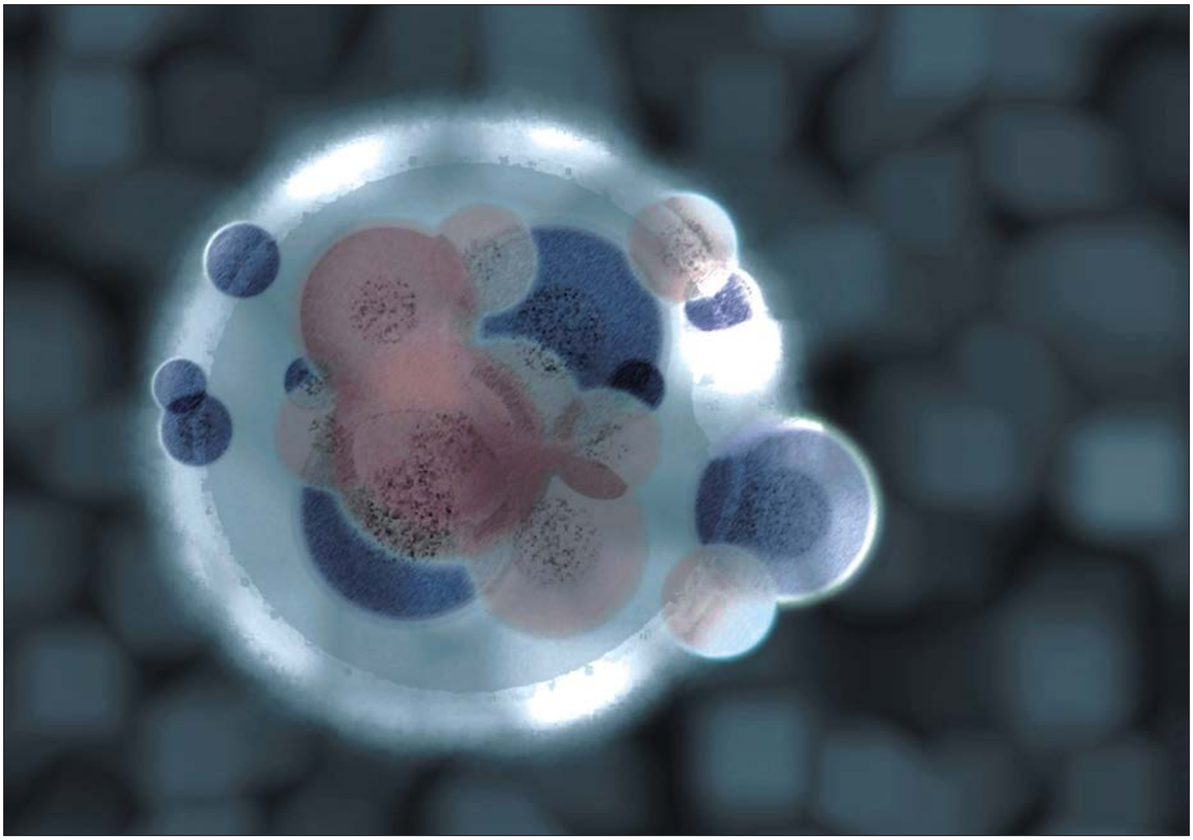

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Effect of Biomethanated Distillery Spentwash and Pressmud Biocompost on Microbial and Enzyme Dynamics in Sugarcane Grown Soil

