Decomposition of Sugarcane Trash by Selected Microbes and their Biofilms: A Laboratory Investigation

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ABSTRACT

Natural decomposition of sugarcane trash is slow, taking more than three months, and hence, it becomes a problem with inter-cultivation and restricts the growth of the ratoon of the sugarcane crop. Trash blanketing is a common management practice carried out but does not solve the above problems. This study investigates the potential of using selected microbial combinations, including fungal-bacterial biofilms (FBB), for rapid decomposition of sugarcane trash. Two studies were carried out at laboratory level, namely, to identify the sugarcane trash decomposition process and then to evaluate selected microbial combinations in enhancing sugarcane trash decomposition. In the first study, sugarcane trash samples were placed in 24 wells of a tissue culture plate separately, and evaluated the surface functional groups of organic compounds by Fourier transform infrared (FTIR) spectroscopy. In the second study, 24 treatments in a completely randomised design were used to identify selected microbial combinations that could be effective in sugarcane trash decomposition. When considering the results of the first study, there was a positive correlation between weight loss and FTIR peak degradation of organic molecular functional groups, particularly O-H of the carboxylic group, C-H of the aromatic methyl group, and Si-O of the cuticle wax layer. The results of the second study showed that urea being a chemical treatment, was significantly effective in reducing the C/N ratio of decomposed trash. In the microbial treatments, bacterium B2 (yet to be identified) was effective on trash fragmentation, and the FBB coded as F1F2B1B2 was effective on both trash fragmentation and reducing its C/N ratio. The combination should therefore be tested for trash decomposition in the long run of the sugarcane crop cycle under field conditions.

INTRODUCTION

Sugarcane trash, an important source potentially for soil improvement, is left on the field following the harvest of sugarcane stalks. However, its slow decomposition process has become a problem with inter-cultivation as it takes more than three months. This may be due to the chemical composition of trash, which includes organic compounds, cellulose, hemicellulose, and lignin in percentages 45.1, 25.6, and 12.7, respectively, along with other minor components such as inorganic materials, ash, silicon, chlorine, and metals (Woytiuk, 2006).

Effective management of trash leads to greater profits for the sugarcane grower. Trash blanketing is a common management practice that improves the soil properties like water holding capacity, organic matter, crumb structures, and total exchange capacity (Thompson, 1966). But there are drawbacks of trash blanketing where it can restrict the growth of ratoon crops by causing temporal nutrient immobilisation and interfering with inter-cultivation practices.

The decomposition of organic matter is the process by which they are converted into smaller and simpler compounds. It is a biological process carried out by macro and microorganisms (Kuers and

Simmons, 2006). Following initial decomposition, humification takes place, resulting in the formation of humus. During this process, initially degradable carbon sources start to decompose, and then more resistant compounds like lignin degrade into smaller units by the action of extracellular enzymes (Varadachari and Ghosh, 1984). Microorganisms play a major and important role in the decomposition process. Fungi can decompose lignocelluloses, cellulose, and hemicellulose (Tuomela *et al*., 2000). Further, Basidiomycotina is effective on lignin (Eriksson *et al*., 1990). Bacteria contribute by consuming the small molecular weight intermediate compounds which are produced by fungi (Vicuna, 1988 and Ruttimann *et al*., 1991). A study done by Dixon (2013) highlights that both fungi and bacteria are important in plant litter decomposition as they fulfill their nutritional sugar requirement by breaking down plant cell walls. The same study shows that fungi use degradable enzymes called cellulases, which uniformly dissolve the wall from the innermost side. Bacteria use a multiple enzyme complex called cellulosomes, and they start digesting the cell walls away from the middle lamella. Therefore, this clearly shows that the decomposition process could be accelerated when fungi and bacteria are together, like in FBBs, rather than in isolation (Seneviratne *et a*l., 2008).

Biofilms in soils are complex multicellular communities comprising mainly fungi and bacteria, where the bacteria may adhere to the biotic surface of the fungus (Seneviratne et al., 2008). Biofilms adhere to the plant roots of crops and play an important role in improving agricultural production by cycling the nutrients and also by controlling pests and diseases. Therefore, the present study investigates the potential of using selected microbial combinations, including FBBs, for the rapid decomposition of sugarcane trash.

MATERIALS AND METHODS

The investigation was carried out in two studies, 1) to identify the sugarcane trash decomposition process and then, 2) to evaluate selected microbial combinations in enhancing sugarcane trash decomposition.

