Influence of distillery spentwash application on the microbial population dynamics and enzyme activity of maize soil

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Abstract: An investigation was undertaken to evaluate the impact of distillery spentwash on soil enzyme activity and microbial dynamics. The pH of the untreated spentwash was acidic whereas the treated spentwash was alkaline in nature. The total solid contents of the untreated, primary and secondary treated spentwash were 83,000, 46,000 and 31,000 mg l^{-1} respectively. The untreated and treated spentwash were rich in plant nutrients and in addition treated spentwash recorded appreciable amount of microbial load and enhanced enzyme activity. The field experiment which was taken up with various levels of spentwash being applied as one time land application prior to crop cultivation revealed that the application of spentwash to soil grown with maize crop recorded higher amylase and catalase enzyme activity. In addition spentwash application also showed significant increase with respect to microbial population *viz.* bacteria, fungi, actinomycetes and *Azotobacter*. However, the effect of long term application of spentwash on physico-chemical and biological properties of soil and the impact on ground water quality should be carefully monitored.

Key words : Distillery spentwash, Maize, Enzymes, Microbes.

Introduction

Every year distillery industries release enormous amount of spentwash as waste and this when disposed to soil in untreated form causes pollution. Pressmud and distillery spentwash are the two waste materials resulting from sugar and distillery industries respectively. Although these products are considered as wastes by the factories, they are excellent sources of organic carbon and plant nutrients. In addition the waste by products of the distillery industry in which major parts of the nutrients removed from the soils are stored intact and when applied to soil can return the nutrient to same soil from which it is removed and so the nutrients required for subsequent crops could be minimized. In India, there are about 285 distilleries with an installed capacity to produce 1.7 billion litres of alcohol per annum. For every litre of alcohol production about 15 litres of waste water namely spentwash is being generated and at present about 10-15 billion litres of spentwash is being generated per annum from these distilleries.

The distillery spentwash is non toxic and when treated with microbial inoculants can be used as good organic manure. After proper dilution and systematic application it would not cause any harm to soil and treated spentwash has essential nutrients along with exuberant load of microbial population and enzyme activity which can be effectively used for recycling in agriculture (Devarajan *et al.* 1994). Untreated spentwash application to soil might deplete the oxygen level due to higher loads of BOD and COD which in turn would lead to decrease in microbial population and enzyme activity. The treated spentwash application at lower concentration increases enzyme activity due to the increased microbial load in the soil.

Activity of enzymes such as amylase, invertase and cellulase evokes the total organic matter degradation and in addition enzymes catalase and dehydrogenase are responsible for oxidative process in soil. In order to curb the pollution, an eco-friendly recycling technology with spentwash has been initiated which evokes the use of treated spentwash as one time land application prior to crop cultivation to evaluate the effect on soil enzyme and microbial properties.

Materials and Methods

The physical properties such as colour, suspended, dissolved and total solids of untreated, primary and secondary treated spentwash were

Table 1. Characteristics of distillery spentwash

Parameter	Untreated	Primary treated	Secondary treated
Colour	Dark brown	Reddish brown	Reddish brown
Odour	Burnt sugar	Mild molasses	Mild molasses
Turbidity	High	High	High
Temperature	80°C	37°C	32°C
pH	4.0	8.2	8.0
EC (dSm^{-1})	45.8	36.3	32.5
BOD ($mg\ l^{-1}$)	35000	5000	1000
COD ($mg\ l^{-1}$)	90000	35000	21000
Suspended solids ($mg\ l^{-1}$)	5500	5500	2800
Total solids ($mg\ l^{-1}$)	83000	46000	31000
Total dissolved (TDS) solids ($mg\ l^{-1}$)	80000	50000	36000
Sulphate as SO_4 ($mg\ l^{-1}$)	5000	1200	1050
Chloride ($mg\ l^{-1}$)	9000	9400	9000
Sodium ($mg\ l^{-1}$)	400	400	400
Potassium ($mg\ l^{-1}$)	10000	10000	10000
Magnesium ($mg\ l^{-1}$)	1400	1200	900
Calcium ($mg\ l^{-1}$)	2200	1100	900
Nitrogen as total N ($mg\ l^{-1}$)	1200	1200	1000
Phosphorus as PO_4 ($mg\ l^{-1}$)	580	450	400

Table 2. Influence of spentwash application on bacterial population (x 10^6 CFU g^{-1}) in maize soil