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Abstract: A field experiment was conducted to study the effect of different levels and methods of biomethanated distillery spentwash and pressmud biocompost application on soil microbial and enzymatic activity using sugarcane (*Sachharum officinarum*) as a test crop at farms of M/S. Bhavani distilleries and chemicals Ltd., T. Pudur, Thimiri, Vellore District. Sugarcane crop was raised with ten treatments comprising the one-time as pre-plant application (30 days before planting) of BDS at 100, 112.5, 150 and 187.5 m^3 ha^{-1}, pre and post-plant application of BDS at 112.5, 150 and 187.5 m^3 ha^{-1} as split doses at various stages of crop growth and application of biocompost at 2.5 and 5 t ha^{-1} along with control. The soil samples were collected at 90 days intervals and analyzed for the change in soil microbial population and enzyme activities. The results of the study showed that the microbial population and enzymatic activities of the soil were substantially increased throughout the crop growth period due to biomethanated distillery spentwash and bio-compost application. The highest microbial population and enzyme activities were recorded with split application of BDS at 187.5 m^3 ha^{-1} along with balance P fertilizer. This study revealed that the utilization of biomethanated distillery spentwash at 187.5 m^3 ha^{-1} as split doses along with balance phosphorus requirement through inorganic fertilizer could improve the microbial population and enzyme activities in sugarcane grown soil.

Key words: Biomethanated distillery spentwash, pressmud biocompost, microbial population, enzyme activities

INTRODUCTION

Sugar industry is the second largest agro-based industry in India, which contributes substantially to the economic development of the country. The 579 sugar industries in the country produce 14.5 million tonnes of sugar by crushing 145 million tonnes of sugarcane. The annual byproduct production from through these industries is 7 million tonnes of pressmud and 7.5 million tonnes of molasses. Molasses is utilized in the distillery for the production of alcohol. The distilleries are listed at the top in the "Red Category" industries having a high polluting potential by the Ministry of Environment and Forests (MoEF), Government of India (Tewari *et al*., 2007). There are 319 distilleries in India producing 3.25 billion litres of alcohol and generating 40 billion litres of spentwash annually (Uppal, 2004). These spentwash and pressmud cause disposal and pollution problems. The raw spentwash contains very high BOD, COD and solids. It is subjected to biomethanation process where 80 percent of BOD reduction and energy production are obtained. The effluent discharged after biomethanation process is known as Biomethanated Distillery Spentwash (BDS). The BDS is effective organic liquid manure derived from sugar industry waste materials. It contains large amount of organic carbon, K, Ca, Mg, Cl and SO_4 and moderate amounts of N and P and traces of Zn, Cu, Fe and Mn. Pressmud is rich in plant nutrients besides containing luxurious amount of organic matter. Biocompost is produced from the pressmud and BDS by inoculating suitable microbes and aerating through aerotillers in a mechanized open windrow system. Conversion of raw pressmud into biocompost through composting process was increased the available nutrient content like N, P and K. Therefore, upon field application, these wastes enhance the biological properties and enzyme activities of soils and thus influence the fertility of soil significantly. The addition of organic matter through BDS may be favourable for microorganisms and enzymes in soils. Batch *et al*. (1993) observed that the spentwash at 250 m^3 ha^{-1} rate stimulated the soil microorganism and increased the dehydrogenase activity in soil. The spentwash addition increased the activity of phosphatase, dehydrogenase and urease enzymes in dry land black and

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red soils especially at levels of 125 m^3 ha^{-1} (Murugaragavan, 2002).

Microorganisms play an important role in nutrient cycling and organic matter stabilization in soils (Jedidi *et al*., 2004). Kremer and Li (2003) reported that sustainability of soil health is based, in part, on the efficient management of soil microorganisms to improve soil quality. Enzyme activities have been found to be very responsive to addition of organic materials (Miller and Dick, 1995). The enzymatic activities of a soil catalyzes the biochemical activities performed by bacteria and thereby indicates the potential of the soil to permit the basic biochemical processes necessary for maintaining soil fertility. Therefore, the present study was undertaken with a view to studying the effect of distillery wastes viz., biomethanated distillery spentwash and pressmud biocompost application on microbial population and enzyme activities of soil under field condition.

MATERIALS AND METHODS

A field experiment was conducted at Research and Development Farm, M/s. Bhavani Distilleries and Chemicals Limited, T. Pudur, Thimiri, Arcot taluk, Vellore district, Tamil Nadu in randomized block design with three replications using sugarcane (*Sachharum officinarum*) var. CO 86032 as a test crop. The experimental field was laid out and the calculated quantity of BDS (Table 1) was uniformly applied in each plot as per the treatment details given below. Then, the soil was ploughed at 10 days interval for two times providing better soil aeration and consequent reduction of BOD level in the soil-water system. Biocompost (Table 2) was incorporated in the respective treatments at the time of last ploughing.

