



## Effect of Biomethanated Spent Wash on Enzymatic Activities under Irrigated Condition

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The biomethanated distillery spentwash is a nutrient rich liquid organic waste obtained from molasses based distillery industries after biomethanation process. The effect of different levels and methods of spentwash application on soil enzymatic activity was examined through a field experiment using sesame c.var.VRI (Sv) 2 as the test crop at Research and Development Farm, The Sakthi Sugars Pvt. Ltd., Appakkudal, Erode District. The experiment was formulated with six treatments with four replications and laid out in a randomized block design. As per the treatment schedule, the calculated quantity of biomethanated distillery spentwash (DSW) was uniformly applied to the plots @ 25, 50, 75 and 100 % along with recommended dose of NP (19.4, 25.2, 58.3 and 77.77 L, respectively) before sowing as pre-sown application. In addition, one absolute control and a treatment with recommended dose of NPK (35:23:23 kg NPK/ha) have been imposed for comparison. The soil samples were collected at 30 d intervals and analyzed for the changes in soil enzyme activities. The results of the study showed that the enzymatic activities of the soil were substantially increased throughout the crop growth period due to biomethanated DSW application, compared to check. However, application of 100 % N through DSW (77.77 L/1200 m<sup>2</sup>) and 75 % N through DSW + 25 % N through inorganic source (58.3 L/1200 m<sup>2</sup>) was found to be significantly superior by increasing the activities of phosphatase (15.07 µg p-nitrophenol g<sup>-1</sup> soil h<sup>-1</sup>), dehydrogenase (29.92 µg TPF g<sup>-1</sup> soil h<sup>-1</sup>) and urease (10.97 µg NH<sub>4</sub>-N g<sup>-1</sup> soil h<sup>-1</sup>), respectively.

**Key words:** Post methanated distillery spent wash, Enzyme activities, Irrigated soil

Molasses, one of the important byproducts of sugar industry, is the chief source for the production of ethanol in distilleries by fermentation method. The Indian distillery units mainly use sugarcane molasses as a preferred raw material because of its easy and large scale availability. Alcohol is produced from molasses by two types of fermentation processes i.e., Praj type and Alfa Laval distillation. In Praj type, for the production of one liter of alcohol, about 12–15 liters of spentwash is generated; whereas, in the Alfa Laval continuous fermentation and distillation process, only 7–8 liters of wastewater per liter of alcohol is produced; where, it uses evaporators for concentrating the effluent. The spentwash is acidic (pH 3.94 - 4.30), dark brown liquid with high BOD (45,000 – 1,00,000 mg l<sup>-1</sup>), COD (90,000 – 2,10,000 mg l<sup>-1</sup>) and produce obnoxious odour. Although it does not contain toxic substances, its discharge without any treatment brings about immediate discolouration and depletion of dissolved oxygen in the receiving water streams, in turn posing serious threat to the aquatic flora and fauna (Mane *et al.*, 2006). Distillery waste is rich in organic matter and nutrients especially nitrogen and potassium and also can be utilized as a source of irrigation water in water scarcity areas. However, they are also characterized by high soluble salts coupled with high BOD and COD. Hence, while aiming for better crop production; their utilization has

to be optimized for sustaining the environment. On an average, distillery effluent release 80 million kg of nitrogen and 520 million kg of potassium annually. Thus, the availability of nutrients in distillery effluents and the possibility of substituting these for inorganic fertilizer in agriculture have a great promise (Joshi and Singh, 2010). The addition of organic matter through the BDS may be favourable for enzymes in soils. Batch *et al.* (1993) observed that the spentwash at 250 m<sup>3</sup> ha<sup>-1</sup> rate stimulated the soil microorganisms and increased the dehydrogenase activity in soil. The spentwash addition increased the phosphatase, dehydrogenase and urease enzymes in dry land black and red soils especially, at levels of 125 m<sup>3</sup> ha<sup>-1</sup> (Murugaragavan, 2002).

