Physico-Chemical and Microbiological Changes in Soil Amended with Agro-Industrial Waste

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ABSTRACT

Laboratory experiments were conducted for 10, 30 and 60 days to test the effect of industrial brewer's waste on soil physico-chemical and biological properties. The treatment consists of industrial waste applied at the rate of 50g/3kg soil. The experiment was laid out in a completely randomised design with 3 replications. The soil physico-chemical properties, bacteria and fungi count, and biochemical activities were determined both in the treated and untreated (control) soil. All data were analysed using ANOVA. Soil physico-chemical properties increase significantly (P>0.05) with days of incubation in both treated and untreated soil but the treatment lower the soil pH when compared with the untreated (control) soil. Bacteria count for the treated soil ranged from 11.4 – 41.6 X 10^{-7} CFU/g while in the untreated soil the bacteria count ranged from 9.0 – 13.0 X 10^{-7} CFU/g. The fungi count also ranged from 5.0 – 13.7 X 10^{-7} CFU/g for the treated soil and 7.0 – 16.0 X 10^{-7} CFU/g for the untreated soil. The biochemical enzyme activities increase significantly (P>0.05) with days of incubation in the treated soil except amylase enzyme activity that gave steady increase with days of incubation in the untreated soil.

KeyWords: Physico-chemical properties, Microbiological changes, Agro-industrial waste

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INTRODUCTION

Waste (solid or liquid) are the major source of pollution. When generation is in large quantities, they pose environmental problems. Agro-industrial wastes like spent grain is a by-product of the brewery industry. The brewing industry generates relatively large amount of by-products and wastes. However, this agricultural products can be easily recycled and used (Grasser and Fadel, 1995).

Nutrients contents of spent grain are among the highest of all agro-industrial wastes. The use of spent grains as a soil amendment for cultivated soils to provide appreciable quantities of all important plant nutrients is documented (Mussatto, et. al., 2006 and Sims and Vance,2001). Research has shown that spent grains contain very high nitrogen content comparable with agro-industrial wastes like cocoa husk, rice bran and sawdust. It is rich in potassium, calcium, magnesium and sulphur and considerable amount of trace elements (Sommerfedt and Chang, 1992).

Biochemical reactions are important in nutrient cycling and are catalysed by soil enzymes (Tabatabai, 1982). Soil enzymes are constantly being synthesised, accumulated, inactivated and or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrient cycling (Makoi and Ndakidemi, 2008). All soils contain a group of enzymes that determine soil metabolic processes which in turn depend on its physical, chemical, microbiological and biochemical properties. This study therefore evaluate the effect of spent grain from Guiness company located at Lagos State of Nigeria as a soil amendment on physico-chemical properties, microbial population and enzyme activities of soil under laboratory condition.

MATERIALS AND METHODS

Bulk soil for the experiment was collected from the teaching and research farm of the University of Agriculture, Abeokuta, Ogun State at a depth of 0-20cm. The soil sample was passed through a 2mm sieve. A brewer industrial waste (spent grain) was collected from Guiness company at Agbara industrial estate, Ogun State. Three kilogram (3kg) soil were measure into different five litres bucket, 50g of the spent grain were applied to the soil. An homogenous 100g

of soil was measured into sample flask for analysis at 0, 10, 30 and 60days after application. The experiment was laid out in a complete randomised design with 3 replications. The treatments were moistened with water and incubated under laboratory condition.

Physico-chemical analysis:

The initial physicochemical analysis of the soil and of the spent grain before application was determined. The physico-chemical properties of the soil at 10, 30 and 60 days of application were also determined.

pH was measured using a soil:double distilled water suspension (in ratio of 1:5) by using a pH meter Unicam 9450, Orion model according to Sarangi et. al.(2001). Organic C was determined as described by Walkley and Black (1934), total nitrogen as described by Bremner (1966), total phosphorus was analysed by the Vanado-Molubdo phosphoric acid method using colorimeter Total K, Ca and Na were determined using flame photometer (AOAC,1984), total Mg, Pb, Fe, Cd, Cu, Zn and Mn were done using Buck 200 absorption spectrophotometer AAS (AOAC,1984).