Treatments:

T_1: RDF- NPK (275:62.5: 112.5 kg of N: P_2O_5: K_2O ha^{-1})
T_2: Application of biocompost at 2.5 t ha^{-1} + RDF- NPK
T_3: Application of biocompost at 5 t ha^{-1} + RDF- NPK
T_4: Pre-sown application of BDS at 100 m^3 ha^{-1} + RDF- NP alone
T_5: Pre-sown application of BDS at 112.5 m^3 ha^{-1} (= 75% RDF- N) + balance N and P through inorganic fertilizer
T_6: Pre-sown application of BDS at 150 m^3 ha^{-1} (= 100% RDF- N) + balance P through inorganic fertilizer
T_7: Pre-sown application of BDS at 187.5 m^3 ha^{-1} (= 125% RDF- N) + balance P through inorganic fertilizer
T_8: Pre-sown application of BDS at 75 m^3 ha^{-1} (= 50% RDF- N) + Post-sown application of BDS at 37.5 m^3 ha^{-1} (= 25% RDF- N) on 45 DAP + balance N and P through inorganic fertilizer
T_9: Pre-sown application of BDS at 75 m^3 ha^{-1} (= 50% RDF- N) + Post-sown application of BDS at 75 m^3 ha^{-1} (= 50% RDF- N) on 45 DAP + balance P through inorganic fertilizer
T_{10}: Pre-sown application of BDS at 75 m^3 ha^{-1} (= 50% RDF- N) + Post-sown application of BDS at 75 m^3 ha^{-1} (= 50% RDF N) on 45 DAP + Post-sown application of BDS at 37.5 m^3 ha^{-1} (= 25% RDF- N) on 90 DAP + balance P through inorganic fertilizer

On 30th day of BDS application, the individual plot was formed with ridges and furrows and sugarcane setts were planted in the ridges by adopting the spacing of 80×40 cm^2. All cultural practices including gap filling, weeding, plant protection measures and other cultural practices were done as per Tamil Nadu Agricultural University recommendations.

Collection and analysis of soil samples: Soil samples were collected at 90 DAP (S_1), 180 DAP (S_2), 270 DAP (S_3) and after harvest of sugarcane (S_4) and the number of fungi, bacteria and actinomycetes colonies were assessed by plating dilution technique (Waksman and Fred, 1922). The activities of urease, phosphatase and dehydrogenase enzymes were assayed as per the standard procedures (Tabatabai and Bremner, 1972).

Table 1: Characteristics of biomethanated distillery spentwash

Parameters	Values
Physical properties	
Colour	Dark brown
Odour	Unpleasant
Total dissolved solids (mg L^{-1})	42300
Total suspended solids (mg L^{-1})	6100
Total solids (mg L^{-1})	48400
Physico-chemical properties	
pH	7.57
EC (dS m^{-1})	33.45
BOD (mg L^{-1})	11400
COD (mg L^{-1})	42800
Organic carbon (mg L^{-1})	25200
Nitrogen (mg L^{-1})	1700
Phosphorus (mg L^{-1})	248
Potassium (mg L^{-1})	10680
Sodium (mg L^{-1})	480
Calcium (mg L^{-1})	2120
Magnesium (mg L^{-1})	1680
Biological properties	
Bacteria (×10^6 CFU mL^{-1})	21
Fungi (×10^4 CFU mL^{-1})	11
Actinomycetes (×10^2 CFU mL^{-1})	3