Study 1: Identification of sugarcane trash decomposition process

Sugarcane-growing soil from Uda Walawe was used, and it was air-dried and sieved through a 0.5 mm sieve before the experiment. Fresh sugarcane trash pieces (1 cm x 1 cm) were cut from the middle part of the trash in between the edge and the midrib, and twenty-four-well tissue culture plates were used for the experiment. Soil (0.5 g) was weighed and put into each well of the tissue culture plates. A synthetic mesh was cut and placed above the soil layer (Figure 1). The initial weights of fresh trash pieces were recorded, and then they were placed on the synthetic mesh and kept in the incubator at 37 ^{o}C. Another 3 - 4 fresh trash pieces were taken, and their initial weights and oven-dried weights were recorded to calculate the moisture factor for calculating the dry weights of the trash pieces.

Figure 1: The layout of a tissue culture plate after the establishment of the 1st study

Analysis

Trash samples were ground into powder by using a mortar and pestle. Potassium bromide (KBr) pellets were prepared with the weight ratio of 1:100 of sample:KBr pellets were subjected to spectral analysis

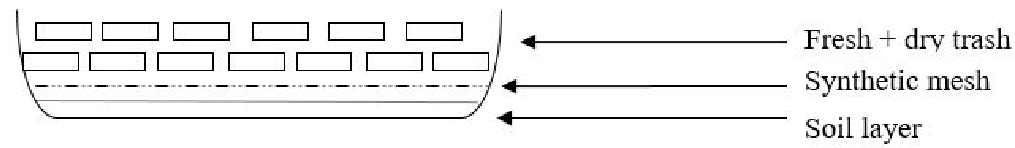

Figure 2: A cross section of Petri plates after establishment of the 2nd study

of Fourier transform infrared (FTIR) spectrophotometer for evaluation of surface functional groups of organic compounds.

Study 2: Evaluation of selected microbial combinations in enhancing sugarcane trash decomposition

Disposable sterilized plastic petri plates were used for the experiment. One gram of 2 mm sieved soil was put into the petri plates, and a synthetic mesh was placed on it. Both fresh and dry trash pieces were cut into 3 cm x 1 cm pieces (Figure 2). According to the fresh/dry trash weight ratio at the harvesting of mature sugarcane in the field, 1.4 g of fresh trash and 1 g of dry trash were measured separately and laid above the synthetic mesh.

Experimental design and treatments

The plates were arranged in a completely randomized design (CRD) with 24 treatments and 3 replicates under laboratory conditions. was used with DMRT mean separation method at 5% probability level for comparing the effects of the treatment on the crop parameters and the moisture parameters.

Microbial treatment combinations

Fungi and bacteria previously isolated from sugarcane fresh and dry trash and their combinations (FBBs) were used for this study. The microbial cultures included two fungi (F1 = Mucor spp and F2 = un-identified) and two bacteria (B1 and B2 both un-identified). The microbial combinations included three FBBs and two mixed cultures. The microbial treatments were also separately treated with a known dilution of molasses (Table 1).

Table 1. Microbial treatments used in the 2nd study

Treatment no.	Treatments (monocultures, biofilms, and mixed cultures)
1	Fungi-1 (F1)
2	Fungi-2 (F2)
3	Bacteria-1 (B1)
4	Bacteria-2 (B2)
5	F1F2B1B2
6	F1B1B2
7	F2B1B2
8	F1F2
9	B1B2
10	100% urea (basal dressing of 50 kg/ha)
11	50% of urea from the basal dressing
12	Control
Treatment no	**Treatments mixed with molasses**
13	F1 + Molasses
14	F2 + Molasses
15	B1 + Molasses
16	B2 + Molasses
17	F1F2B1B2 + Molasses
18	F1B1B2 + Molasses
19	F2B1B2 + Molasses
20	F1F2 + Molasses
21	B1B2 + Molasses
22	100% Urea + Molasses
23	50% Urea + Molasses
24	Molasses

Treatment application

Microbial monocultures, mixed cultures, and FBBs were applied at the rate of 50 ml per 0.1 ha. Urea was added to the rate of 50 kg/ha for 100 % urea-added treatments and exactly half for the 50 % urea application. One gram of molasses was diluted 1000 times, and 2 ml was applied for appropriate treatments.

Data analysis

Data were analysed by ANOVA procedure using SAS® software, and mean separation was done by Tukey's test. All the interpretations were made at 95 % probability ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Study no 1: Identification of the sugarcane trash decomposition process.

Dry mass of trash during decomposition reduced with time, as given in Figure 3. The highest sugarcane trash decomposition occurred during the initial three weeks, and out of that, the third week was prominent. This can be attributed to the higher microbial activity coupled with higher nutrient content. The activity declined with the depletion of nutrients. The weekly fluctuation of the decomposition is a result of microbial succession.