Biological analysis:

All biological analyses were conducted on the moist samples. Total microbial population (bacteria and fungi) in the soil were determined by serial dilution technique and pour plate method. Bacteria were isolated and enumerated on nutrient agar and fungi were isolated and enumeration on potato dextrose agar. Plates were incubated at 35 ± 1^0C for 3 days for bacteria and fungi plates were incubated at 28+10C for 5 days. Colonies were counted and expressed as colony forming units (CFU) per gram. Isolates were purified and subcultured for identification. Biochemical characterisation was carried out according to the methods of Buchanan and Gibbons (1974) and Holding and Colle (1971). Fungal isolates were identified with reference to Gilman (1975) and Barnett et al., (1990).

Phosphatase activity was measured using the method of Eivazi and Tabatabai (1994). Buffered p-nitrophenyl phosphate solution was added to soil samples and incubated for 1h at 37^0C. The p-nitrophenol released by phosphomonoesterase activity was extracted and coloured with sodium hydroxide and determined photometrically at 400nm using a systronics spectrophotometer -106.

Dehydrogenase activity (µg formazan g soil $^{-1}$ h^{-1}) was determined using the method of (Casida et al. 1964), The incubation mixture contained 2 g fresh soil, 2 ml of 1% 2,3,5-triphenyl

tetrazolium chloride (TTC) and 0.5 ml of 1% glucose in screw cap test tube and was incubated at 32^0C for 24 h. Methanol was added (10 ml) and the resulting slurry was washed into a buchner funnel (Whatmann no 30, Whatmann, England). The absorbance was read at 484nm using a systronics spectrophotometer -106.and methanol as blank

Soil amylase and invertase activities (µg glucose g soil $^{-1}h^{-1}$) were determined according to the method of Mishra et al. (1979). The incubation mixture containing 3g of fresh soil, 0.2ml of toluene, 6ml of Sorensen's buffer (0.06M, pH 5.5) and 6ml substrate solution (1% of soluble starch for amylase and 5% sucrose for invertase) was sealed and incubated at 30^0C for 24h. A 3,5-dinitrosalicylic acid solution (2ml) was added to 1ml of supernatant of the incubated mixture and heated in water bath for 5min. at 37^0C. Colour was read at 540nm using a systronics spectrophotometer -106.

The method of Reese and Mandels (1963) was used to determine cellulase activity. About 5 ml of 1% carboxymethyl cellulose (CMC) in 0.1 M citrate buffer pH 5.5 was mixed with 5 ml of the enzyme solution. The reaction was incubated at room temperature for 1 hr. At the end of incubation period 5 ml of Dinitrosalicylic acid (DNSA) reagent was added to stop the reaction and to estimate the amount of reducing sugar released. Absorbance was read at 575nm using a systronics spectrophotometer -106.

Soil protease activity (µg throsine g soil^{-1} h^{-1}) was determined according to Spear and Ross (1975). The incubated mixture contained 2 g of fresh soil. 0.2 ml of toluene, 10 ml Tris-HCl buffer (0.1 M, pH 8.1) containing 1% sodium caseinate and was incubated at room temperature for 2 h. A trichloroacetic acid (TCA) solution (17.5 w/v) was added (4 ml) and the mixture was centrifuged. A 2 ml solution of supernatant liquid was treated with 3 ml of sodium carbonate solution (1.4 M) and 1 ml of folin's reagent (33% w/v) with rapid swirling. The colour was read after 30 min. at 700nm using a systronics spectrophotometer-106

RESULTS AND DISCUSSION

The physico-chemical propertites of soil before treatment is shown in table 1. The soil is slightly acidic and sandy with low level of organic carbon, nitrogen, phosphorus and exchangeable bases. The physico-chemical propertities of the brewer's spent grain before mixing with soil is also shown in table 1. The brewer's spent grain is alkaline in nature with pH of 6.85

and contain both micro and macro nutrients in varying proportions. The organic carbon and nitrogen is high, this is in agreement with Sommerfedt and Chang 1992.

