Eco. Env. & Cons. 30 (February Suppl. Issue) : 2024; pp. (S21-S26)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i02s.005

Identification and evaluation of microbial consortium for accelerated sugarcane trash decomposition

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(Received 8 July, 2023; Accepted 2 September, 2023)

ABSTRACT

The present study was carried out to evaluate the microbial consortium developed at Agricultural Research Station, Perumallapalle for accelerating the sugarcane trash decomposition during 2020-2023. Application of the microbial consortium @2kg/ton of sugarcane trash showed least decomposition time ie., 89 days with C:N ratio of 18.77:1 while it took about 147 days in control treatment with C: N ratio of 30: 1. Maximum loss in mass (37.34%) and volume (44.55%) was observed in T3 treatment as against the control treatment with 21.23 % loss in mass and 30.11%loss in volume. The nutrient content in terms of total nitrogen, total phosphorus and total potassium content was also estimated in each treatment and maximum values was observed in T3 treatment with 0.93% N, 0.45% P and 1.12% K.

Key words: *Sugarcane, Trash decomposition, Microbial consortium, C:N ratio*

Introduction

Sugarcane (*Saccharium officinarum*) is an important commercial crop of the world and is principal sources of sugar, ethanol, and jaggery globally. It is cultivated in more than 100 countries viz., Brazil, India, China, Thailand and Pakistan. India is the largest consumer of sugar and second largest producer of sugarcane after Brazil. In India, sugarcane is grown Sugarcane is one of the important cash crops in India and plays pivotal role in both agricultural and industrial economy of the country. About 50 million farmers' families are dependent on sugarcane cultivation and our country produces approximately 30 to 33 million tons of sugar annually which makes as one of the largest producers of the world, (Economic times). Sugarcane produces nearly 8–10 tonnes of trash per ha and approximately 6.5 million tonnes of sugar cane trash are being produced every year. The utilization of this large quantity of trash for beneficial use is not possible for the farmers and hence, farmers usually burn the trash to clean the field for next crop, leading to pollution and energy waste and most of the residues are usually burnt in the field due to lack of proper composing techniques. The current practice of open air burning of post-harvest sugarcane residue is under very close scrutiny by various regulatory agencies because of the recent amendment to the clean air act, 1998. The amendment stipulates that release at the source of particulate matter greater than 2:5 µm is prohibited, whether it is from industry or open air burning. Currently, the open air burning of sugarcane crop residue is exempted from this regulation. Due to ever increasing residential development close to sugarcane fields and smoke-related public health risks associated with open air burning of sugarcane cropresidue, the indefinite continuance of this exemption is in doubt. From the past few years, greater attention is given only in improving the sugar cane yield and not much in managing the cane trash. The possible reasons for reduced yields in

sugarcane are depletion of soil health and crop productivity in the sugarcane cultivating areas. Besides the loss of organic matter and plant nutrients, burning of crop residues also causes atmospheric pollution due to the emission of toxic gases methane, carbon dioxide that poses threat to human and ecosystem.In this context, in situ composting of cane trash can be a good alternate to mitigate these problems. Though the composting is better option for sugarcane decomposition but the time taken is little high. In recent years integrated system of composting, with bioinoculants and subsequent vermicomposting, to overcome the problem of lignocellulosic waste degradation of different crop residues and waste industrial by-products is receiving worldwide attention of scientists (Shweta *et al*., 2010).

Sugarcane trash contains around 60% of the total above ground plant N (Chapman *et al.,* 1994) and when it is burnt, >70% of the carbon (C) and N are lost to the atmosphere (Mitchell *et al.* 2000). Consequently, with retention of trash, N and C may be accumulating in the soil. However, little is known about the effects of sugarcane trash retention on the dynamics of soil C and N, despite the importance of these processes for soil fertility, crop nutrient availability, fertiliser requirements, and risks of N losses off-site.

Sugarcane trash is chemically composed of cellulose, hemicellulose and lignin and can be easily composted by using the fungi like *Pleurotussajor-caju, Aspergillus niger, A. flavipus, Penicillium chrysogenum* and *Trichoderma viridae, Rhizopus oryzae.* Earlier studies confirmed that *Aspergillus flavipes, Penicillium, Chrysogenum, Cochliolous speifer, Rhizopus oryzae* and *Trichoderma viride* were found to be effective in sugarcane trash decomposition (Shweta *et al*., 2010). *Trichoderma* spp., common inhabitants of the rhizosphere, besides accelerating decomposition of organic residues can act as biocontrol agents of soil-borne plant pathogens (Harman, 2000).

