicompost after a period of six weeks. This parameter indicated a slightly longer period of organic waste

stabilization with an additional week. The results implied that a combination of maturity and stability methods were required to ensure high quality of vermicompost before agronomic application.

KEYWORDS: dehydrogenase activity; hydrolytic enzymes; microbial population; nitrogen transformation; waste reuse

Introduction

Valorization refers to any chemical and biotechnological processes aimed at recovering value-added products from by-products or waste [1]. An example of waste valorization technology is a vermicomposting process. Vermicomposting is a well-known process for transforming organic waste biologically into vermicompost, which is a nutrient-rich fertilizer and soil conditioner [2]. Vermicomposting involves the interactions between earthworms and microorganisms in the organic waste decomposition process. Earthworms will condition and fragment the organic waste, altering its biological activity for further biochemical decomposition by the microorganisms [3,4]. The potential of vermicomposting technology to manage a wide variety of biodegradable solid waste is well documented, such as agro-industrial waste [5,6], industrial waste [7,8] and municipal waste [9,10]. The efficiency of the vermicomposting process is characterized by earthworm growth, C/N ratio and available nutrients content [11,12].

However, biological parameters such as dehydrogenase and hydrolytic enzyme activities are not commonly evaluated but they are also useful indicators of biological activity in various ecosystems. Enzyme activities can provide insights into the evolution of the vermicomposting process, allowing the determination of vermicompost stability and maturity [10]. For example, the decrease in dehydrogenase and hydrolytic enzyme activities is known to be related to the stabilization of organic matter during the vermicomposting process [13]. Dehydrogenase activity represents the overall microbial activity while hydrolytic enzymes metabolize high organic polymers into smaller ones. Thus, analyzing them helps to track the evolution of organic matter during the vermicomposting process [14]. Hydrolytic enzymes like cellulase and β-glucosidase are

associated to the C-cycle and therefore responsible for the changes of carbon in the organic waste. Cellulase catalyzes the release of the substrate for β-glucosidase which in turn catalyzes the hydrolysis of cellobiose and other disaccharides compounds [15,16]. Protease and urease activities are enzymes related to the N-cycle, and finally, phosphatase activity is the enzyme of P-cycle [15]. Also, other biological parameters like microbial population can also be used to indicate the quality and maturity of the vermicomposted material [17].

In this study, the evolution of enzyme activities such as dehydrogenase, β-glucosidase, cellulase, amylase, acid phosphatase, urease, and protease was investigated during the vermicomposting of palm oil mill effluent (POME). POME is a polluted agro-industrial wastewater generated in the significant amount due to a wet process for palm oil milling. This effluent originates from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater [18]. It contains high biochemical oxygen demand, chemical oxygen demand, oil and grease, total solids and suspended solids which can cause severe environmental problems if the untreated effluent is released into the environment. The conventional treatment methods for POME such as ponding system is economically viable but has the highest environmental pollution with the lowest utilization of renewable resources [19]. On the other hand, biological decomposition process like vermicomposting gives rise to opportunities for resource recovery at a lesser cost as compared to other more sophisticated treatment technologies [20].

The feasibility of using vermicomposting technology to transform POME into organic fertilizer has been demonstrated through various chemical [20,21] and instrumental [21,22] parameters. However, the studies on using biological parameters, especially enzymatic activities in the determination of vermicompost maturity are

generally very limited. Currently, no study is done on the evolution of enzymatic activities during vermicomposting of POME which has been absorbed onto the rice straw. Therefore, the present study focused on analyzing the evolution of organic matter during vermicomposting process via enzyme activities. Also, the microbial populations were also evaluated so that a more comprehensive study on the overall vermicompost maturity and stability could be obtained.