Table 2: Characteristics of biocompost

Parameters	Values
Physico-chemical properties	
PH	7.50
EC (dS m^{-1})	8.80
Organic carbon (%)	24.80
Total nitrogen (%)	1.54
Total phosphorous (%)	1.08
Total potassium (%)	2.95
Total calcium (%)	3.20
Total magnesium (%)	2.00
Sodium (%)	1.05
C/N ratio	16.10
Copper (mg kg^{-1})	45.00
Zinc (mg kg^{-1})	105.00
Iron (mg kg^{-1})	2000.00
Manganese (mg kg^{-1})	190.00
Biological properties	
Bacteria ($\times 10^6$ CFU g^{-1})	31.00
Fungi ($\times 10^4$ CFU g^{-1})	17.00
Actinomycetes ($\times 10^3$ CFU g^{-1})	11.00

RESULTS AND DISCUSSION

Soil microflora: The impact of different concentrations of BDS and biocompost on the population of soil bacteria, fungi and actinomycetes at various sampling periods was very well pronounced compared to RDF-NPK (Table 3). In general, irrespective of the treatments, population of bacteria, fungi and actinomycetes was mostly appeared to have increased at the initial stages and thereafter, progressively decreased with crop growth stages. The degradation of the organic matter and depletion of plant nutrients might be the reason for the reduction of microbial population. In general, the soil microbial population was increased from the initial level by the application of BDS and biocompost. BDS containing higher levels of nutrients and organic matter might be the reason for the increase in soil microbial population. Among the treatments, higher microbial population was observed in split application of BDS at 187.5 $m^3 ha^{-1}$ along with balance phosphorus requirement through inorganic fertilizer. Mattiazo and Ada Gloria (1985) who reported an increase in soil microbial activity due to oxidation of organic matter in BDS applied soil. According to Patil *et al*. (1982) BDS contains about 42.7% polysaccharides which might have served as a source of carbon, sulphate for microbial proliferation. Tauk *et al*. (1990) also lend support for the increased number of fungi, bacteria and actinomycetes owing to the addition of BDS. The application of BDS improved the physical and chemical properties of the soil and further increased soil microflora (Kaushik *et al*., 2005).

The results of this study are in the agreement with results obtained by Lee *et al*. (2004) who stated that the addition of organic matter increased the microbial population in soil. Such effect of BDS on the population of bacteria, fungi and actinomycetes in the soil was also reported by Goyal *et al*. (1995) and the results of Valliappan (1998) are in good agreement with the present findings.

Phosphatase activity: Significant variation in soil phosphatase activity was noticed due to application of graded dose of BDS and biocompost at various crop growth stages. Interaction effect also was found significant (Fig. 1). The highest soil phosphatase activity (μg p-nitrophenol g^{-1} soil h^{-1}) was detected in treatment that received higher doses of BDS at 187.5 $m^3 ha^{-1}$ as split doses along with balance phosphorus requirement through inorganic fertilizer (82.15) followed by BDS application as pre-plant at 187.5 m^3 ha^{-1} along with balance phosphorus requirement through inorganic fertilizer (81.79) while, RDF-NPK registered significantly lower phosphatase activity (10.71). Among graded dose of biocompost, application at 5 t ha^{-1} registered the higher activity of 56.76 μg p-nitrophenol g^{-1} soil h^{-1} compared to application of 2.5 t ha^{-1} (30.45 μg p-nitrophenol g^{-1} soil h^{-1}). The increase in the soil phosphatase activity might be due to BDS and biocompost application.

Earlier study indicated that phosphatase activity of soil increase after the application of BDS and biocompost (Latha and Valliappan, 2010) while soil organic matter content and soil microbial population, vital for the nutrient turnover and long term productivity of soil, are enhanced by the application of BDS and biocompost. Similar increase in soil phosphatase activity with application of BDS has been reported by Kalaiselvi and Mahimairaja (2009).

