

## BIOLOGICAL MATURITY AND CHEMICAL STABILITY OF MICROBIAL INFLUENCED TAPIOCA SOLID WASTE VERMICOMPOST

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*Abstract: Tapioca solid waste (TSW), a major biodegradable solid waste having high amounts of organic matter, act as a better source and cow dung (CD) used as bulking material in the proportion of 3:1 for polyculture earthworm composting. Aim of the present study was to evaluate the biological maturity and chemical stability (vermicompost aging) by analyzing the humic acid level, microbial biomass, earthworm biomass, cocoon formation and worm mortality rate in microbial (*Trichoderma viridae* and *Bacillus polymyxa*) aided TSW vermicomposting, because these parameters can control the quality of the resulting vermicompost. Analysis of the composts in control, microbes, earthworms and earthworms with microbes treated media were done on the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> days. Significant changes were observed in all media except control. Significant changes, such as: 1. increased humic acid content, 2. increased microbial biomass (CO<sub>2</sub>) up to 50<sup>th</sup> day then decline on the 75<sup>th</sup> day, 3. decreased earthworm biomass, 4. increased earthworm biomass and 5. cocoon formation, were recorded. The yield of the final compost was high in earthworms with microbes treated medium. Thus, our results reveal that the use of selected microorganisms with polyculture earthworms enhances the composting process and chemical stability of Tapioca solid waste vermicomposting.*

Key words: Vermicomposting, Tapioca solid waste, Microorganisms

### INTRODUCTION

Vermicomposting could be an adequate technology for the transformation of organic wastes into valuable products [1]. Several kinds of wastes can be used such as solid organic waste, sewage sludge, agricultural waste, animal manure and some sorts of industrial waste as source of organic matter. Earthworm accelerates composting process [2], control potential environmental risks, improve soil structure and physico-chemical and biological properties of the soil [3]. The result of the composting process

through earthworms (vermicomposting) is a high quality humic product (earthworm casting) used as soil organic amendment [4].

Maturation is the critical step during vermicomposting process, which begins once earthworms leave the substrate, which therefore, can be claimed as microbial-driven process. The microbiological properties of vermicompost, such as microbial biomass, regulate nutrient dynamics, and leading to immobilization or release of nutrients are important. It promotes plant uptake if they are applied to the soil [5].

The degradation of organic waste results in the formation of high quality humified product which can be used as soil organic amendment [6]. The amount and quality of humic acid in vermicompost considered as an indicator of their biological maturity, chemical stability, and warranty for safe impact and successful performance in soil [7]. The effects of vermicompost aging, especially patterns of humic acid level, microbial biomass, worm biomass, cocoon formation, worm mortality and yield of the compost were investigated because these parameters can control the quality of the resulting vermicompost.

Tapioca or cassava is a widely cultivating cash crop of South India (Tamil Nadu). More than 1000 small and large scale sago industries functions in this area. Tapioca tuber processing generate large amounts of solid waste consists of cellulose, hemi-cellulose and lignin, which need extensive treatment to reduce its pollution potential [8]. The generation and accumulation of the sago waste in and around sago processing industries pose threat to environment [9]. The utilization of this material for vermicomposting may give a solution to solid waste management in the sago industry. Our previous study investigated the suitable ratio of TSW and CD to obtain high quality vermicompost. The incorporation of bulking materials as co-substrate modifies the physical structure of waste and also accelerates the waste mineralization rate in vermibeds. Cow dung is considered as efficient bulking agent for rapid decomposition of waste through vermicomposting. It contains a variety of microbes which may accelerates mineralization process through enzyme synthesis [10]. Earthworm can mineralize cow dung more easily than other organic wastes because which contains greater population of decomposing communities, e.g., bacteria, protozoans, nematodes, fungi and actinomycetes [11].