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	3.66	12.0	36.33	37.33	29.66	26.0	24.16
T2	5.33	12.33	36.0	41.66	35.33	28.66	26.55
T3	7.66	15.0	26.0	45.0	41.33	31.0	27.66
T4	8.33	16.33	18.33	43.66	42.33	33.33	27.05
T5(C)	5.33	12.66	24.66	22.33	19.0	30.66	19.11
T6	4.33	16.33	45.0	54.0	47.66	31.66	33.16
T7	5.66	12.66	35.0	41.66	37.33	27.0	26.55
Mean	5.76	13.9	31.61	40.80	36.09	29.76	26.32
	SEd			CD (0.05)			
D	0.45			0.91			
T	0.49			0.98			
DxT	1.213			2.41			

estimated as suggested by Anon, 1989. Chemical properties like pH, ammoniacal and nitrate nitrogen, available calcium and magnesium (Jackson, 1967), electrical conductivity, chloride, available potassium (Jackson, 1973), in addition dissolved oxygen, biochemical oxygen demand and chemical oxygen demand were also quantified.

Microbial properties such as bacteria, fungi, actinomycetes and *Azotobacter* were also characterized (Jenson, 1968). Amylase activity of soil sample was assayed by following the method described by Ross (1966). Catalase activity of soil sample was assayed by following the method described by Skujins (1976).

Fig.2. Microbial load in distillery spentwash

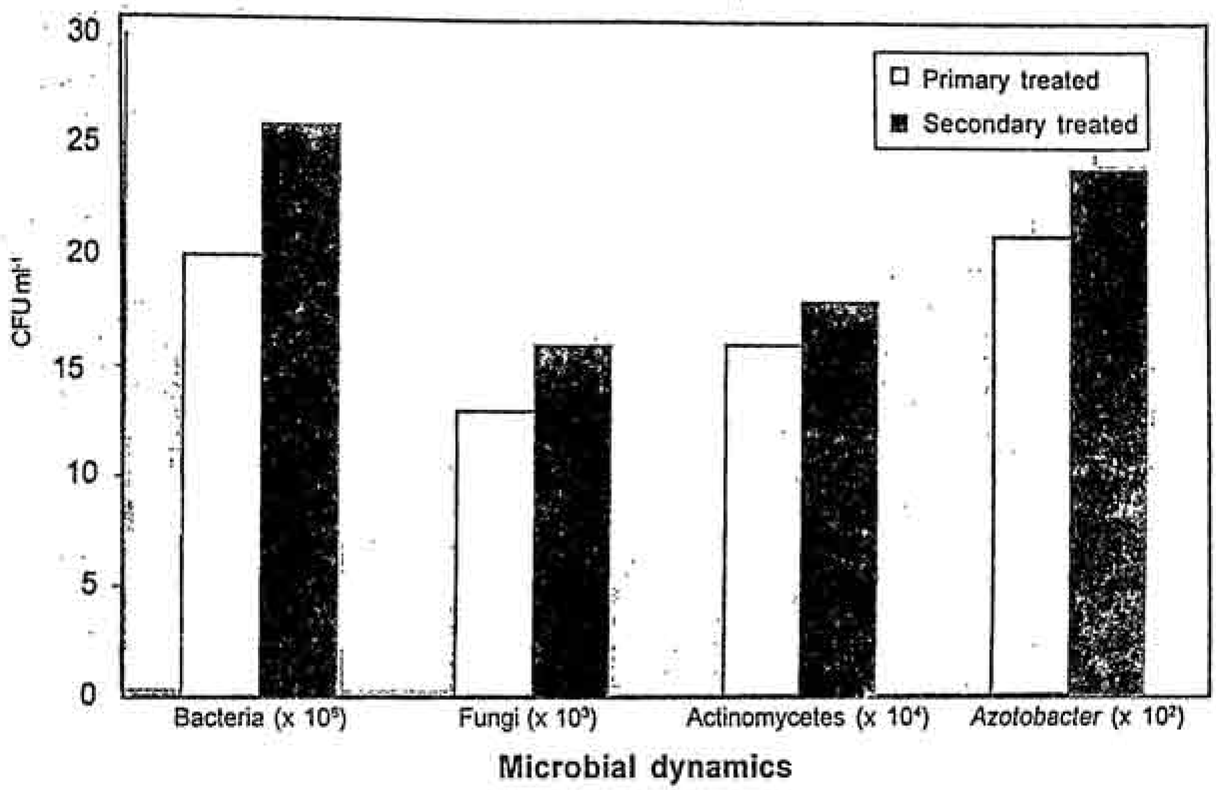

Table 3. Influence of spentwash application on *Azotobacter* population (x 10^2 CFU g^{-1}) in maize soil

