Research article

Long-Term Effects of Spent Wash Liquor on Soil Bacterial Population in Sugar Cane Production Systems

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Abstract Spent wash liquor (or Distillery Slop) is an agro-industrial wastes generated during alcohol distillery production. Its application to the soil for agriculture is highly beneficial due to rich in plant nutrients, organic carbon, less toxic and easily amenable for microorganisms. An advantage is made to direct apply the spent wash liquor as a supplement fertilizer to enhance crop yield in agricultural field. Therefore, long-term effects of spent wash liquor on soil bacterial population in agricultural crop production system is still poorly understood. In this research, the effect of long-term application of spent wash liquor on bacterial decomposers was investigated in sugar cane production systems in Nam Phong watershed. Eight treatments in this research including soil with no spent wash liquor applied (control), soil applied with spent wash liquor for 1, 2, 3, 4, 5, 7, and 8 years were conducted. The results showed that bacterial population at 1 year after application was significantly higher than those from control soil then, the population declined until 8 years after application the population can reach up to 2.04 x 10^6 CFU/g soil. This study suggested that application of spent wash liquor can affect the bacterial decomposer under sugar cane field. However, the application of spent wash liquor need to be tested in longer time under farmer's field conditions before further recommendation can be given.

Keywords spent wash liquor, sugar cane, bacterial population

INTRODUCTION

Nam Phong watershed is the most important watershed in the upper northeast region of Thailand. Its catchment covers approximately 1,518,900 ha, extending to the five provinces of Chaiyaphum, Khon Kaen, Loei, Nong Bua Lumphu, and Petchaboon (Sthiannopkao et al., 2007). Nowadays, Nam Phong watershed is threatened by a variety of human activities. Besides problem of soil

erosion from agricultural areas around Nam Phong watershed, sediments and wastewater from industrial areas consist of large and medium scale factories such as sugar mill, distillery mill, paperboard mill, tapioca mill are also become a pollutant in the watershed.

Removing pollutants from wastewater by wastewater treatment is the preliminary way to remediate water and reduce soils contamination before discharging it into the river and watershed. Among wastewater from industrial areas, spent wash liquor, agro-industrial wastes generated during alcohol distillery production, becomes the most promising wastewater using for agricultural production due to it contains high concentration of organic load, high amount of nutrients such as nitrogen, phosphorous, potassium, sulphur and a large amount of micronutrients (Suganya and Rajannan, 2009). There have been many studies done investigation the effect of spent wash liquor on plant growth and yield (Rath et al., 2011; Suganya and Rajannan, 2009). However, there is limited knowledge on how spent wash liquor can shape the abundance of bacterial communities in agricultural area. Therefore, this study was designed to investigate how long-term spent wash liquor affected the bacterial populations as well as soil properties for successful application of spent wash liquor under sugar cane production systems to reduce chemical fertilizer application and also recycle treated wastewater for agricultural use.

OBJECTIVE

The purpose of this study was to investigate the influence of spent wash liquor on the bacterial population in sugar cane production system in Nam Phong watershed.

METHODOLOGY

Study Sites and Soil Sampling

Study sites were selected from Nam Phong watershed, Nam Phong district, Khon Kean province between latitude 16°42' N to 16°48' N and longitude 102°53' E to 102°57' E. The soil is characterized as a Chakkarat soil series. Soil samples were collected from sugar cane plantation which cultivated sugar cane cultivar K92 on soil depth 0-15 cm. Year of the experiment was in 2013. Eight treatments were used in this research, including soil with no spent wash liquor applied (control), soil applied with spent wash liquor for 1 year (1Y), soil applied with spent wash liquor consecutively for 2 years (2Y), soil applied with spent wash liquor consecutively for 3 years (3Y), soil applied with spent wash liquor consecutively for 4 years (4Y), soil applied with spent wash liquor consecutively for 5 years (5Y), soil applied with spent wash liquor consecutively for 7 years (7Y) and soil applied with spent wash liquor consecutively for 8 years (8Y). The spent wash liquor was used for pre-sowing irrigation at rates of 6.4 m^3/ha (40 m^3/1600 m^2).

Analysis of Chemical Properties of Spent Wash Liquor

Spent wash liquor characteristic analyses were done by studying the chemical properties of spent wash liquor (Table 1). Chemical properties studied include pH, electric conductivity (EC), total alkalinity (as $CaCO_3$), total dissolved solids (TDS), total solid, total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO) and salinity were investigated by APHA standard methods (APHA, 1995). Organic matter was studied by wet oxidation method (Walkley and Black, 1934), total nitrogen (as N) by micro Kjedahl method (Bremner, 1960), total phosphorus (as P_2O_5) by Bray II and colorimetric method (Bray and Kurtz, 1945), total potash (K_2O) by extracting with ammonium acetate 1*N*, pH 7.0 and measured by flame photometer.