The three species of earthworms (*Eisenia fetida*, *Perionyx excavatus*, and *Lampito mauritii*) have been widely used for commercial composting due to their high tolerance of environmental variations [12]. These species are highly

adaptable and can tolerate varying degree of moisture. To exploit the organic resources, the microorganisms mutually associate with (macro-organisms) earthworms. Microorganism aided the chemical transformation during decomposition of organic materials. Previous study reported that numerous microorganisms in the earthworm cast than in surrounding soil [13]. In addition to the residual microbial population in the substrate, selected microorganisms such as *Tricoderma viridae*, a cellulase producing fungi and *Bacillus polymyxa*, a free living nitrogen fixing bacteria used as effective inoculants for composting and increases the nitrogen content of the stabilized product [14,15]. The nitrogen fixing ability of bacteria depends on the organic wastes used. Microbes in the organic waste are not killed during passage through earthworm gut [16].

In the present study, the effect of selected microorganisms on biological maturity and chemical stability (vermicompost aging) of TSW vermicompost by analyzing the humic acid level, microbial biomass (CO<sub>2</sub> evolution), earthworm biomass, cocoon formation and the rate of earthworm mortality on the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day of the experiment. The yield of the final compost was also determined on the 75<sup>th</sup> day of the experiment.

## MATERIALS AND METHODS

TSW was collected from Logesh Sago factory, H. Pudupatti, Dharmapuri District, Tamilnadu. TSW was dried and powdered to reduce the particle size. CD used as bulking material. Each experimental cistern contained 5 kg of feed mixture with the proportion of 3:1 ratio of TSW and CD; our previous study proved this ratio is suitable for composting [17]. The substrate mixture in the cisterns were maintained at 50% to 60% moisture content and kept in darkness at room temperature (22-26°C). The cisterns were allowed to pre-compost for 20 days. Then the experimental cisterns were divided in to four media. Medium-1(C) served as control. Medium-2 (M) both microbes were introduced (50 ml of fresh culture/Kg of the substrate mix). Medium-

3 (E) received a mixture of three species of earthworms (*Eisenia fetida*, *Perionyx excavatus* and *Lampito mauritii*) (10 earthworms/Kg of the substrate mix). Medium-4 (E+M) received three species of earthworms and two types of microbes (*T. viridae* and *B. polymyxa*, 50 ml of each fresh culture/Kg of the substrate mix). The sieved vermicompost was used for the experiment. For each analysis triplicate were maintained. The compost maturity analysis, such as humic acid level, microbial biomass, earthworms biomass, cocoon formation and mortality of earthworms were carried out at the end of pre-compost (20 days), vermicomposting stage 25<sup>th</sup>, 50<sup>th</sup> and finally on the 75<sup>th</sup> day. The yield of the sieved compost was also weighed at the end of the experimental period.

Humic acid was extracted using alkali acid fractionation procedure [18]. The vermicompost was digested in 0.1N KOH (1:10W/V) for 24 hrs at room temperature. The undigested bulk residue from each vermicompost was then separated from the solute fraction by centrifugation at 5000 rpm for 30 min followed by vacuum filtration through a glass filter paper. The filtered supernatant was acidified to pH2 with 6.0 N H<sub>2</sub>SO<sub>4</sub> and kept in a cold room in the dark for 24 hrs in order to obtain flocculation of humic acids. After acidification the humic precipitate (humate) was collected by centrifuging at 5000 rpm for 30 min, washed three times with distilled water to remove residual H<sub>2</sub>SO<sub>4</sub>. It was freeze-dried, then ground with a mortar and a pestle into a brown powder.

Microbial activity was assessed by measuring the amount of CO<sub>2</sub> evolution from samples during 6 hrs incubation. The evolved CO<sub>2</sub> was trapped in 0.02 M NaOH and then measured by titration with HCl to a phenolphthalein endpoint, after adding excess BaCl<sub>2</sub> [19].

Growth and cocoon production in each experimental cistern was measured on 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day. Earthworms and cocoons produced during experiment were separated from the substrate material by hand sorting, after which earthworms were washed in tap water to remove

the adhering material from their body and subsequently weighed on a live weight basis. Then all measured earthworms were returned to the concerned cistern. Separated cocoons were counted and introduced in the same way in which their parent was reared. On this basis, worm biomass, cocoon numbers and mortality rate were calculated. The total number of earthworms in the media was analyzed at the end of the experiment by hand sorting method. On 75<sup>th</sup> day the composts were collected, air dried, sieved and weighed to observe the yield of the compost.