Materials and methods

Vermicomposting process

POME was obtained from Ulu Langat Palm Oil Mill, Selangor, Malaysia and stored at 4°C before being used. Rice straw was obtained from a paddy field in Sekinchan, Selangor, Malaysia. The chemical characteristics of POME and rice straw are given in Table 1. The earthworm species, *Eudrilus eugeniae* were obtained from ESI Agrotech, Malaysia. The feedstock for the vermicomposting process was prepared by adding 600 mL of POME to 200 g of rice straw [20] and placed in a rectangular plastic container (17 cm × 14 cm × 12 cm). Control bins (without earthworms) were also set up with the same feedstock. Before the addition of earthworms, the feedstock was turned periodically for two weeks to eliminate any volatile gasses potentially toxic to earthworms [23]. The moisture content was maintained at 70-75% throughout the six weeks of the vermicomposting process. All experimental containers were kept in triplicate and placed in a dark laboratory at an ambient temperature of 25±2°C.

Enzyme activities analysis

For cellulase activity, 0.4 g of sample were mixed with 1% carboxymethyl cellulose sodium salt (CMC) and citrate buffer. The mixture was then incubated at 50°C for 24 h. As for amylase activity, 1 % of the starch solution and acetate buffer were added to 0.4 g of samples before the incubation at 37°C for 2 h. Finally, the cellulase and amylase activities were measured spectrophotometrically at 540 nm via determination of reducing sugars calorimetrically by DNS method [24].

β-glucosidase activity was determined by adding the substrate, 4-nitrophenyl-β-D-glucanopyranoside (PNG) and modified universal buffer (MUB) to 0.2 g of samples. After the incubation for 1 h at 37°C, $CaCl_2$ solution and Tris buffer were added. The p-nitrophenol produced was measured spectrophotometrically at 410 nm [25]. Assays of acid phosphatase activity were assessed by incubating (1 h at 37°C) 0.25 g samples mixed with MUB, toluene and 0.115M p-nitrophenyl phosphate. After the incubation, $CaCl_2$ and NaOH solutions were added. Acid phosphatase activity was then measured spectrophotometrically at 420 nm [26].

Urease activity was estimated by incubating 0.5 g of samples with the urea solution and borate buffer for 2 h at 37°C. KCl solution was then added and mix thoroughly, followed by the additions of sodium salicylate and sodium dichloroisocyanide solutions to determine the amount of ammonium in the samples. The released ammonium was measured spectrophotometrically at 690 nm [26]. To determine protease activity, casein solution was used as a substrate. The substrate and phosphate buffer was added to 0.5 g of samples for incubation at 50°C up to 2 h. After the incubation, 10% of trichloroacetic acid was added to stop the reaction. The amount of tyrosine in the

samples were then determined via the Lowry method and measured spectrophotometrically at 750 nm [26].

Dehydrogenase activity was determined using the method as described by Chaperon and Sauvé [27]. Briefly, 1 mL of 1% 2,3,5 triphenyltetrazolium (TTC), 5 mL of deionized water and 2 mL of Tris buffer (pH 8) was added to 0.2 g of samples. The mixture was then incubated for 24 h at 37°C. After the incubation, 10 mL of methanol were added, the samples were centrifuged, and its supernatant was measured using spectrophotometer for dehydrogenase activity at $\lambda = 485$ nm.

Microbial analysis

Initial, vermicompost and control samples were analyzed for bacterial, fungal and actinomycetes population. One gram of sample was mixed with distilled water using a horizontal shaker for 30 min. The mixture was diluted serially, and 1-µL of aliquots were spread-plated in Nutrient Agar, Potato Dextrose Agar and Starch-Casein Agar for determination of bacteria, fungi and actinomycetes population, respectively. The plates were incubated for 24 h (bacteria), three days (fungi) and one week (actinomycetes) to count the colony forming units (CFUs) of the microbes.

Statistical analysis

One-way ANOVA was used to analyze the significant differences between initial, vermicompost and control samples. Tukey's-b test was used as a post hoc analysis to

compare the means of the samples. IBM SPSS Statistics (Version 20) was used for data analysis. All statements reported in this study were at the $p < 0.05$ levels.