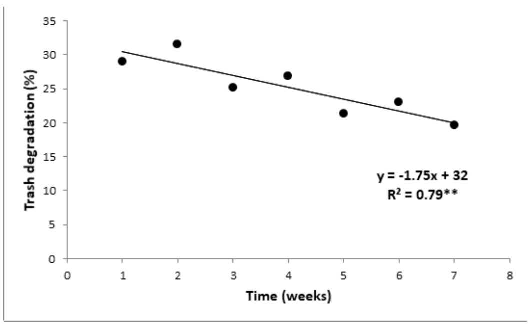

Figure 3: Sugarcane trash decomposition with time

Correlation between percentage dry weight loss and FTIR peak degradation during sugarcane trash decomposition showed fairly significant relations for several peak numbers (Table 2). The Peak at 3410 cm^{-1} resulted in the highest correlation, followed by peaks at 2918, 1046, and 1383 cm^{-1}. These peaks reflected the presence of O-H, C-H, Si-O, and CH_3-R bonds (Table 3), which are generally present in biochemical compounds such as carbohydrates (cellulose, hemicellulose), cuticle wax layer of grasses and amino acids of proteins, respectively.

Table 2: Pearson correlation coefficients between percentage dry weight loss and FTIR peak degradation during sugarcane trash litter degradation from 0 to 8 weeks

Peak number (cm^{-1})	**Pearson Correlation Coefficients(r)**	**Probability (P)**
3410	0.7652	0.0269
2918	0.6569	0.0768
2850	0.2520	0.5471
2360	0.3988	0.3277
1716	-0.1018	0.8104
1735	0.3890	0.3409
1637	0.2241	0.5937
1515	0.1747	0.679
1540	-0.1018	0.8104
1557	-0.0104	0.9804
1458	-0.0075	0.986
1421	-0.0243	0.9545
1383	0.4948	0.2126
1246	0.4857	0.2224
1160	0.1335	0.7527
1073	-0.0466	0.9128
1046	0.6143	0.1052
897	0.1795	0.6707
799	0.2010	0.6332
668	0.4482	0.2653
561	0.4795	0.2292
515	-0.0788	0.8528
470	0.4229	0.2966

Table 3: FTIR peak numbers and respective functional groups of organic molecules (Smidt *et al*., 2011)

Peak Number (cm^{-1})	**Functional group**
3410	O-H stretching in hydroxyl group
2918	C-H stretching in anti-symmetric aliphatic methylene group
2850	C-H stretching in symmetric aliphatic methylene group
1735	C=O stretching in symmetric aldehyde, ketone, carboxylic acid, esters
1515	Aromatic skeletal lignin, lignocellulosic materials
1383	CH_3-R stretch in nitromethane
1246	C-O-C stretching vibration in esters C-N stretching vibration in amide-iii
1046	Si-O stretching vibration Si-O-Si stretching vibration in Silica
668	S=O bending vibration in sulphate
561	P=O stretching of phosphate
470	NH^+ torsion, COO^- stretching

There were positive correlations between total weight loss during 0 to 8 weeks and FTIR peak degradation at peak numbers 3410 cm^{-1}, 2918 cm^{-1}, and 1046 cm^{-1} during the 3rd, 4th, 5th, and 7th weeks (Figure 4). Thus, total weight loss was reflected by total peak degradation of the abovementioned peaks, meaning that breakage of litter surface functional groups of biochemical compounds can be used to predict long-term trash decomposition. The major part of weight loss occurred during the 3rd, 4th, and 5th weeks.

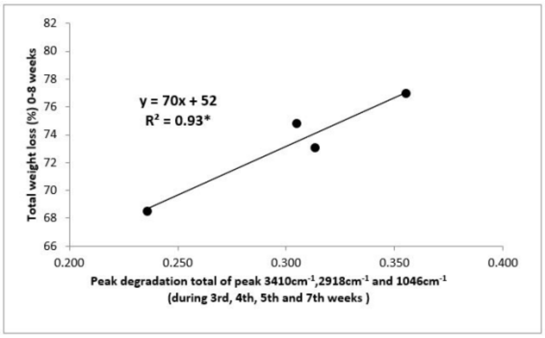

Figure 4: Relationship between total FTIR peak degradation and total weight loss of sugarcane trash.

Study no 2: Evaluation of biofilm activity on enhancing sugarcane trash decomposition.