Hence, a study was attempted for sugarcane trash decomposition using the microbial consortium developed at Agricultural Research Station, Perumallapalle during 2019-20. Five different fungal (*Trichoderma viride, T. harzianum*) and bacterial strains (*Pseudomonas fluorescens* and *Bacillus subtilis*) isolated from organic wastes and rhizospheric soils and found effective for cellulase activity were selected for developing the consortium. These fungal and bacterial isolates were also studied for their efficacy against in producing potential against different pathogens were used for developing the consortium. These isolates also showed maximum cellulase production and PGPR activity viz., HCN production, phosphatase production, siderophore production and protease production. About eight treatments were imposed including the consortium developed at ARS, Amaravathi, Bio-D, Waste decomposer, Single super phosphate etc.

Materials and Methods

Screening of fungi and bacteria for cellulase activity

The cellulase activity of fungal and bacterial cultures were tested on carboxymethyl cellulose (CMC) agar plates containing 0.5 g KH2PO4, 0.25 g MgSO4, 0.25 g cellulose. After inoculating the cultures on the medium, the plates were incubated at 37 °C for overnight and preserved at 4 °C. (Yin *et al.*, 2010). Cellulolytic activity was measured as a diameter of clear zone after the CMC plate was poured by 1% congo red. (Ando *et al.*, 1980; Hong *et al.,* 2005). Cellulolytic index was calculated using formula as follow.

Cellulolytic index = (Diameter of zone - Diameter of bacterial colony)/Diameter of bacterial colony

The microbial consortium developed at Agricultural Research Station, Perumallapalle was used for the study @ 2 kg/ton of sugarcane trash. The experiment was conducted in cement pits of 1m X1m X 1m with three replications. Eight treatments were imposed including the reference cultures obtained from ARS, Amaravathi and Rajasree sugars, Tamil Nadu. Each cement trough was filled with 2 kg sugarcane trash collected from sugarcane fields of ARS, Perumallapalle. The microbial consortium was applied @2 kg/ton of trash along with handful of earthen soil and cow dung slurry @ 200 ml. The treatments under the study include application of microbial consortium with bacterial isolates, with fungal isolates, combination of fungal and bacterial isolates, Bio-D, Amaravathi culture, Waste decomposer, Single super phosphate and uninoculated control. The troughs were watered frequently so as to maintain 60-65% moisture level. Turnings were given at 15 days interval to facilitate proper decomposition. Estimation of microbial population in the composting samples was carried out by counting

the Colony Forming Units (CFU) platted on specific media (Potato Dextrose Agar and Nutrient Agar) for overnight incubation at 37°C. The samples were serially diluted in autoclaved distilled water and plated, then the microbial colonies were calculated by CFU method and expressed in CFU /g unit respectively.

Temperature in the troughs was measured by hand thermometer weekly at fixed time. For initial and final pH, samples were taken in 100 ml beaker and diluted 1:10 (1 part sample in 10 parts of distilled water) and placed on shaker for 1 hr. The samples were centrifuged at 4000 rpm for 30 min. and filtered through Whatman No.1 filter paper. pH of the suspension was measured potentiometrically using a combined glass electrode. Periodical samples were drawn once in 30 days to observe the fungal and bacterial count. C: N ratio was estimated at the early stages of the experiment and at 80 days after incubation and continued till end of the experiment. At the end of the experiment, percent loss in mass and volume of the trash was recorded. The major mineral composition (N, P and K) of the final end product was determined. Organic carbon content of substrates was determined by ignition method (Bremner, 1970). Total nitrogen content of the substrates was determined by modified Kjeldhals method (Piper, 1966). Total phosphorus content was estimated by following the procedure given by Jackson (1973). Total potassium content in an aliquot of tri acid mixture with suitable dilution was estimated using flame photometer (Jackson, 1973). Maturity of compost was recorded on the basis of preestablished maturity and stability parameters of compost (Ranalli *et al.,* 2001; Goyal *et al.,* 2005 and Raj and Antil, 2011)

Results and Discussion

Cellulolytic activity of fungal and bacterial isolates was determined based on clear zone of degraded CMC area around the colony. Cellulolytic activity test showed that all the four isolates were superior in producing cellulase activity ranging from 1.65 - 2.25. Maximum cellulolytic index was observed in *Bacillus subtilis* (2.25) and minimum with *Trichoderma harzianum* (1.65) (Table 1).