### Material and Methods

A field experiment was conducted at Research and Development Farm, The Sakthi Sugars Pvt. Ltd., Appakkudal, Erode District, TamilNadu in randomized block design with three replications using sesame (*Sesamum indicum*) var .VRI (Sv) 2 as the 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 providing better soil aeration and consequent reduction of BOD level in the soil-water system.

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### Treatment details

T<sub>1</sub>: Absolute control, T<sub>2</sub>: Control - 100% recommended dose of NPK, T<sub>3</sub>: 25 % N through DSW and 75 % N through inorganic source based on crop requirement, T<sub>4</sub>: 50 % N through DSW and 50 % N through inorganic source based on crop requirement, T<sub>5</sub>: 75 % N through DSW and 25 % N through inorganic source based on crop requirement, T<sub>6</sub>: 100 % N through DSW. While applying P, the available P in DSW and inorganic P has been taken together to meet the P requirement of crop. Potassium has been skipped in DSW applied treatments.

Soil samples were collected at 30(R1), 60(R2), 90(R3) and 120 DAS (R4). The activities of urease, phosphatase and dehydrogenase enzymes were assayed as per the standard procedures (Tabatabai and Bremner, 1972).

## Results and Discussion

### Dehydrogenase activity

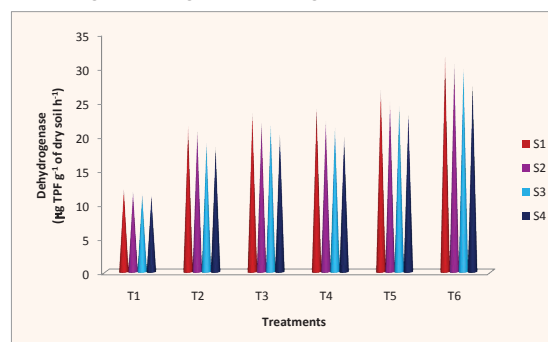
The DSW application increased the activities of dehydrogenase, phosphatase and urease with different doses of DSW viz., 75 per cent N through

**Table 1. Characteristics of post methanated distillery spentwash (PMDSW)**

Characters	Unit	Values
<b>Physical properties</b>		
Colour	-	Greenish brown
Odour	-	Unpleasant
Moisture	%	82
Total suspended solids	mg L <sup>-1</sup>	6850
Total dissolved solids	mg L <sup>-1</sup>	45,120
Total solids	mg L <sup>-1</sup>	51,970
Specific gravity	g cc <sup>-1</sup>	1.12
<b>Physico-chemical properties</b>		
pH	-	7.75
EC	dS m <sup>-1</sup>	37.8
Biological oxygen demand	mg L <sup>-1</sup>	8,740
Chemical oxygen demand	mg L <sup>-1</sup>	37,476
Organic carbon	mg L <sup>-1</sup>	26,110
Total Nitrogen	mg L <sup>-1</sup>	1,700
Total Phosphorus	mg L <sup>-1</sup>	450
Total Potassium	mg L <sup>-1</sup>	11,550
Total Sodium	mg L <sup>-1</sup>	845
Total Calcium	mg L <sup>-1</sup>	2,272
Total Magnesium	mg L <sup>-1</sup>	1,580
<b>Water soluble cations</b>		
Calcium	m.e. L <sup>-1</sup>	52.89
Magnesium	m.e. L <sup>-1</sup>	61.25
Sodium	m.e. L <sup>-1</sup>	32.87
Potassium	m.e. L <sup>-1</sup>	227.35
<b>Water soluble anions</b>		
Carbonate	m.e. L <sup>-1</sup>	Absent
Bicarbonate	m.e. L <sup>-1</sup>	54.12
Chloride	m.e. L <sup>-1</sup>	240.82
Sulphate	meq L <sup>-1</sup>	75.70
SAR		4.56
RSC	meq L <sup>-1</sup>	-61.20
SSP	Per cent	9.87
Potential salinity	meq L <sup>-1</sup>	258.24
<b>Biological properties</b>		
Bacteria	× 10 <sup>6</sup> CFU ml <sup>-1</sup>	23.6
Fungi	× 10 <sup>4</sup> CFU ml <sup>-1</sup>	11.2
Actinomycetes	× 10 <sup>2</sup> CFU ml <sup>-1</sup>	7.2