Dehydrogenase activity: The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). It appears to be more related to the metabolic state of soil microbial population than to be activity of specific free enzymes activity on a particular substrate. Like phosphatase, the dehydrogenase activity of soil also differed significantly among the various doses of BDS and biocompost application with different crop growth stages besides significant interaction between them (Fig. 2). The soil dehydrogenase activity was highest in BDS application at 187.5 m^3 ha^{-1} as split doses along with balance phosphorus requirement through inorganic fertilizer (12.02 μg TPF g^{-1} soil h^{-1}) followed by pre-plant application of BDS at 187.5 m^3 ha^{-1}along with balance phosphorus requirement through inorganic fertilizer (11.64 μg TPF g^{-1} soil h^{-1}). Significantly lower soil dehydrogenase activity was noticed in RDF-NPK (2.17 μg TPF g^{-1} soil h^{-1}). Dehydrogenases are considered to play an essential role in the initial stages of

Table 3: Effect of biomethanated distillery spentwash and biocompost application on soil microbial population at various stages

	Bacteria ($\times10^6$ cfu g^{-1} of soil)					Fungi ($\times10^4$ cfu g^{-1} of soil)					Actinomycetes ($\times10^3$ cfu g^{-1} of soil)				
Treatments	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
T_1	23.1	20.4	19.8	18.4	20.4	12.6	12.0	11.4	10.2	11.6	7.2	6.4	5.6	4.5	5.9
T_2	27.4	22.6	21.0	19.6	22.7	17.7	15.4	13.8	12.0	14.7	8.8	7.8	6.7	4.8	7.0
T_3	39.7	32.3	30.2	25.4	31.9	23.8	22.8	19.2	15.6	20.4	10.4	9.0	8.0	4.9	8.1
T_4	40.0	33.8	31.4	25.8	32.8	25.4	23.2	19.6	15.8	21.0	10.3	8.9	7.8	5.2	8.1
T_5	40.4	34.0	31.6	26.4	33.1	24.8	23.4	20.2	16.0	21.1	10.4	9.0	8.0	5.2	8.2
T_6	46.8	42.2	39.7	29.2	39.5	25.4	25.0	23.5	16.2	22.5	10.8	9.6	8.2	5.5	8.5
T_7	48.0	45.4	43.7	30.4	41.9	28.7	26.3	24.5	18.1	24.4	11.0	10.0	8.6	5.8	8.9
T_8	41.4	33.4	31.4	26.3	33.1	24.6	23.7	21.0	15.5	21.2	10.1	8.8	8.0	5.2	8.0
T_9	50.0	42.8	39.3	29.6	40.4	26.5	25.8	24.2	16.8	23.3	10.5	9.6	8.8	5.7	8.7
T_{10}	51.0	44.5	41.6	34.7	43.0	27.6	26.8	25.8	20.0	25.1	10.8	10.0	9.6	6.2	9.2
Mean	40.8	35.1	33.0	26.6	33.9	23.7	22.4	20.3	15.6	20.5	10.0	8.9	7.9	5.3	8.1

Sov	SEd	CD (0.05)	Sed	CD (0.05)	Sed	CD (0.05)
T	2.99	5.96	2.57	5.12	1.55	3.08
S	1.92	3.81	1.65	3.28	0.99	1.97
TXS	5.99	11.92	5.15	10.24	3.10	6.16

S_1: 90 DAP; S_2: 180 DAP; S_3: 270 DAP; S_4: After harvest of sugarcane

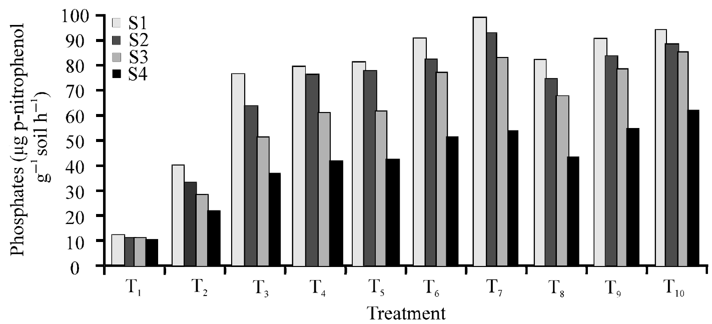

Fig. 1: Effect of biomethanated distillery spentwash and biocompost application on soil phosphatase activity at various stages

