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# Studies on the Effect of Post Biomethanated Distillery Spentwash Application on Soil Biological Activities

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# ABSTRACT

The biomethanated distillery spentwash is a nutrient rich liquid organic waste obtained from molasses based distillery industries after biomethanation process. The spentwash, being loaded with organic compounds could bring remarkable changes on the biological properties of soils and thus influences the fertility of soil significantly. The effect of different levels and methods of spentwash application on soil microbial activity was examined through a field experiment. The field experiment was conducted on groundnut (*Arachis hypogaea* L.) as a test crop at Research and Development Cane Farm, The Salem Co-operative Sugar Mills Ltd., Mohanur, Namakkal District. Application of distillery spentwash significantly increased the microbial population *viz.*, bacteria, fungi and actinomycetes in soil. The application of spentwash at the rate of 120 m<sup>3</sup>/ ha plus NP fertilizers recorded the highest microbial population. The continuous application of split doses was found to be better than one time application of spentwash in promoting the microbial activities.

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## INTRODUCTION

The distillery spentwash is rich in nutrients and organic components with high BOD. Therefore, upon field application, it increases the soil organic matter content, pH, nutrient content and mineral content, which may be favourable for number of microorganisms and enzymes in soils. Sugarcane crop irrigated with treated distillery effluent with varying dilutions significantly altered the microbial population in the rhizosphere zone. The microbial population was found to be high in soil irrigated with 50 times diluted effluent (Lakshmanan & Gopal 1996). The population dynamics of bacteria, actinomycetes, fungi, *Azospirillum* and *Azotobacter* in the field soils grown with turmeric, paddy, gingelly, cotton, banana and groundnut showed that, 40 and 50 times diluted effluent irrigations enhanced or maintained the microbial populations in the soils.

The population of different groups of microorganisms was enumerated in the distillery spentwash (Murugaragavan 2002). He reported that the populations of bacteria, fungi and yeast were higher ranging from 16 to  $33.5 \times 10^6$  CFU/mL, 4.33 to  $12.5 \times 10^3$  CFU/mL and 3 to  $4 \times 10^4$  CFU/mL of spentwash, respectively. The application of distillery wastewater to a field soil at rates equivalent to 40, 80 and 160 m<sup>3</sup>/ha of irrigation increased soil microbial biomass and dehydrogenase activity (Goyal et al. 1995). The catalase and dehydrogenase are considered as important enzymes for the oxidative process in soil. The catalase activity was enhanced

with 40 times and 50 times dilutions of spentwash (Rajannan et al. 1998).

#### MATERIALS AND METHODS

**Collection and characterization of distillery spentwash:** The biomethanated distillery spentwash was characterized for its nutritive value and pollution potential by following the standard methods (APHA 1989) (Table 1). The biomethanated distillery spentwash sample was collected from The Salem Co-operative Sugar Mills Ltd, Mohanur, Tamil Nadu, India.

**Field experiment:** A field experiment was conducted using groundnut (*Arachis hypogaea* L.) as a test crop to examine the effect of spentwash on nutrient dynamics at Research and Development Cane Farm, The Salem Co-operative Sugar Mills Ltd., Mohanur. The experimental soil was sandy loam in texture. Taxonomically the soil belongs to the family Typic Rhodustalfs. The experiment was laid out in a split plot design with two main plots and eight sub plot treatments with three replications consisting of different levels of spentwash with and without NP fertilizers allotted to the plots of 13.5 m<sup>2</sup> size (6 m × 2.25 m) leaving 1 m between each replication for irrigation purpose following random principles.

#### **Treatment Details**

**Main plots:**  $M_1$ -One time application;  $M_2$ -Continuous split doses of application.

Sub plots: T<sub>1</sub> - Control; T<sub>2</sub> - RD of NP; T<sub>3</sub> - Spentwash @

40 m<sup>3</sup>/ha; T<sub>4</sub> - Spentwash @ 40 m<sup>3</sup>/ ha + RD of NP; T<sub>5</sub> - Spentwash @ 80 m<sup>3</sup>/ha; T<sub>6</sub> - Spentwash @ 80 m<sup>3</sup>/ha + RD of NP; T<sub>7</sub> - Spentwash @ 120 m<sup>3</sup>/ha; T<sub>8</sub> - Spentwash @ 120 m<sup>3</sup>/ ha + RD of NP (RD-Recommended Dose)