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	14.66	17.0	19.0	23.66	17.0	12.33	17.27
T2	13.66	20.0	21.66	22.0	14.33	11.33	17.16
T3	16.33	21.33	25.0	25.3	16.33	16.0	20.05
T4	20.66	17.0	26.66	31.33	24.66	19.66	23.33
T5(C)	19.33	19.33	31.66	24.33	19.66	15.33	21.61
T6	17.33	25.0	18.33	23.66	17.66	14.33	19.38
T7	16.0	17.0	13.0	21.33	15.66	10.6	15.61
Mean	16.85	19.52	22.19	24.52	17.9	14.23	19.20
	SEd			CD (0.05)			
D	0.44			0.87			
T	0.47			0.94			
DxT	1.16			2.31			

To evaluate the effect of tertiary treated spentwash on enzyme and microbial properties of soil grown with maize (CO 1), a field trial was conducted in Bannari Amman Sugars-Distillery unit field site during August 2000, with land application of treated spentwash as single dose prior to crop cultivation. The treatment details of the experiment are given below.

Treatment details

T_1 = 20 kilolitres of treated spentwash ac^{-1} + 20 kilolitres of treated spentwash ac^{-1} on standing crop (two split doses)

T_2 = 40 kilolitres of treated spentwash ac^{-1}

T_3 = 60 kilolitres of treated spentwash ac^{-1}

T_4 = 80 kilolitres of treated spentwash ac^{-1}

T_5 = Absolute control (no fertilizers)

T_6 = 40 kilolitres of treated spentwash ac^{-1} + Nitrogen and phosphorus (50% recommended dose)

T_7 = Control (100% recommended NPK)

Table 4. Influence of spentwash application on fungal population (x 10^3 CFU g^{-1}) in maize soil

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	6.33	3.66	8.33	10.33	8.66	6.66	7.33
T2	4.66	4.33	17.0	15.33	11.0	8.66	10.16
T3	4.33	6.66	16.0	15.33	13.0	10.0	10.88
T4	4.0	5.66	27.3	21.33	16.33	12.33	14.5
T5(C)	6.66	8.0	8.66	11.33	9.33	7.66	8.61
T6	5.0	7.66	20.0	19.33	17.66	14.66	14.05
T7	2.66	5.66	13.0	12.33	9.66	7.0	8.38
Mean	4.8	5.95	15.76	15.04	12.23	9.57	10.56
	SEd			CD (0.05)			
D	0.43			0.87			
T	0.47			0.94			
DxT	1.16			2.30			

Table 5. Influence of spentwash application on actinomycetes population (x 10^4 CFU g^{-1}) in maize sc

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	9.33	10.33	7.0	8.33	7.0	5.33	7.88
T2	13.33	13.0	10.0	10.66	8.66	6.66	10.38
T3	13.66	10.66	9.33	14.66	12.66	9.33	11.72
T4	12.66	13.0	5.66	16.33	12.33	10.0	11.83
T5(C)	7.66	11.0	9.33	18.66	11.33	10.0	11.33
T6	5.33	14.0	6.0	15.66	11.66	8.0	10.11
T7	14.33	12.3	6.33	10.66	8.23	6.33	9.72
Mean	10.90	12.04	7.8	13.57	10.28	7.95	10.42
	SEd			CD (0.05)			
D	0.49			0.97			
T	0.53			1.05			
DxT	1.30			2.58			

Table 6. Influence of spentwash application on amylase enzyme activity (µg of glucose g^{-1}) in maize soil

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	1010	1310	1220	995	980	628	1023
T2	1040	1340	1245	1010	995	965	1099
T3	1100	1400	1265	1025	1010	995	1132
T4	1145	1445	1310	1055	1040	1010	1167
T5(C)	800	1145	998	965	935	905	958
T6	1130	1475	1295	1130	1055	1010	1182
T7	1055	1325	1235	1100	1055	1025	1132
Mean	1040	1348	1224	1040	1010	934	1099
	SEd			CD (0.05)			
D	25.3			50.3			
T	27.3			54.4			
DxT	66.9			133.2			

Table 7. Influence of spentwash application on catalase enzyme activity (µmol of H_2O_2 g^{-1}) in maize soil

Treatments	Sampling Periods (days)						Mean
	D1 (15)	D2 (30)	D3 (45)	D4 (60)	D5 (90)	D6 (post harvest)	
T1	66.6	166.6	416.6	233.3	133.3	83.3	183.3
T2	133.3	233.3	500.0	250.0	83.3	100.0	216.6
T3	133.3	266.6	483.3	283.3	133.3	116.6	236.1
T4	183.3	283.3	583.3	416.6	166.6	133.3	294.4
T5(C)	66.6	116.6	233.3	183.3	83.3	66.6	125.0
T6	166.6	233.3	483.3	283.3	166.6	150.0	247.2
T7	83.3	150.0	433.3	200.0	133.3	116.6	186.1
Mean	119	207.1	447.6	264.2	128.5	109.5	212.6
	SEd			CD (0.05)			
D	10.28			20.4			
T	11.10			22.0			
DxT	27.2			54.1			