DSW+25 per cent N through inorganic source in the field experiments with sesame at irrigated condition, respectively. The dehydrogenase activity of the soil

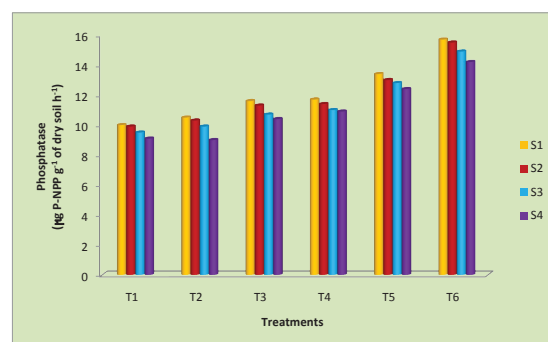
was also influenced by other doses of DSW application. Significantly higher dehydrogenase activity of 29.92 and 24.87 µg of TPF g<sup>-1</sup> of soil was recorded in T<sub>6</sub> and T<sub>5</sub>, which were on par with each other. The lowest enzyme activity of 11.70 µg of TPF g<sup>-1</sup> of soil was recorded in T<sub>1</sub> (Control). The soil dehydrogenase activity significantly differed at all stages of crop growth. The dehydrogenase enzyme activity was found to be the lowest at S<sub>4</sub> (at harvest stage) of 20.05 µg of TPF g<sup>-1</sup> of soil and the highest at S<sub>1</sub> (vegetative stage) of 23.35 µg of TPF g<sup>-1</sup> of soil (Fig 1).



**Fig 1. Effect of distillery spent wash application on soil dehydrogenase activity**

### Phosphatase activity

The phosphatase activity of the soil was highly influenced by different doses of DSW application. Significantly higher phosphatase activity of 15.07 µg of PNPP g<sup>-1</sup> of soil was recorded in T<sub>6</sub> (100 per cent N through distillery spentwash) followed by the treatments T<sub>5</sub>, T<sub>4</sub> and T<sub>3</sub>. The lowest enzyme activity of 9.62 µg of PNPP g<sup>-1</sup> of soil was recorded in T<sub>1</sub> (Control). The soil phosphatase activity significantly differed at all stages of sesame crop growth. The enzyme activity was the lowest at S<sub>4</sub> (harvest stage) of 11.00 µg of PNPP g<sup>-1</sup> of soil and the highest at S<sub>1</sub> (Vegetative stage) of 12.15 µg of PNPP g<sup>-1</sup> of soil (Fig 2).

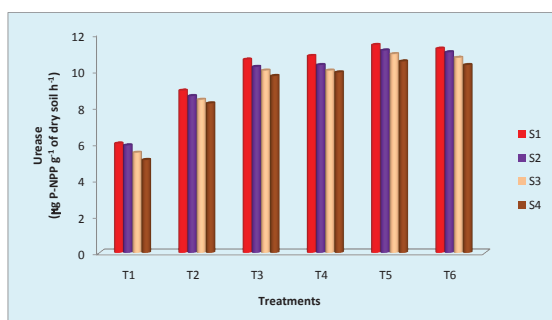


**Fig 2. Effect of distillery spent wash application on soil phosphatase activity**

### Urease activity

Urease activity of the soil was measured in distillery spentwash applied field. Significantly higher urease activity of 10.97 µg of ammonia released g<sup>-1</sup> of soil h<sup>-1</sup> was recorded in T<sub>5</sub> (75 per cent N through distillery spentwash + 25 per cent N through inorganic

source), which was on par with  $T_6$  (100 per cent N through distillery spentwash) of  $10.80 \mu\text{g}$  of ammonia released  $\text{g}^{-1}$  of soil  $\text{h}^{-1}$ . The lowest enzyme activity of  $5.62 \mu\text{g}$  of ammonia released  $\text{g}^{-1}$  of soil  $\text{h}^{-1}$  was recorded with  $T_1$  (Control). The soil urease activity significantly differed at all stages of crop growth. The enzyme activity was the lowest at  $S_4$  (at harvest stage) of  $8.95 \mu\text{g}$  of ammonia released  $\text{g}^{-1}$  of soil  $\text{h}^{-1}$  and the highest at  $S_1$  (vegetative stage) of  $9.81 \mu\text{g}$  of ammonia released  $\text{g}^{-1}$  of soil  $\text{h}^{-1}$  (Fig 3).