**Statistical analysis:** The data are expressed as the mean  $\pm$  SD. Statistical comparisons were performed by two way analysis of variance (ANOVA) [20]. The means for each treatment were compared for statistical significance by the CD (critical difference) value at the 0.05 level. The difference between all possible pairs of treatment means were compared to the CD value. If the difference was equal to or greater than the CD value, it was considered as significant. Statistical comparisons were also performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p values were 0.05 or less.

## RESULTS

Tables 1 to 5 present the humic acid content, microbial biomass (CO<sub>2</sub> evolution), worm biomass, cocoon formation and worm mortality rate on the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> days, and yield of the compost on the 75<sup>th</sup> day in control, treatment media.

Humic acid content increases gradually with duration (25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day) of the compost in control and treatment media (table1). On 75<sup>th</sup> day, the per cent of humic acid content increased significantly high in earthworms with microbes treated medium (900%) compared to other media control (70%) and microbes (360%) earthworms (610%).

The CO<sub>2</sub> evolution was steadily increased with duration (25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day) of the compost

**Table 1:** Humic acid levels in control and treatment media of TSW: CD composting. Unit: mg/gram vermicompost. TSW: Tapioca solid waste, CD: Cow dung C: Control, M: Microbes E: Earthworms, E+M: Earthworms and Microbes Values are expressed as mean  $\pm$  SD for triplet in each medium () indicates the percent increase from pre-compost stage. \*CD: Critical difference

	C	M	E	E+M
Precompost	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	0.09 $\pm$ 0.004
25 <sup>th</sup> Day	0.14 $\pm$ 0.01 (40 %)	0.20 $\pm$ 0.02 (100 %)	0.21 $\pm$ 0.02 (110 %)	0.40 $\pm$ 0.02 (344%)
50 <sup>th</sup> Day	0.14 $\pm$ 0.04 (40 %)	0.35 $\pm$ 0.03 (250 %)	0.46 $\pm$ 0.03 (360 %)	0.87 $\pm$ 0.04 (866%)
75 <sup>th</sup> Day	0.17 $\pm$ 0.01 (70 %)	0.46 $\pm$ 0.03 (360 %)	0.71 $\pm$ 0.04 (610%)	0.90 $\pm$ 0.03 (900%)
*CD	-----0.03-----			

**Table 2:** CO<sub>2</sub> evolution in control and treatment media of TSW:CD composting. Unit: mg/gram vermicompost /day. TSW: Tapioca solid waste, CD: Cow dung . C: Control, M: Microbes E: Earthworms, E+M: Earthworms and Microbes Values are expressed as mean  $\pm$  SD for triplet in each medium. () indicates the percent increase from pre-compost stage \*CD: Critical difference

	C	M	E	E+M
Precompost	4.1 $\pm$ 0.2	4.0 $\pm$ 0.2	4.0 $\pm$ 0.2	4.4 $\pm$ 0.3
25 <sup>th</sup> Day	4.3 $\pm$ 0.2 (4.9%)	5.5 $\pm$ 0.5 (37.5 %)	4.7 $\pm$ 0.3 (17.5%)	6.5 $\pm$ 0.5 (47.7%)
50 <sup>th</sup> Day	4.8 $\pm$ 0.3 (17.1%)	7.0 $\pm$ 0.6 (75 %)	6.0 $\pm$ 0.4 (50%)	9.3 $\pm$ 1.4 (111.3%)
75 <sup>th</sup> Day	5.3 $\pm$ 0.3 (29.3%)	8.3 $\pm$ 0.4 (107.5%)	9.0 $\pm$ 0.4 (125%)	3.2 $\pm$ 0.3 (-27.2%)
*CD	-----0.96-----			

**Table 3:** Gross Biomass (Earthworms) treatment media of TSW: CD composting. Unit: Total Number of Earthworms used in each medium 50 . TSW: Tapioca solid waste, CD: Cow dung C: Control, M: Microbes E: Earthworms, E+M: Earthworms and Microbes. Values are expressed as mean  $\pm$  SD for triplet in each medium. () indicates the percent increase from pre-compost stage. \*CD: Critical difference