The different levels of spentwash were applied to the field uniformly by spraying manually to each plot 15 days before sowing for first main plot treatment  $(M_1)$ . In the second main plot treatment  $(M_{2})$ , the spentwash was applied in three equal splits along with irrigation water. The first split dose of spentwash was applied 15 days after sowing. The crop was supplied with N and P fertilizers as per the treatments at the recommended dose of 17 and 34 kg/ha, respectively. The fertilizers were applied in the form of urea and single super phosphate. The K was entirely supplied through the spentwash. Sowing was done with groundnut seeds of TMV 7 by adopting a seed rate of 125 kg/ha and a spacing of 30  $cm \times 10$  cm. All other routine cultural operations until the harvest of the crop were followed as per the recommendations of crop production guide of Tamil Nadu Agricultural University.

**Microbial population:** The populations of different groups of microorganisms namely bacteria, fungi and actinomycetes in the field soils were enumerated by using standard serial dilution plating techniques (Waksman & Fred 1922).

# RESULTS

### Effect of Spentwash on Microbial Population

**Bacterial population:** The changes in the soil bacterial population due to different levels and methods of spentwash application are presented in Table 2. The bacterial population ranged from 20.00 to  $56.33 \times 106$  CFU/g of soil initially before sowing under one time application ( $M_1$ ) and from 14.00 to  $18.00 \times 106$  CFU/g of soil under continuous application ( $M_2$ ).

The lowest bacterial population was observed in control  $(T_1)$ , whereas, the highest was recorded in the soil that received 120 m<sup>3</sup> of spentwash with NP fertilizers  $(T_8)$ . While the bacterial population was found gradually decreased during crop growth with one time application, it increased significantly up to pod formation and decreased at post harvest stage with continuous application. Such increase was more due to NP fertilizers.

**Fungi population:** Before sowing, the fungal population in soil ranged from 9.67 to  $18.00 \times 10^4$  CFU/g of soil under one time application (M<sub>1</sub>) and from 9.33 to  $15.00 \times 10^4$  CFU/g of soil under continuous application of spentwash (M<sub>2</sub>). Increase in the rate of application recorded considerable increase in the fungal population and such increase was more due to NP fertilizers (Table 3). With the one time application of spentwash the fungal population was found to be decreased

Table 1: Characteristics of biomethanated distillery spentwash.

S. No.	Characteristics Bi	iomethanated spentwash*
1.	Colour	Dark brown
2.	Odour	Unpleasant burnt sugar
3.	pH	7.1
4.	EC (dS/m)	38
5.	Total dissolved solids	50000
6.	Total suspended solids	3300
7.	Total solids	53300
8.	Biochemical oxygen demand	12800
9.	Chemical oxygen demand	35000
10.	Carbon (g/L)	24
11.	Nitrogen	420
12.	Phosphorus	40
13.	Potassium	9097
14.	Total sugars (%)	3.49
15.	Reducing sugars (%)	1.77
16.	Total phenols	84
17.	Zinc	7.20
18.	Iron	78
19.	Manganese	5.3
20.	Copper	5.5
21.	Bacteria (× $10^6$ CFU/mL of effluent)	12
22.	Fungi (× 10 <sup>4</sup> CFU/mL of effluent)	19
23.	Actinomycetes (× $10^3$ CFU/mL of eff	luent) Nil

\*Mean of triplicate samples (values are in mg/L unless otherwise stated).

both at pod formation and at post harvest stages. However, with the continuous application, it was found increased at pod formation stage and decreased at harvest stage. At all times, irrespective of methods of application the fungal population was the lowest in soil with no spentwash application  $(T_1)$ ; the highest was with the application of spentwash at a rate of 120 m<sup>3</sup>/ha plus NP fertilizers  $(T_o)$ .

Actinomycetes population: The actinomycetes population was markedly affected due to different levels and methods of spentwash application (Table 4). The actinomycetes population in soil ranged from 8.33 to  $13.33 \times 10^3$  CFU/g of soil initially before sowing under one time application (M<sub>1</sub>) and from 7.33 to  $11.33 \times 10^3$  CFU/g of soil under continuous application  $(M_{2})$ . The lowest actinomycetes population was in control  $(T_1)$ , whereas the highest was recorded in soil that received 120 m<sup>3</sup> of spentwash with NP fertilizers (T<sub>a</sub>). Similar to bacteria and fungi, the actinomycetes population was found reduced during the crop growth due to one time application; whereas, it was found increased at pod formation stage, but decreased at post harvest stage with continuous application. Increase in the rate of spentwash application has significantly increased the actinomycetes population in soil and such increase was more due to NP fertilizers.