Soil Analyses

Soil studies were conducted after spent wash liquor was used for pre-sowing irrigation 6 months by

digging for three replications in each treatments of sugar cane production system. Soil characteristic analyses were done by studying the physical and chemical properties of the soil (Table 2). Physical properties studied include soil moisture and bulk density through the core method. Particle-size distribution and soil texture by hydrometer method. Chemical properties studied include soil reaction by pH meter in ratio of 1:2.5 with water, EC by EC meter, cation exchange capacity (CEC) by the 1.0 M ammonium acetate (NH_4OAC) method (Chapman, 1965), total nitrogen (Total N) by micro Kjedahl method (Bremner, 1960), available phosphorus concentration by colorimetric method (Bray and Kurtz, 1945), exchangeable potassium level measured by flame photometer, organic carbon and organic matter in soil by wet oxidation method (Walkley and Black, 1934).

Bacterial Population Analysis

Bacterial population analysis was done by serial dilution plating technique on nutrient agar medium (NAM) (Dubey and Maheshwari, 2002). Plates with serial dilution plating were incubated at 28 $^{\circ}$C for 1-2 days. Bacterial populations were enumerated from the plates and expressed as CFU (colony forming units) per gram soil.

Statistical Analysis

This experiment has been designed as the completely randomized design (CRD). F-test along with method of Duncan's multiple range test (DMRT) was used to analyze the differences of the average on each experiment.

RESULTS AND DISCUSSION

Spent Wash Liquor Characteristic Analysis

The data of chemical properties of spent wash liquor are shown in Table 1. The results showed that spent wash liquor used in this study were rich in organic matter and available certain nutrients. In addition, spent wash liquor contained high pH and showed low values of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) suggesting that using of spent wash liquor is safe for agricultural application.

Table 1 Chemical properties of spent wash liquor

Spent wash liquor	Results	Unit
pH	7.83	-
Electric Conductivity; EC	6.89	dS/m
Total Nitrogen (as N)	0.11	%
Total Phosphorus (as P_2O_5)	0.01	%
Total Potash (K_2O)	0.36	%
Total Alkalinity (as $CaCO_3$)	2647.19	mg/L
Total Dissolved Solids (TDS)	10503.33	mg/L
Total Solid	12900.00	mg/L
Total Suspended Solids (TSS)	373.33	mg/L
Organic matter (OM)	0.58	%
Biochemical Oxygen Demand (BOD)	<5	mg/L
Chemical Oxygen Demand (COD)	45	mg/L
Dissolved oxygen (DO)	0.33	mg/L
Salinity	3.44	ppt

Soil Analysis

The data of physical and chemical properties of soil are shown in Table 2. Being very rich in organic matters, the utilization of spent wash liquor in agricultural fields creates organic

fertilization in the soil which raises the pH of the soil, increases availability of soil nutrients and capability to retain water and also improves the physical structure of soil. The results of nutrient contents analysis showed that application of spent wash liquor into soil significantly increased the exchangeable potassium content in the soil. Likewise, the previous report found that the application of spent wash liquor into Sansai soil series significantly increased the available potassium content in the paddy field (Chatchaisiri et al., 2003).

Table 2 Physical and chemical properties of soil

Soil properties	Treatments								F-test	CV (%)
	Control	1Y	2Y	3Y	4Y	5Y	7Y	8Y		
Organic matter; OM (%)	0.603^{a}	0.333^{e}	0.473^{c}	0.358^{e}	0.337^{e}	0.535^{b}	0.515^{b}	0.440^{d}	*	21.64
Total N (%)	0.027^{a}	0.013^{d}	0.02c	0.014^{d}	0.013^{d}	0.023^{b}	0.023^{b}	0.018^{c}	*	29.14
Available P (mg/kg)	48.64^{a}	36.19^{c}	24.96^{e}	36.53^{c}	42.3^{b}	20.37^{f}	13.44^{g}	33.58^{d}	*	35.26
Exchange-able K (mg/kg)	79.10^{c}	80.48^{c}	38.85^{e}	72.29^{d}	39.32^{e}	116.34^{a}	81.87^{c}	108.91^{b}	*	35.20
Organic carbon; OC (%)	0.344^{a}	0.194^{e}	0.256^{c}	0.215^{d}	0.198^{e}	0.309^{b}	0.306^{b}	0.263^{c}	*	20.85
Soil pH	4.85^{d}	5.50^{b}	5.30^{c}	6.41^{a}	5.63^{b}	5.69^{b}	4.85^{d}	4.74^{d}	*	10.19
Electrical conductivity; EC (dS/m)	0.042^{a}	0.029^{d}	0.015^{g}	0.020^{f}	0.039^{b}	0.035^{c}	0.039^{b}	0.023^{e}	*	32.43
Cation exchange capacity; CEC (cmol/kg)	3.371^{b}	3.627^{b}	5.051^{b}	3.559^{b}	4.612^{b}	1.190^{c}	8.088^{a}	1.272^{c}	*	57.59
Bulk density (g/cm^3)	0.890^{bc}	0.960^{a}	0.910^{b}	0.910^{b}	0.950^{a}	0.950^{a}	0.860^{c}	0.855^{c}	*	4.59
Soil moisture (%)	3.860^{d}	4.420^{b}	3.300^{f}	3.550^{e}	4.150^{c}	5.880^{a}	3.415^{ef}	4.265^{bc}	*	19.48
Soil texture	sand	sand	sand	sand	sand	sand	sand	sand	-	-