Results and Discussion

Enzymes of the C-cycle: Amylase, cellulase and β-glucosidase

Trends of enzymes active in the C-cycle, namely amylase, cellulase and β-glucosidase are shown in Figs. 1a to 1c. The three enzymes in the vermicompost treatment were characterized by an initial increase to a maximum before decreasing towards the end of the vermicomposting process. Both amylase and β-glucosidase activities peaked at week 2 while cellulase activity reached a maximum at week 4. The increasing and subsequent decreasing trend in these enzymatic activities were also reported by Sen and Chandra [8] and Cunha-Queda et al. [28]. For the control treatments, only the enzyme amylase showed an initial increase up to week 2 and was followed by a decreasing trend. The enzyme β-glucosidase did not show any significant peak throughout the experimental process while the cellulase activity showed an increase towards the end of the process.

During organic waste biodegradation process, microbial communities did not decompose organic waste directly but produce hydrolytic extracellular enzymes to breakdown large organic compounds into smaller fragments. The evolution of enzyme activities during the biodegradation of organic waste was dependent on the growth of microbial communities which increased with substrate concentrations [8]. Earthworm activities are also known to be instrumental in promoting enzymatic activities via increasing substrate availability and activation of microbial metabolism [15]. This would

explain the higher enzyme activities obtained in the vermicompost treatments as compared to the control. Furthermore, the enzyme β-glucosidase could be activated in the worm's gut resulting in higher cellulolytic activity in the vermicast [29]. Fresh vermicast from *E. eugeniae* is known to increase enzymatic activities [8]. Thus, the enzyme activity would decrease as the earthworm biomass decreases due to the lower production of vermicast [29]. The decreasing enzymatic activities observed in this study was corroborated by the decline in earthworm biomass (results not shown) at the end of the vermicomposting process. The declining trend in all three enzymes towards the end of the vermicomposting process also indicated the stabilization of organic matter [10].

Enzymes of the N-cycle: Protease and urease

Protease and urease are hydrolytic enzymes involved in the N-cycle as they catalyse the hydrolysis of protein N to dissolved organic nitrogen [15] and urea to CO_2 and NH_4^+ [10], respectively. Protease activity showed a continuous decrease in both vermicompost and control treatments throughout the biodegradation process (Fig. 1d). On the other hand, urease activity increased significantly to a maximum at week 4 and then decreased until week 6 in the vermicompost. However, the urease activity in control treatment did not show any noticeable increase or decrease for six weeks (Fig. 1e). The protease and urease activities trend were similar to those obtained by Benitez et al. [10]. The reduction in protease activity would suggest the decrease in the available organic substrate [10]. The maximum value for urease activity was achieved after the maximum for protease activity which agrees with the general premise that urease activity acts after the hydrolysis promoted by the protease [28]. The increase in urease activity during the first four weeks

was probably due to the low NH_4^+ content in the organic waste. High NH_4^+ content is known to inhibit the urease activity [29]. Thus, there was a significant decrease until the end of the vermicomposting process. The differences in urease activity between the vermicompost and control treatments suggest that earthworms were instrumental in promoting the enzyme activity. According to Pramanik et al. [30], earthworms enhanced the activity of microorganisms in the organic waste as it passed through the gut of earthworms. The microorganisms will then increase the urease activity in the vermicompost.