### DISCUSSION

Application of distillery spentwash significantly increased the microbial population *viz.*, bacteria, fungi and

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Treatments	Stage I			Stage II			Stage III		
	$M_1$	M <sub>2</sub>	Mean	M <sub>1</sub>	$M_2$	Mean	M <sub>1</sub>	$M_2$	Mean
TOTAL	20.00	14.22	17.17	11.00	10.00	11.67	7.00	7.00	. 1.5
T <sub>1</sub> - Control	20.00	14.33	17.17	11.00	12.33	11.67	7.33	7.00	7.17
T <sub>2</sub> - NP alone	26.33	14.67	20.50	23.00	23.67	23.33	8.00	7.33	7.67
$T_3$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup>	46.33	15.33	30.83	35.00	39.00	37.00	21.00	23.00	22.00
$T_4$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup> + NP	49.67	18.00	33.83	40.00	39.00	39.50	22.00	24.33	23.17
$T_5$ - Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup>	49.67	16.00	32.83	39.00	43.00	41.00	22.00	24.67	23.33
$T_6$ - Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup> + NP	50.33	15.33	32.83	38.00	44.00	41.00	23.00	25.00	24.00
$T_{7}^{-}$ Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup>	54.00	16.00	35.00	44.33	43.00	43.67	24.00	27.00	25.50
$T_{8}$ - Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup> + NP	56.33	14.00	35.17	46.00	46.00	46.00	25.00	28.67	26.83
Mean	44.08	15.46	29.77	34.54	36.25	35.40	19.04	20.88	19.96
	SEd		CD (0.05)	SEd		CD (0.05)	SEd	l	CD (0.05)
Т	0.99		2.12	1.01		2.16	0.57	7	1.22
М	0.53		1.12	0.58		1.23	0.30	)	0.64
$T \times M$	1.45		3.08	1.54		3.28	0.83	3	1.77
$M\times T$	1.50		3.17	1.65		3.49	0.86	5	1.82

Table 2: Effect of different levels of spentwash application on bacterial population (×10<sup>6</sup> CFU/g of soil) in soil.

M<sub>1</sub> - One time application; M<sub>2</sub> - Continuous application

Stage I - Before sowing; Stage II - Pod formation; Stage III - Post harvest

Table 3. Effect of different levels of spentwash application on fungal population ( $\times 10^4$  CFU/g of soil) in soil.

Treatments	Stage I			Stage II			Stage III		
	$M_1$	M <sub>2</sub>	Mean	M <sub>1</sub>	$\tilde{M}_2$	Mean	M <sub>1</sub>	$M_2$	Mean
T <sub>1</sub> - Control	9.67	9.33	9.50	7.00	6.67	6.83	4.00	5.00	4.50
$T_{2}$ - NP alone	11.67	10.33	11.00	9.33	10.67	10.00	5.67	5.33	5.50
$T_3^2$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup>	13.67	11.00	12.33	10.33	11.67	11.00	5.67	6.00	5.83
$T_4$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup> + NP	15.33	12.33	13.83	12.00	12.33	12.17	6.00	7.67	6.83
$T_5$ - Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup>	15.00	13.67	14.33	13.67	13.33	13.50	5.67	7.67	6.67
$T_6^-$ Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup> + NP	15.67	15.00	15.33	12.67	15.67	14.17	6.33	8.00	7.17
$T_7$ - Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup>	17.00	12.00	14.50	14.67	14.33	14.50	7.00	8.00	7.50
$T_8$ - Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup> + NP	18.00	12.67	15.33	15.33	16.33	15.83	7.67	9.33	8.50
Mean	14.50	12.04	13.27	11.88	12.63	12.25	6.00	7.13	6.56
	SEd		CD (0.05)	SEd		CD (0.05)	SEd	CD	(0.05)
Т	0.39		0.84	0.38		0.81	0.27	0.5	· /
М	0.25		0.53	0.16		0.34	0.12	0.2	5
ТхМ	0.64		1.35	0.50		1.06	0.35	0.7	6
M x T	0.71		1.50	0.46		0.97	0.33	0.7	1