	C	M	E	E+M
Precompost	0	0	107.2 $\pm$ 7.0	102 $\pm$ 9.5
25 <sup>th</sup> Day	0	0	169.3 $\pm$ 5.0 (57.9%)	215 $\pm$ 0.02 (110.8%)
50 <sup>th</sup> Day	0	0	266 $\pm$ 13.4 (148.1%)	321 $\pm$ 6.5 (214.7%)
75 <sup>th</sup> Day	0	0	303.3 $\pm$ 7.4 (182.9%)	291 $\pm$ 7.0 (185.3%)
*CD	-----13.05-----			

**Table 4** Total number of cocoons in control and treatment media of TSW: CD composting. TSW: Tapioca solid waste, CD: Cow dung . C: Control, M: Microbes E: Earthworms, E+M: Earthworms and Microbes Values are expressed as mean  $\pm$  SD for triplet in each medium. () indicates the percent increase from pre-compost stage. \*CD: Critical difference

	C	M	E	E+M
Precompost	0	0	0	0
25 <sup>th</sup> Day	0	0	17.0 $\pm$ 0.8 (34 %)	24.0 $\pm$ 2.0 (48 %)
50 <sup>th</sup> Day	0	0	31.0 $\pm$ 3.0 (62 %)	57.0 $\pm$ 5.0 (114 %)
75 <sup>th</sup> Day	0	0	62.0 $\pm$ 4.0 (124 %)	51.0 $\pm$ 4.0 (102.0 %)
*CD	-----8.14-----			

**Table 5:** Earthworms mortality, Total number of earthworms and Yield of the vermicast at the end of 75<sup>th</sup> day in TSW: CD composting. Unit: gram of compost / kilogram substrate. TSW: Tapioca solid waste, CD: Cow dung. C: Control, M: Microbes E: Earthworms, E+M: Earthworms and Microbes. Values are expressed as mean  $\pm$  SD for triplet in each medium. Values in each row not sharing a common superscript letter differ significantly at P<0.05 (DMRT)

	C	M	E	E+M
Earthworms mortality on 25 <sup>th</sup> Day	-	-	5.0 $\pm$ 1.0 <sup>a</sup> (10%)	3.0 $\pm$ 1.0 <sup>b</sup> (6%)
Total number of earthworms	0	0	153 $\pm$ 6.0 <sup>a</sup>	202 $\pm$ 10.6 <sup>b</sup>
Yield	80 <sup>a</sup>	147 <sup>b</sup>	279 $\pm$ 4.0 <sup>c</sup>	330 $\pm$ 13 <sup>d</sup>

in control, microbes, and earthworms. On 75<sup>th</sup> day, the per cent of CO<sub>2</sub> evolution in the medium of control, microbes, earthworms are (29.3%), (107.5%), (125%) respectively. Whereas earthworms with microbes treated medium, the CO<sub>2</sub> evolution was high on 25<sup>th</sup> (47.7%) and 50<sup>th</sup> (111.3%) days and drastic decrease on the 75<sup>th</sup> day (-27.2%) (table2).

Biomass of earthworms in the earthworms, earthworms with microbes treated medium were presented in the table3. Percentage of biomass increases gradually in the earthworms medium 57.9%, 148.1%, and 182.9% on 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day respectively. Whereas earthworms with

microbes treated medium earthworms biomass was high on 25<sup>th</sup> (110.8%) and 50<sup>th</sup> (214.7%) days and drastic decrease on 75<sup>th</sup> day (185.3 %).

The number of cocoon in the earthworms, earthworms with microbes treated medium steadily increased with duration (25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> day) of the compost. On 75<sup>th</sup> day, the percentage of cocoon was significantly high in earthworms treated medium (124%) compared to earthworms with microbes medium (102.0 %) (table 4).

The mortality of earthworms was significantly high in earthworms alone treated medium on 25<sup>th</sup> day (10%), whereas earthworms with microbes treated medium mortality of earthworms on 25<sup>th</sup> (6%). The number of earthworms in the final compost was significantly high in earthworms with microbes treated medium (202) than earthworms treated medium (153). The yield of the cast on 75<sup>th</sup> day in control, microbes, earthworms, earthworms with microbes treated media are 80g, 147g, 279g and 330g respectively (table5).