Enzyme of the P-cycle: Acid phosphatase

Acid phosphatase activity is related to the P-cycle as it catalyzes the hydrolysis of organic phosphomonoester into inorganic phosphate [16]. The phosphatase activity in the vermicompost showed an increase up to week 3 before decreasing till the end of the vermicomposting process. However, the control treatment showed maximum phosphatase activity at week 6 (Fig. 1f). The differences between vermicompost and control treatments suggested that earthworms played a crucial role in phosphatase activities during organic waste decomposition. The increase of phosphatase activity in the vermicompost could be caused by the consumption of P-cycle metabolizable substrates by the earthworms and microorganisms [14]. The decline in phosphatase activity indicated the disappearance of easily decomposable organic compounds which occurred during the stabilization of organic matter [10]. Furthermore, the release of inorganic phosphate during the mineralization of organic waste is known as an inhibitor of

phosphatase activity [28]. A similar trend in phosphatase activity was also observed by Castillo et al. [29] during the vermicomposting of vine shoots and biosolid vinasse.

Dehydrogenase activity

Dehydrogenase activity is used as an assessment of overall microbial activity during the biotransformation process [14] and provides a clearer indication of the dynamics of organic matter degradation [16]. The dehydrogenase activities in both vermicompost and control treatments increased markedly during the six weeks of the vermicomposting process (Fig. 2). The higher increase in the vermicompost could be caused by the release of metabolizable substances for microorganisms due to the earthworm activities [14]. After the initial increase, the dehydrogenase activity will generally decrease towards the end of the vermicomposting process due to the decomposition of available organic matter [10]. However, this trend was not observed during the first six weeks of the vermicomposting process even though the C/N ratio of vermicompost was less than 20. Therefore, the vermicomposting process was extended to determine the duration required for the decrease in dehydrogenase activity. An additional week was necessary for the dehydrogenase activity to show a significant reduction in the vermicompost treatment but not the control. This reduction indicated the mineralization of organic matter which reduced available carbon substrates for the microorganisms [16]. The drop of dehydrogenase activity in the vermicompost as opposed to the control treatments could be explained by higher mineralization rate [10] which could be due to the presence of earthworms. The results also implied that control treatments required a longer time to achieve maturity as compared to the vermicompost treatments.

Microbial population

Microbial population was measured to determine the impact of the vermicomposting process on compost microbial richness [17]. The bacterial, fungal and actinomycetes population were significantly higher in vermicompost than those in control and initial (Fig. 3). There was no significant difference between the control and initial. This result suggested that the presence of earthworms increased the microbial population during the vermicomposting process. The positive impact of earthworm on bacterial, fungal and actinomycetes population were also reported by Singh and Suthar [7] during vermicomposting of cow dung and herbal pharmaceutical waste. Microbes present in the organic waste were not killed in the intestinal tract of the earthworms; instead, the microbial population increased in the ejected earthworm casts [30]. John Paul et al. [9] suggested that earthworms were instrumental in increasing microbial colonization which led to higher microbial activity and population. As the vermicomposting process progresses, the increasing availability of bio-transformed organic matter and soluble nutrients could serve as a media for the growth of fungal and actinomycetes population [17].

Conclusion

In the present study, the decrease in enzymatic activities towards the end of the vermicomposting process indicated the decomposition and stabilization of organic waste. The significantly higher enzymatic activities and microbial populations in the

vermicompost as compared to the control suggested that earthworms helped increase the rate of POME degradation process. This study also showed that vermicompost was deemed matured after 6 weeks based on the hydrolytic enzyme activities but not dehydrogenase activity. Thus, the results further confirmed the importance of using multiple biological parameters to ensure the overall vermicompost stability and maturity.

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List of Table

Table 1 Chemical characteristics of POME and rice straw (means ± SD, *n* = 3).