M<sub>1</sub> - One time application; M<sub>2</sub> - Continuous application

Stage I - Before sowing; Stage II - Pod formation; Stage III - Post harvest

actinomycetes in soil. The microbial population gradually increased with advancement of the crop growth. The results of the field experiment clearly indicated that the microbial populations were not affected by the spentwash application even at higher doses. The treatment, which received the spentwash equivalent to  $120 \text{ m}^3/\text{ha} + \text{NP}$  fertilizers, recorded the highest microbial populations. Being rich in nutrients and organic material, particularly easily oxidizable and soluble organic carbon, the spentwash might have favoured the proliferation of microbial population in soil. By comparing the one time application, the continuous split application of spentwash promotes the growth of the microbial population throughout the crop growth by steady supply of nutrients and build up of organic matter. This was in line with the findings, that the sugarcane crop irrigated with treated distillery effluent with varying dilutions significantly altered the microbial population in the rhizosphere zone (Lakshmanan & Gopal 1996).

The microbial population was found to be high in soil irrigated with 50 times diluted effluent. A high evolution of  $CO_2$  from the spentwash treated soil was observed, which was five times greater than FYM due to greater microbial activities (Patil & Shinde 1995). The reduction in the microbial activities during the crop growth, particularly at har-

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Treatments	Stage I			Stage II			Stage III		
	$M_1$	$M_2$	Mean	M <sub>1</sub>	$M_2$	Mean	$\mathbf{M}_{1}$	$M_2$	Mean
T <sub>1</sub> - Control	8.33	7.33	7.83	6.00	5.67	5.83	3.00	3.00	3.00
$T_2^{1}$ - NP alone	8.67	7.67	8.17	8.33	9.00	8.67	4.00	3.00	3.50
$T_3^2$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup>	11.00	8.67	9.83	9.00	11.33	10.17	4.00	5.00	4.50
$T_4$ - Spentwash @ 40 m <sup>3</sup> ha <sup>-1</sup> + NP	11.33	9.67	10.50	10.33	12.00	11.17	4.33	5.67	5.00
T <sub>5</sub> - Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup>	11.33	10.33	10.83	9.33	11.67	10.50	5.33	5.67	5.50
$T_6$ - Spentwash @ 80 m <sup>3</sup> ha <sup>-1</sup> + NP	12.00	11.33	11.67	10.67	12.00	11.33	5.67	6.33	6.00
T <sub>7</sub> - Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup>	12.67	9.33	11.00	11.00	12.33	11.67	4.00	6.33	5.17
T <sub>8</sub> - Spentwash @ 120 m <sup>3</sup> ha <sup>-1</sup> + NP	13.33	9.67	11.50	11.67	13.67	12.67	4.67	7.00	5.83
Mean	11.08	9.25	10.17	9.54	10.96	10.25	4.38	5.25	4.81
	SEd		CD (0.05)	SEd		CD (0.05)	SEd	CD	(0.05)
Т	0.34		0.73	0.38		0.81	0.23	0.5	0
M 0.20			0.42	0.18		0.39	0.12	0.2	5
T x M	0.53		1.12	0.52		1.12	0.33	0.7	1
M x T	0.57		1.20	0.51		1.09	0.33	0.7	1

Table 4. Effect of different levels of spentwash application on actinomycetes population ( $\times$  10<sup>3</sup> CFU/g of soil) in soil.

M<sub>1</sub> - One time application; M<sub>2</sub> - Continuous application

Stage I - Before sowing; Stage II - Pod formation; Stage III - Post harvest

vest was probably due to the exhaustation of nutrients and organic matter as a result of intense microbial activity and crop uptake during the active stage of crop growth. This finding corroborates with the results of Valliappan (1998), Goyal et al. (1995) and Murugaragavan (2002). Therefore, the spentwash application appeared to have promoted the microbial populations in the soil. The highest microbial populations were resulted due to the application of spentwash at the rate of 120 m<sup>3</sup>/ha plus NP fertilizers. The continuous application of spentwash in promoting the growth of microbial populations throughout the crop growth, mainly by providing steady supply of nutrients and organic matter.

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