Results and Discussion

The physical, chemical and biological properties of distillery spentwash are furnished in Table 1 and Fig.1. The untreated spentwash was dark brown in colour while primary and secondary treated spentwash were reddish brown in colour, with high loads of suspended and dissolved solids. The pH of the untreated spentwash was acidic whereas the treated spentwash was alkaline in nature. The electrical conductivity was high in untreated spentwash than the treated spentwash. The total solid contents of the untreated, primary and secondary treated spentwash were 83,000, 46,000 and 31,000 mg l^{-1} respectively.

The BOD and COD of secondary treated spentwash, which was used for field application, were 1000 and 21,000 mg l^{-1} respectively. The untreated and treated spentwash were rich in plant nutrients. The nitrogen content of untreated, primary and secondary treated spentwash was 1200, 1200 and 1000 mg l^{-1} respectively, while the phosphorus content was 580,450 and 400 mg l^{-1} respectively. The potassium content was very high and it was about 10,000 mg l^{-1} in all the three types of spentwash.

The secondary treated spentwash also contained sulphates, chloride, sodium and magnesium to an extent of 1050, 9000, 400 and 900 mg l^{-1} respectively. The primary and secondary treated spentwash also had appreciable counts of bacteria, fungi, actinomycetes and *Azotobacter* (Fig.1).

The bacterial population of the secondary treated spentwash was 26 x 10^6 CFU ml^{-1}, the fungal population was 16x10^3 CFU ml^{-1}, and actinomycetes population was 18 x 10^4 CFU ml^{-1}. The relatively reduced microbial load might be due to the elevated levels of biological oxygen demand and chemical oxygen demand. The presence of *Azotobacter* was also noticed in the secondary treated distillery spentwash (24 x 10^2 CFU ml^{-1}). Niemelae and Vacaetaenen (1982) reported the presence of *Azotobacter* species in the spentwash.

The mean bacterial population during 15th day after the application of spentwash was 5.76 x 10^6 CFU g^{-1} (Table 2). The bacterial population recorded gradual increase over periods and the mean bacterial population during 60th day was 40.8 x 10^6 CFU g^{-1} and the population started decreasing during post harvest period (29.76 x 10^6 CFU g^{-1}).

The population of actinomycetes and fungi were also high during 60th day which was 13.57 x 10^4 CFU g^{-1} and 15.04 x 10^3 CFU g^{-1} respectively (Table 4 & 5). Decrease in microbial population in post harvest stage might be due to decreased nutrient availability as a result of plant uptake and leaching losses.

The rhizosphere region could be influenced by various environmental factors and physiological conditions of plant which might have been responsible for variation in the population dynamics of different groups of microflora.

Goyal *et al.* (1995) reported increased soil microbial dynamics and dehydrogenase enzyme activity upto 60 days of spentwash application and after that there was reduction in later stages.

Devarajan *et al.* (1998) reported a reductio in the microbial population in the post harves stage in banana. The population of the diazotroph *Azotobacter* recorded a steady increase as th sampling proceeded and a maximum mea *Azotobacter* population of 24.52 x 10^2 CFU g^{-1} (Table 3) was recorded during 60th day Increase in population of actinomycetes, yeast fungi and *Azotobacter* was recorded in soi irrigated with 50 times diluted spentwash (Rovira 1959). The amylase enzyme activity showe significant difference among treatments and activit (Table 6) was comparatively low during 15t day after application of spentwash which wa 1040 mg of glucose g^{-1} and the activity increase during 30th and 45th day of sampling.

The activity of catalase enzyn (Table 7) in soil was also significantly increas over period and the 45th day sampling record maximum catalase activity of 447.6 μ m of H_2O_2 g^{-1}, while the 15th day soil samp recorded 119 μ mol of H_2O_2 g^{-1}. Increas enzyme activity over periods might be d to increased microbial activity which wou have enhanced the organic matter degradati and mineralization. This is in confirmation wi results by Rajannan *et al.* (1998) who report that the catalase, dehydrogenase are consider as important enzymes for oxidative process the soil and presence of high concentrati of salts in spentwash might be responsibel f the reduction in activity of catalase in s immediately after the application of spentwa

Hence, the study revealed that the c time application of treated distillery spentwa in soil grown with maize had no adverse eff on biological properties and yield attribut

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