Parameters	POME	Rice straw
pH	4.51 ± 0.00	7.43 ± 0.02
EC (μS/cm)	1400 ± 28	521 ± 39
C/N	27.21 ± 0.73	55.60 ± 11.54
Ca (g/kg dry wt)	0.50 ± 0.00	1.09 ± 0.10
K (g/kg dry wt)	4.36 ± 0.18	14.42 ± 1.70
Mg (g/kg dry wt)	0.81 ± 0.04	2.19 ± 0.40
P (g/kg dry wt)*	0.26 ± 0.00	1.34 ± 0.09
COD (mg/L)*	65667 ± 1803	-

*Lim et al. [20]

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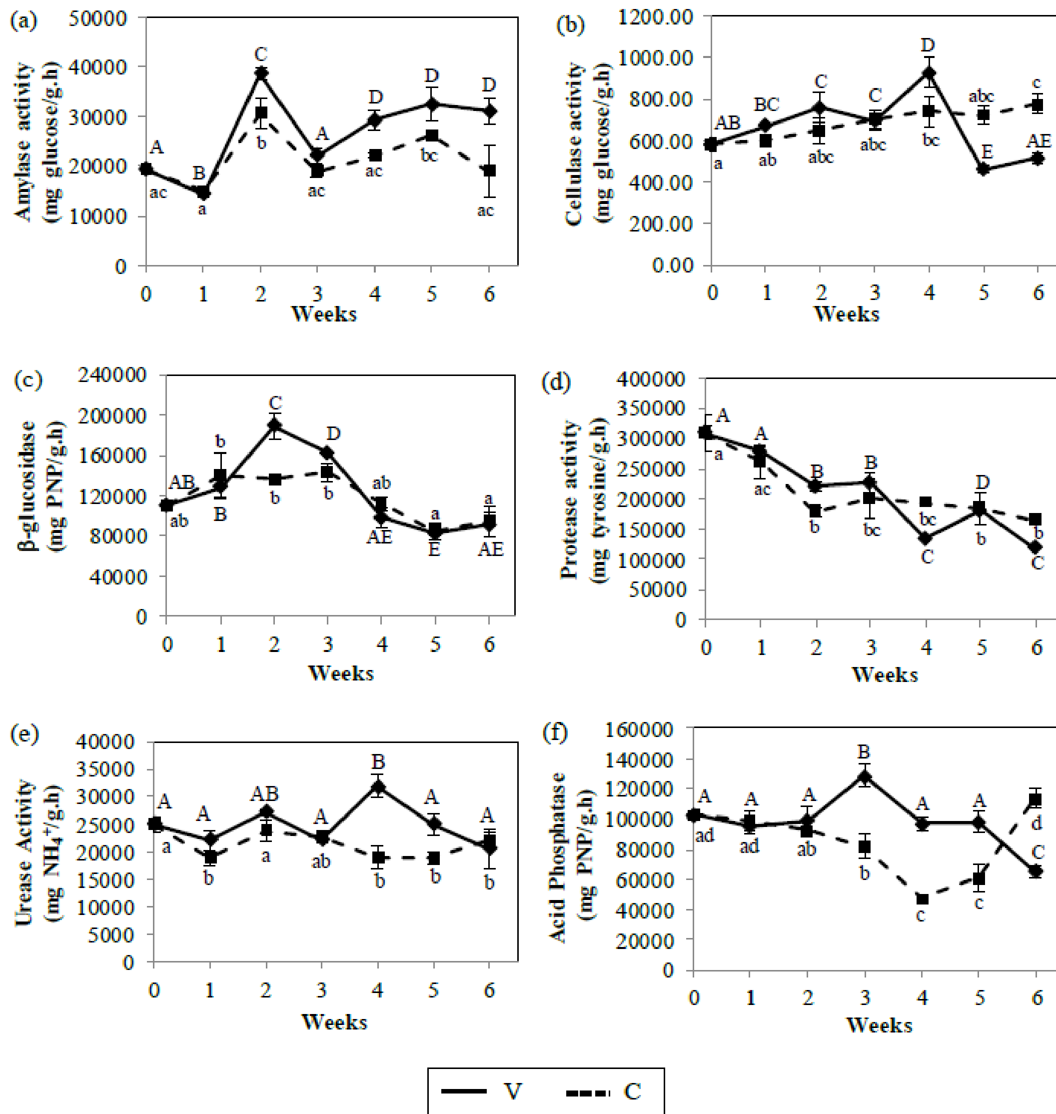