## DISCUSSION

Vermicomposting process increases the humic acid content, the quantity of the humic acid considered as an important indicator of the biological maturity and chemical stability of vermicompost [21]. The important property of the humic acid is the large buffering capacity in a wide pH range, which arises essentially from the dissociation of acidic functional groups [22]. The high level humic acid in M medium may be due to continuous microbial growth and its metabolic action. The humification action of earthworms may be responsible for the increased humic acid level in E medium. Humification action of earthworms and metabolic activities of microbe increases degradation rate of organic matter has been linked with significantly high content of humic acid in E+M medium. Inoculation of *B. polymyxa* was one of the most effective inoculants in increasing the humic acid content [23].

The evolution of CO<sub>2</sub> is an indicator of microbial population in the medium. High CO<sub>2</sub> evolution in the initial stages of composting and decreased in later stage in E+M medium indicates the microbial growth and substrate availability to the organisms. The results were correlated with the observations of Aira [24], a sudden increase in CO<sub>2</sub> release was observed on 30<sup>th</sup> day and their activity was maintained up to 45<sup>th</sup> day. After 60 days the CO<sub>2</sub> evolution was strongly dropped. About 75% reduction of CO<sub>2</sub> release with polyculture earthworms were found on 75<sup>th</sup> day, the decrease in CO<sub>2</sub> evolution was very low after 45 days of composting in the worm worked reactors indicating the stability of the finished compost [25].

Analysis of earthworm biomass, reproductive and mortality is important because these parameters can control quality of the resulting compost. The increased biomass in the vermicompost was due to the continuous growth of earthworms till the experimental period. Whereas the reduced biomass in microbes inoculated vermicompost medium on the 75<sup>th</sup> day may be due to the absence of substrate for the earthworm growth indirectly indicating the maturity of the compost. It was supported by the observations of Benitz [26], where the total biomass of earthworm reached a maximum at 7<sup>th</sup> week and a marked increase occurred between 4<sup>th</sup> and 7<sup>th</sup> week. The maximum growth of earthworm in E+M medium concludes the dual function of bacteria i.e. in having been utilized as food material and enriching the substrate through the nitrogen fixation process [27].

An increased biomass of earthworms was also reported by Anshu and Satyawati [28]. After the 11<sup>th</sup> week biomass decrease was observed, it may be due to the exhaustion of food. Earthworms gained weight when reared in cultures of certain fungal species [11].

The rate of cocoon formation was high in the initial hydrolytic phase and little drop in the final maturation phase. It is the best indicator to evaluate the vermicomposting process [29]. The

cocoon formation in vermicomposting showed the substrate utilization for the growth and reproduction of the earthworms. The results are equal to the reports of Anshu and Satyawati [28], a pronounced increase in the number of earthworms as well as the cocoons was observed during vermicomposting. The reduced cocoon formation in microbial inoculated medium on the 75<sup>th</sup> day represents the depletion of the substrate, attaining the stability and maturity of the compost. It was supported by the fact that the rate of cocoon production was increased followed by decrease on later stage accompanied due to loss of their reproductive condition [1].

The food consumption rate of earthworms during initial critical period (period of acclimatization of earthworms in waste system) determines the survival rate of earthworms in vermibeds. The C: N ratio of initial feedstuff may also be a limiting factor for the feed consumption rate of earthworms [30]. The duration required for the adaptation of earthworms into a new media may be the causative factor for the mortality of the earthworms in vermicompost during the initial period. Growth retarding substances in feedstock may also affect the earthworm population in vermibeds [31], and consequently affects earthworm survival in vermibeds.

The number of earthworms and the yield of the final compost from microbes inoculated vermicompost confirms that inoculation of selected microorganisms hasten the degradation of organic matter and promote earthworms growth. The protozoa and fungi are assumed to form a substantial part of their diet [32]

### CONCLUSION

The determination of humic acid level, microbial biomass (CO<sub>2</sub> evolution), earthworm biomass, cocoon formation, earthworm mortality, and yield of the final compost in the vermicompost prepared from tapioca solid waste provides information about substrate availability, which reflects nutritional transformation and maturation. The use of suitable microbes along with earthworms increases the rate of composting

process. The present study supports our previous study on physico-chemical properties and microbial influence on 3:1 ratio of TSW: CD for the production of high quality vermicompost.

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