**Fig. 3. Effect of distillery spentwash application on soil urease activity**

This might be due to tremendous increase in the microbial population, availability of most of the essential nutrients and organic carbon content of the soil applied with different levels of DSW. This 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 for enzyme activities of the soil. The work of Goyal *et al.* (1995) and Rajannan *et al.* (1998), Murugaragavan (2002) lend support for the increased activities of soil enzymes owing to the addition of spentwash. Similar results were obtained by Sivashankari (2009) and Nandha Kumar (2009).

### Conclusion

Results of the present study indicated that application of post methanated distillery spentwash increased the enzyme activities of the soil throughout the crop growth period of sesame. The enzyme activities *viz.*, phosphatase, dehydrogenase and urease recorded the highest value of  $15.07 \mu\text{g}$  p-nitro phenol  $\text{g}^{-1}$  soil  $\text{h}^{-1}$ ,  $29.92 \mu\text{g}$  TPF  $\text{g}^{-1}$  soil  $\text{h}^{-1}$  and  $10.97 \mu\text{g}$   $\text{NH}_4\text{-N}$   $\text{g}^{-1}$  soil  $\text{h}^{-1}$  respectively, in the treatment  $T_6$  (100 per cent N through distillery spentwash) and  $T_5$  (75 per cent N through DSW and 25 per cent N through inorganic source), respectively under

irrigated condition. Enhancement of the activities of phosphatase, dehydrogenase and urease enzyme was observed in the soil that received different doses of post methanated distillery spentwash and maintained stable enzyme activities till the harvest stage of the crop.

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### References

- Batch T, Martinez A, Mustelier-L-del A, Villegas D R and Ponce-de- Leon D.1993. Agricultural use of vinasse mixed with effluent from brown sugar production. *Quimica Fisca y Biologia de Suelos*, **1**: 229-232.
- Goyal S, Chander K and Kapoor K K.1995.Effect of distillery wastewater application on soil microbial properties and plant growth. *Environ.Eco.*, **13**(1): 89-93.
- Joshi H C and Singh G.2010.Use of distillery effluent in agriculture. *J. Socio Envl. Res. Org.*, **1**(3): 21-24.
- Kamalakumari K and Singaram P.1995.Relationship among soil chemical biochemical properties and enzyme activities. *Madras Agric. J.*, **82**: 69-70.
- Mane J D, Modi S, Nagawade S, Phadnis S P and Bhandari V M. 2006. Treatment of spentwash using modified bagasse and colour removal studies, *Bioresource Technol.*, **53**: 80-89.
- Murugaragavan R .2002. Distillery spentwash on crop production in dryland soils M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore.
- Nandakumar N B.2009. Eco-friendly utilization of post biomethanated distillery spentwash for enhanced crop productivity.Ph.D. (Env.Sci.), Thesis, Tamil Nadu Agric Univ., Coimbatore.
- Rajannan G, Helkiah J, Parwin Banu K S and Ramasami P P.1998. Preparation of quality compost from pressmud and distillery effluent.In: Proc. of National seminar on use of distillery and sugar industry wastes in agriculture, 28 – 29 Oct., ADAC & RI, Tiruchirappalli. pp:149-151.
- Sivasankari J. 2009. Studies on impact of post methanated distillery spentwash on the physiology and productivity of crop plants. *M.Sc. (Env. Sci.) Thesis*, TNAU, Coimbatore.
- Tabatabai MA and Bremner J M. 1969.Use of p-nitrophenyl phosphate for the assay of soil phosphatase activity. *Soil Biol. Biochem.*, **1** :301-307.