The results of trash fragmentation percentage, carbon to nitrogen (C/N) ratio, and weight loss were elaborated on in detail

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Trash fragmentation percentage

The highest trash fragmentation percentage was shown in the 50% urea + Molasses treatment. However, they were not significantly different from other treatments at a 5% probability level. When considering microbial treatments alone, the highest trash fragmentation percentages were recorded with B2, followed by F1F2B1B2. With molasses, B2 showed the highest fragmentation, followed by F2B1B2. It was observed that microbial monocultures and FBBs' had greater ability in trash fragmentation (Figure 5). Generally, the FBBs are more effective in litter decomposition as they have better growth and colonization abilities compared to their monocultures (Seneviratne *et al*., 2008).

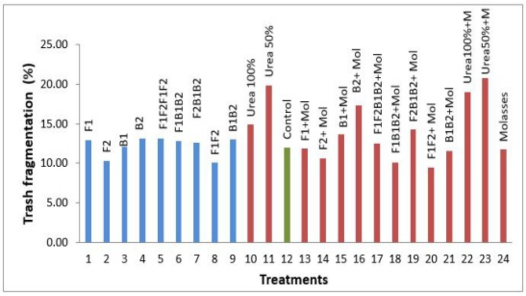

Figure 5. The trash fragmentation percentage of the 24 treatments applied to sugarcane trash, observed after one month.

Carbon to nitrogen ratio

During trash decomposition, the C/N ratio decreased as carbon-containing complex molecules were broken down into simpler molecules. If decomposition results in a lower C/N ratio, it indicates increased decomposition. The lowest C/N ratio was observed in 100% urea, followed by 50% urea (Fig. 6). In the microbial treatments, F1F2B1B2 showed the lowest C/N ratio, followed by F1B1B2.

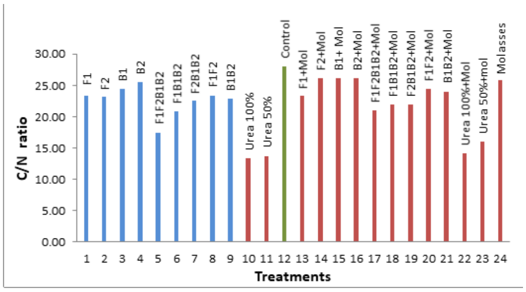

Figure 6: C/N ratio of sugarcane trash in 24 treatments after one month.

Trash decomposition is greatly affected by both endogenous and exogenous N. Endogenous N is the N in the trash, and exogenous N is the N available in the surroundings (Berg and McClaugherty, 2008). Also, it is affected by the type of N (mineral or organic) added, and the amounts of N applied (Fog, 1988). Biofilms are a natural way to get the benefits of synthetic fertilizers without risking the quality of soil health. Diazotrophic bacteria in a FBB help in fixing atmospheric N, thus reducing the C/N ratio (Seneviratne *et al.*, 2008).

According to Figure 7, there is an inverse relationship between the C/N ratio and weight loss which means an increment in weight loss of sugarcane trash with the reduction in the C/N ratio.

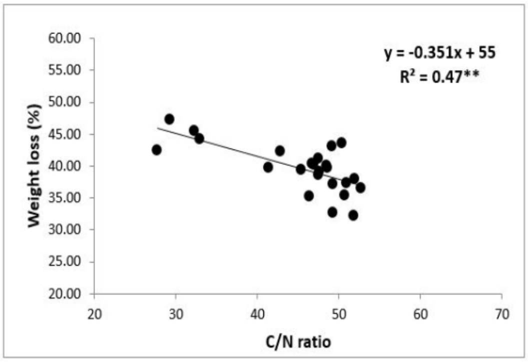

Figure 7: The relationship between C/N ratio and weight loss percentage of decomposed sugarcane trash in 24 treatments after one month.

Results in identifying the sugarcane trash decomposition process conclude that there is a positive correlation between weight loss and FTIR peak degradation of organic molecular functional groups, particularly O-H of the carboxylic group, C-H of the aromatic methyl group, and Si-O of cuticle wax layer. Therefore, sugarcane trash weight loss after two months can be predicted by the breakage of functional groups of organic molecules at 3rd week by using the FTIR peak degradation.

CONCLUSION

In the study to evaluate selected microbial combinations in enhancing sugarcane trash decomposition, the microbial combinations, including FBBs showed promising results even though the urea treatments on C/N ratio were significantly higher than that of the other treatments under laboratory conditions. If more effective N_2 fixing bacteria were identified and incorporated into the microbial combinations as FBBs, it could be more effective on trash decomposition in the long run of the sugarcane crop cycle.

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