The physico-chemical properties of treated and untreated soils changes with days of incubation. The pH of treated soil increased up to 30^{th} day and later decreased by the 60^{th} day (Table 2). This support the findings of Olayinka (2001). The apparent increase could be attributed to the buffering ability of the brewer's spent grain. The decrease in soil pH could probably due to acidification resulting from nitrification (Soretire and Ojo, 2008). The spent grain applied was able to neutralise the pH of the soil to a certain level table 2. Addition of the brewer's spent grain increase the mineral elements Na^{2+}, K^{+}, Mg^{2+}, Ca^{2+}, P, OC, N_2, Fe, Zn, Cu, Mn, Pb and cd appreciable as the days of incubation increase. There was significant increase in organic carbon (OC), N_2 and Mn as a result of the treatment (Table 2).Brewer's spent grain had significant ($p<0.05$) effect on the bacteria and fungi count (table 3). Both treated and untreated bulk soil significantly increased as days of incubation increases but the treatment was able to increase the bacteria count as compared to the fungi count. Microbial biomass in soil is related to the soil's organic C content (Sparling et al., 1986). Hasebe et al. (1985) observed the greatest microbial biomass in soil treated with organic manure. Similar relationship was also found between organic C and microbial population when soil was treated with industrial waste in this experiment

The following bacteria and fungi were isolated and identified from the bulk soil treated and untreated with brewer's spent grain table 4. The following bacteria and fungi were absent in the untreated soil but present in the treated soil. *Streptococcus sp., Pseudomonas nigrificans, Proteus morganii, Micrococcus leteus* and *Saccharomyces cerevisae*. The presences of these organisms in the treated soil could be from the brewer's spent grain. Biochemical reactions are important in nutrient cycling and catalysed by soil enzymes (Tabatabai, 1982). Soil enzymes are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or be released into soil solution from dead cells (Tabatabai, 1994).

Table 1: Physico-chemical properties of soil before treatment and of the brewer's spent grain before mixing with the soil.

Parameters	Soil	Spent grain
Ph	6.5	6.85
Na(Cmol/kg)	0.24	0.61
K(Cmol/kg)	0.37	0.77
Mg_2(Cmol/kg)	3.60	4.31
Ca_2(Cmol/kg)	1.75	2.20
P(mg/kg)	3.24	7.8
OC(g/kg)	130	180
N_2(g/kg)	5.2	9.4
Fe(mg/kg)	23.70	28.12
Zn(mg/kg)	23.15	28.59
Cu(mg/kg)	3.21	6.03
Mn(mg/kg)	52.4	74.7
Pb(mg/kg)	6.5	11.89
Cd(mg/kg)	0.25	0.15

Table 2: Physico-chemical properties of the soil treated with brewer's spent grain at different days of incubation.

Parameters	CONTROL	10days	30days	60days	Mean	LSD (p= 0.05)
pH	6.5	6.1	6.3	6.2	6.3	0.3
Na^{+}(Cmol/kg)	0.24	0.26	0.40	0.33	0.31	0.07
K^{+}(Cmol/kg)	0.37	0.41	0.64	0.76	0.55	0.30
Mg^{2+}(Cmol/kg)	3.60	3.80	3.84	3.93	3.79	0.42
Ca^{2+}(Cmol/kg)	1.75	1.82	1.91	1.89	1.84	0.11
P (mg/kg)	3.24	3.76	4.09	4.20	3.82	0.55
OC (mg/kg)	130	152	179	182	160.75	23
N_2(mg/kg)	5.2	10.3	15.2	16.6	11.83	6.25
Fe (mg/kg)	23.7	23.6	25.0	25.1	24.35	1.92
Zn (mg/kg)	23.15	28.20	30.60	30.10	28.01	2.53
Cu (mg/kg)	3.21	6.20	6.10	6.20	5.43	1.05
Mn (mg/kg)	52.4	74.9	77.8	76.5	70.4	3.25
Pb (mg/kg)	6.5	11.8	11.9	11.8	10.5	0.37
Cd (mg/kg)	0.25	0.2	0.2	0.2	0.2	0.11