Effect of microbial consortium on microbial population

The microbial population was recorded at 30 days interval up to 150 days (Table 2). From the data it is evident that the bacterialpopulation was relatively higher than the fungal population in all the treatments and increased at early stages of decomposition and later the fungal population showed the in-

Table 1. Cellulolytic index of isolates used in present study

Isolates	Diameter of colony (mm)	Diameter of cellulolytic zone (mm)	Cellulolytic index
Trichoderma viride (T1)	1.9	5.5	1.89
Trichoderma harzianum (T2)	2.0	5.3	1.65
Pseudomonas fluorescens (P-7)	1.93	5.7	1.95
Bacillus subtilis (B-10)	2.0	6.5	2.25

Table 2. Microbial population recorded during sugarcane trash decomposition using different sources.

Treatments	30 days		60 days		90 days		120 days		150 days	
	B	F	B	F	B	F	B	F	B	F
T1:Consortium with bacterial cultures	39.9	39.2	54.3	43.5	54.5	55.8	46.25	54.7	43.2	41.0
T2: Consortium with bacterial cultures	37.5	46.8	47.0	57.5	55.25	46.3	52.0	34.0	49.7	30.1
T3: Consortium with bacterial cultures	41.3	48.0	49.2	51.0	45.3	37.5	36.0	29.8	23.0	19.3
T4:Bio-D	23.0	13.7	25.1	18.3	28.9	22.0	29.0	22.0	22.5	20.0
T5: Amaravathi decompo culture	36.5	31.5	46.3	41.5	44.0	36.5	42.5	35.0	38.1	29.7
T6:Waste Decomposer	39.4	21.5	41.5	31.5	42.0	34.3	37.7	34.0	30.1	31.0
T7:SSP	13.2	10.0	16.0	14.7	19.7	19.4	17.9	21.7	17.0	20.0
T8: Control	12.2	10.3	11.2	17.7	19.4	19.9	21.7	21.1	20.0	19.0

Bacterial population : ($x10^7$cfu/g of dry matter)
Fungal population: ($x10^4$cfu/g of dry matter)

creased trend. Higher microbial activity was observed in the treatment T3 at 30 days after inoculation compared to other treatments. The microbial population showed an increase up to 60 days and thereafter decreased in T3 and T5 while the population increased up to 90 days in other treatments, thus showing that the decomposition is almost completed in T3 treatment. The temperature recorded during the experimentation ranged between 47.8 (at the initial stages) to 28^{0}C at the end of the experiment. The bacterial counts peaked upto 120 days and remained constant for most of the study and began to fall towards the end of the 150th day of monitoring period.

Effect of treatments in days taken for maturity of compost, pH, C: N ratio and % loss in mass and volume

From the results of three years, it was revealed that decomposition of sugarcane trash treated with test consortium (T3) showed least time for decomposition i.e. 89 days, reducing the composting time over control by 39.45%. This treatment was followed by T5 which showed 106 days for decomposition with 27.80% reduction decomposition time over uninoculated control. The physical observation of trash decomposition was clearly noted from 20 days onwards in the T3 treatment. The decomposition of trash in un inoculated control took almost 150 days and showed C: N value of 30:1.

The pH of the sugarcane trash was found to be normal ie., 7-7.5 at the completion of the decomposition process. This indicates the maturity stage of the compost. C:N ratio is an essential parameter for determining the extent of degree of maturity of the compost. The pre treatment of microbial consortium significantly reduced the C/N ratio of sugarcane trash after 89 days onwards in case of T3 followed by106 days in T5 treatment (Fig. 1). The C:N ratio of the undecomposed sugarcane trash at the initial stage of the experiment was found to be 48:1. The per cent decrease in C: N ratio of trash over initial was about 60.89% in treatment,T3 while it was 60.64% with Amravati culture (T5) treatment (Table 3). Maximum loss in mass (37.34%) and volume (44.55%) was observed in T3 treatment as against the control treatment with 21.23 % loss in mass and 30.11%loss in volume (Fig. 2)

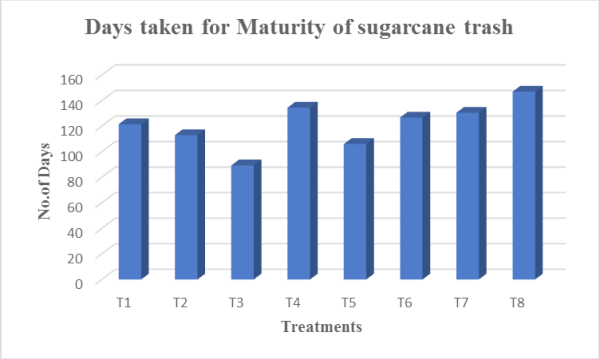

Fig. 1. Days taken for maturity of sugarcane trash in different treatments

The present results are in conformity with the results of research workers who revealed from their studies that the organic matter decomposes gradually with time, stabilizes with final pH of compost between 7 to 8 with reduction in C: N ratio (Ranalli *et al*., 2001, Gade *et al.,* 2010; Raj and Antil, 2011; Himanen and Hanninen, 2011; Sarker *et al*., 2013). Similarly, reduction in composting period due to inoculation of cellulolytic microorganisms has also been reported by Raut *et al*., (2008), Iqbal *et al*., (2010) and Sarkar *et al*. (2011).