Fig. 1 Changes in (a) amylase, (b) cellulase, (c) β-glucosidase, (d) protease, (e) urease and (f) acid phosphatase activities in the vermicompost and control samples. The different upper and lower case letters indicate statistically significant differences ($P < 0.05$) within vermicompost and control samples, respectively

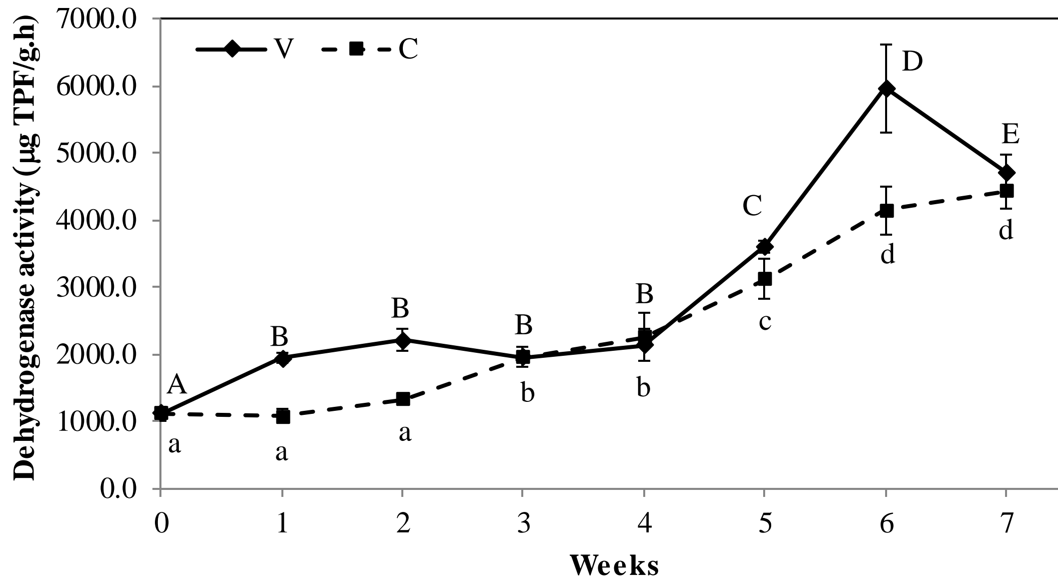
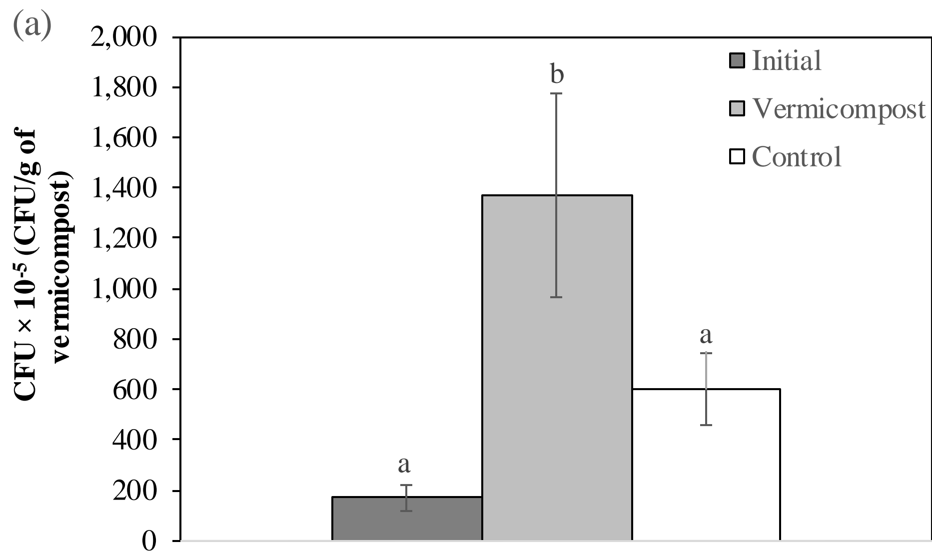
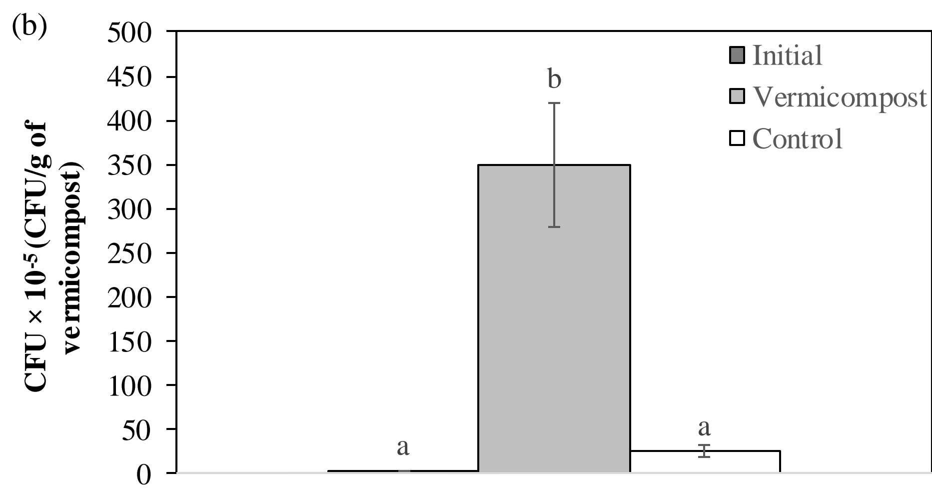