Table 3: Bacteria and fungi count in the soil treated with brewer's spent grain at different days of incubation.

Days of treatment	Bacteria count (10^7cfu/g)	Fungi count(10^7cfu/g)
10days	11.4^{c}	$5.0b^{c}$
30days	18.6^{b}	9.0^{b}
60days	41.6^{a}	13.7^{a}
Control	10.0^{c}	6.0^{b}

Means followed by the same letter within the same row are not significantly different from each other by DMRT (p=0.05).

Table 4: Bacteria and fungi isolated and identified from the treated and untreated soil sample.

Microorganisms	Treated soil	Untreated soil
Bacillus cereus	+	+
Streptococcus sp.	+	-
Staphylococcus aureus	+	+
Pseudomonas aeruginosa	+	+
Pseudomonas nigrificans	+	-
Proteus morganii	+	-
Micrococcus leteus	+	-
Streptomyces sp.	+	+
Penicillium chrysogenum	+	+
Fusarium oxysporum	+	+
Aspergillus niger	+	+
Aspergillus flavus	+	+
Saccharomyces cerevisae	+	-

+ = Present - = Absent

Table 5: Biochemical activities in the soil treated with brewer's spent grain at different days of incubation.

Parameters	Control	10 da*ys*	30 days	60 days
Cellulase activity (μg g soil $^{-1}$ h^{-1})	15.0^{c}	18.3^{b}	38.0^{a}	34.6^{a}
Amylase activity (μg glucose g soil $^{-1}$ h^{-1})	10.0^{c}	17.0^{b}	35.3^{a}	33.0^{a}
Dehydrogenase activity (μg formazan g soil $^{-1}$ h^{-1})	25.0^{b}	29.16^{b}	44.7^{a}	49.6^{a}
Phosphatase activity (μg p-NP g soil $^{-1}$ h^{-1})	23.1^{bc}	26.6^{b}	54.0^{a}	57.3^{a}
Protease activity (μg tyrosine g soil $^{-1}$ h^{-1})	15.0^{c}	21.6^{b}	43.0^{a}	45.0^{a}
Invertaseactivity (μg p-NP g soil $^{-1}$ h^{-1})	7.0^{d}	11.6^{c}	25.0^{b}	32.3^{a}

Means followed by the same letter within the same column are not significantly different from each other by DMRT (p=0.05).

In Table 5, the cellulase and amylase activities of the treated soil increased up to 30th day of incubation and decreased to 60th day but for the untreated soil the cellulase and amylase activities increased throughout the days of incubation. The protease, dehydrogenase, phosphatase and invertase activities increased throughout the days of incubation both in the treated and untreated soil. Similar results were obtained by Nur et. al.(2006) in protease, phosphatase, urease and dehydrogenase enzyme activities when soil was amended with organic manure. The increase in protease activity may be due to the accumulation of proteins in the soil. Protease and urease enzymes are involved in the N cycle and are therefore important for what might be termed the economy of N in a soil (Nur et al., 2006). Phosphatases which play a significant role in the hydrolysis of organic P compounds, occur in soils as endocellular enzymes or in a free state as well as being intimately sorbed to organic, silt and clay colloids (Burns, 1982). The highest enzyme activity is seen in phosphatase enzyme.

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