Treatment: soil with no spent wash liquor applied (control), soil applied with spent wash liquor for 1 year (1Y), soil applied with spent wash liquor consecutively for 2 years (2Y), soil applied with spent wash liquor consecutively for 3 years (3Y), soil applied with spent wash liquor consecutively for 4 years (4Y), soil applied with spent wash liquor consecutively for 5 years (5Y), soil applied with spent wash liquor for consecutively 7 years (7Y) and soil applied with spent wash liquor consecutively for 8 years (8Y). In column, means followed by the same letter do not differ statistically from each other at p≤0.05 according to DMRT test.

Effect of Spent Wash Liquor on Bacterial Population in Sugar Cane Production System

The highest total count of bacteria was found in soil sample that taken from first year application of spent wash liquor for 2.11 x 10^6 cfu/g soil (Table 3). However, bacterial population tend to decrease when soil were applied with spent wash liquor consecutively for 2-7 years. Interestingly, after 8 years consecutively application of spent wash liquor, result showed increasing in the bacterial count as compared to sugar cane production system without any spent wash liquor application. Jintaridth et al., 2002 reported that application of spent wash liquor into soil showed increasing in bacterial and fungal populations at the rate of 40 m^3/1600 m^2 compared to treatment using chemical fertilizer. Moreover, Thitakamol and Kaewpinthong (2004) also found that, at the rice post-harvesting period, the number of bacteria increased at the application of 40 m^3/1600 m^2 of spent wash liquor and the number of fungi was increased at the using rate of 20 m^3/1600 m^2. The bacterial populations can change due to longer time of spent wash liquor application. The bacterial populations may be affected by changing in soil moisture and some certain soil nutrient such as potassium. The results revealed that significantly higher in soil moisture and exchangeable K value

were found in soil applied with spent wash liquor for 1 year (1Y) and soil applied with spent wash liquor consecutively for 8 years (8Y) (Table 2). These findings suggested that the growth and population of soil microorganisms can be influenced by chemical and physical properties of the soil and the availability of macro nutrient elements can limit bacterial population growth in a particular soil ecosystem.

Table 3 Effect of spent wash liquor on bacterial population

Treatment	Bacterial count (cfu/g soil)
Control	$1.45 \times 10^{6\,b}$
1Y	$2.11 \times 10^{6\,a}$
2Y	$1.48 \times 10^{6\,b}$
3Y	$7.07 \times 10^{5\,d}$
4Y	$1.08 \times 10^{6\,c}$
5Y	$8.67 \times 10^{5\,cd}$
7Y	$7.73 \times 10^{5\,d}$
8Y	$2.04 \times 10^{6\,a}$
F-test	*
CV (%)	40.97

Treatment: soil with no spent wash liquor applied (control), soil applied with spent wash liquor for 1 year (1Y), soil applied with spent wash liquor consecutively for 2 years (2Y), soil applied with spent wash liquor consecutively for 3 years (3Y), soil applied with spent wash liquor consecutively for 4 years (4Y), soil applied with spent wash liquor consecutively for 5 years (5Y), soil applied with spent wash liquor for consecutively 7 years (7Y) and soil applied with spent wash liquor consecutively for 8 years (8Y). In column, means followed by the same letter do not differ statistically from each other at $p \leq 0.05$ according to DMRT test.

CONCLUSION

The study of long-term effects of spent wash liquor on soil bacterial population in sugar cane production systems has shown that the highest total count of bacteria was found in soil sample that taken from first year application of spent wash liquor and then bacterial population tend to decrease when soil were applied with spent wash liquor consecutively for 2-7 years and raised up in soil sample that taken from eight years consecutively application of spent wash liquor. This finding suggested that bacterial populations may be affected by changing in soil physical and chemical properties such as soil moisture and some certain soil nutrient such as potassium. Therefore, an essential soil element for plant growth influences the microbial population as these nutrient elements are also needed for microbial growth and activity. However, the application of spent wash liquor need to be tested in longer time under farmer's field conditions before further recommendation can be given.

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