Table 3. C:N ratio and nutrient composition of decomposed sugarcane trash in different treatments

Treatment	C:N ratio	Mineral Composition		
		Total Nitrogen (%)	Total Phosphorus (%)	Total Potassium (%)
T1 Fungal	26.30	0.81	0.32	1.09
T2 -Bacterial	28.56	0.88	0.39	1.02
T3 -T1+T2	18.77	0.93	0.45	1.12
T4 -Bio-D	28.40	0.85	0.33	1.01
T5 -AmaravathiDecompo culture	28.69	0.89	0.41	1.10
T6-Waste decomposer	27.24	0.81	0.35	1.03
T7 -SSP	30.06	0.67	0.37	1.01
T8 (Control)	33.27	0.69	0.27	0.99

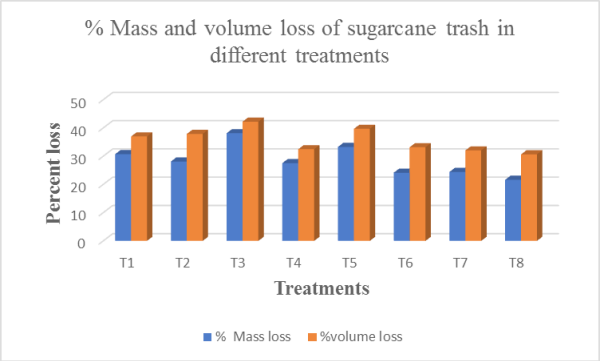

Fig. 2. Percent mass and volume loss of sugarcane trash as influence by different treatments

Nutrient content of sugarcane trash

The nutrient composition of the decomposed sugarcane trash at the end of the experiment is mentioned in the Table 3. Maximum nitrogen content (0.93%), phosphorus (0.45%) and potassium (1.12%) were observed in the treatment which received the combination of fungal and bacterial cultures ie., T3 followed by Treatment, T5. This may be due to the increased metabolic activity by microbes and their secretions resulting in release of soluble nutrients from trash. Cong and Merckx (2005) reported that improved P availability could be due to the reduced P sorption by released organic compounds during the *Trichoderma*-mediated decomposition of trash and root residues.

Application of newly developed microbial consortium consisting of *Trichoderma* sps.(T1 and T2), *Pseudomonas fluorescens* (P-7) and *Bacillus subtilis* (B-10) on sugarcane trash increased the microbial activity, maintained pH and reduced the period of composting and was superior over the commercial consortium and uninoculated control. It is revealed from the results that incorporation of cellulolytic microorganisms enhance the rate of decomposition of organic matter, which will enable to convert the organic matter into valuable compost in short time. Several research workers estimated the nutrient value of compost prepared on inoculation with microbes over the uninoculated control. Game *et al* (2017) and Sarker *et al.*, (2013) revealed that the compost prepared on inoculation of microbes showed the better nutrient levels compared to uninoculated control. This is probably because of quick microbial activity leading to decrease in volume of the material. The present results are thus in conformity with the work done by earlier research workers. This study also suggests that mixing this microbial consortium with sugarcane trash would lessen the acidity of the soil and also aid in increasing crop yields.

Conclusion

The present study indicated that application of microbial consortium used in the present study decreased the time of decomposition by 60.89% than uninoculated control showing C: N ratio of 18.77. The application of decomposed trash or in-situ decomposition of sugarcane trash in the sugarcane fields helps in maintaining the soil pH to normal and also supplement the availability of major nutrients ie., N, P and K to the crop by 22.32%, 20.08% and almost 50.00 respectively. Application of decomposed trash as mulching can give better results in sugarcane crop by reducing the application of the recommended dose of fertilizers and also due to increased microbial activity by beneficial organisms like *Trichoderma viride, T. harzianum, B.subtilis, Pseudomonas fluorescens* results in availability of micronutrients, increases the soil health, thereby increasing the crop yields.

Acknowledgement

Authors are thankful to the Head, Agricultural Research Station, ANGRAU, Perumallapalle for providing necessary facilities during the investigations.

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