Fig. 2 Changes in dehydrogenase activity in the vermicompost and control samples. The different upper and lower case letters indicate statistically significant differences ($P < 0.05$) within vermicompost and control samples, respectively


(a)
CFU × 10-5 (CFU/g of vermicompost)
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
Initial
Vermicompost
Control
a
b
a


(b)
CFU × 10-5 (CFU/g of vermicompost)
500
450
400
350
300
250
200
150
100
50
0
Initial
Vermicompost
Control
a
b
a

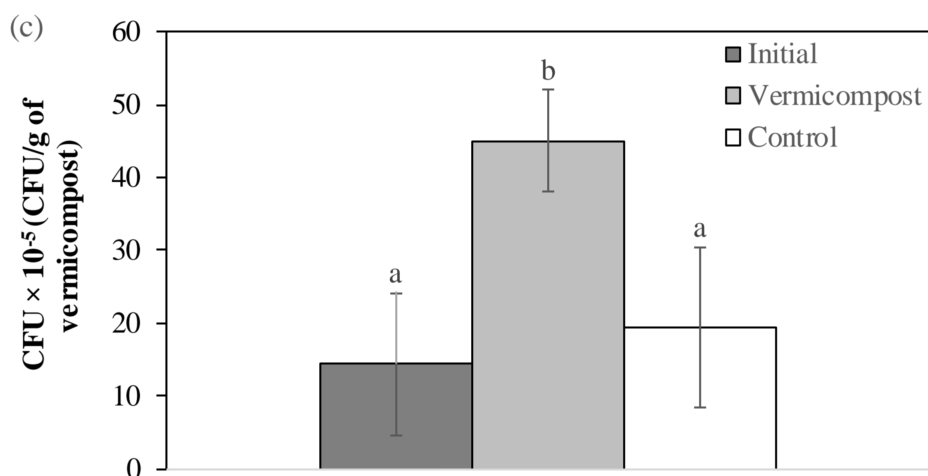


Fig. 3 (a) Bacterial, (b) fungal and (c) actinomycetes population in initial, vermicompost and control samples. The different letters indicate statistically significant differences ($P < 0.05$) between the samples.