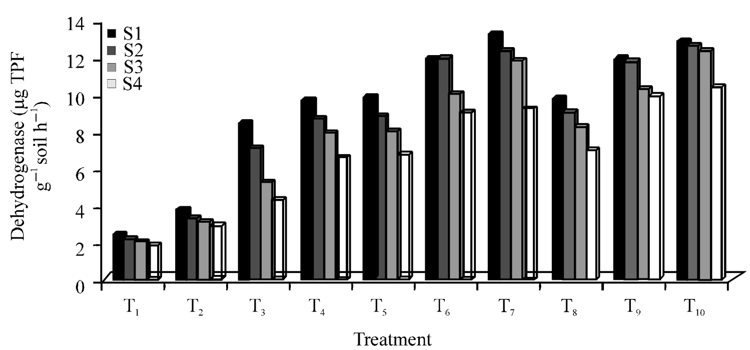

Fig. 2: Effect of biomethanated distillery spentwash and biocompost application on soil dehydrogenase activity at various stages

the oxidation of soil organic matter by transferring hydrogen and electrons from substrates to acceptors. The increase in soil dehydrogenase activity with increasing dose of BDS and biocompost is reflections of organic matter build up which leads to increase in microbial activities (Frankenberger and Dick, 1983).

Urease activity: The BDS and biocompost application has increased the activities of urease in experimental soil (Fig. 3). At higher dose of BDS application at 187.5 m^3 ha^{-1} as split doses along with balance phosphorus requirement through inorganic fertilizer, higher urease activity of soil (23.68 µg NH_4-N g^{-1} soil h^{-1}) was observed over RDF-NPK soil (5.49 µg NH_4-N g^{-1} soil h^{-1}). The several fold increase in the soil urease activity due to BDS application might be ascribed to the addition of organic matter and essential nutrients and subsequent increase in the microbial biomass (Saliha *et al*., 2005). Dinesh *et al*. (2000) also suggested addition of organic material to soil enhanced the microbial activities which in turn favour the

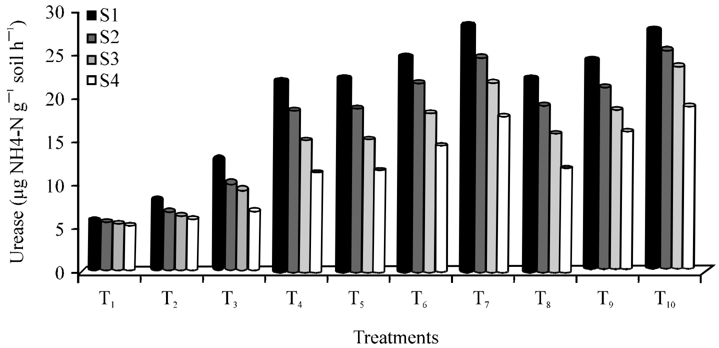

Fig. 3: Effect of biomethanated distillery spentwash and biocompost application on soil urease activity at various stages

synthesis of various enzymes in soil. The results of this study is in close agreement with the findings of Kamalakumari and Singaram (1995) who observed a strong positive relationship among the available NPK and organic carbon and enzyme activities of the soil. Kalaiselvi and Mahimairaja (2009) also reported that the enzyme activities were increased due to the application of BDS. The work of Goyal *et al.* (1995) and Murugaragavan (2002) lend support for the increased urease activity of the soil owing to the addition of BDS.

CONCLUSION

Results of the present study indicated that application of biomethanated distillery spentwash and pressmud biocompost substantially increased the microflora and enzymatic activities of the soil throughout the crop growth period of sugarcane. Application of biomethanated distillery spentwash at 187.5 m^3 ha^{-1} as split doses had a more microflora and enzymatic activities in sugarcane grown soil. The increased microbial biomass and enzymatic activities in sugarcane grown soil expedited mineralization of biomethanated distillery spentwash and pressmud biocompost, nutrient cycling and formation of organic matter and soil structure. The utilization of biomethanated distillery spentwash and pressmud biocompost will increase the activities of soil microbes and enzymes thus enhancing the fertility status besides paving an eco-friendly approach for the disposal of the distillery spentwash.

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