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Effect of application of algal Biochar on soil enzymes

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Abstract
Biochar, a product of the pyrolysis of organic material, has received wide attention as a means to improve soil fertility and crop productivity, absorb pollutants in soil, and sequester carbon to mitigate climate change. Little information exists on the short- and longer-term effects of biochar on soil microbial communities and enzyme activities, relative to other organic amendments such as manure. The present study is mainly focused on algal biochar effects on soil enzymes such as phosphatase, dehydrogenase and soil microbial biomass carbon in soil such as total organic carbon (TOC) and humic acid in potted soil. The results showed that the soil acid phosphatase activity and alkaline phosphates increased in Biochar treatment. During the pot culture experiment, the acid phosphatase level was higher in mixture of Biochar and FYM (1:2) ratio. Dehydrogenase activity was noted higher in a sample treated with FYM alone and lesser in treatment of 2g of Biochar. Soil microbial biomass carbon (SMBC) on potted soil Biochar and FYM mixture with 1:2 ratio has shown higher levels of SMBC content. Humic acid percentage has increased up to 47% with the addition of Biochar 2g + FYM2g (1:1) ratio.

Keywords: bio-char, micro algae, soil enzymes, phosphatase, dehydrogenase, Total organic carbon

Introduction

Biochar has long been used to date archaeological deposits by quantifying its carbon-14 decay **(Arnold and Libby 1951),** since Biochar and other more aromatic black carbons persist in the environment longer than any other form of organic carbon. Finely divided Biochar has even (**Sombroek et al., 2003)** remained in soils in humid tropical climates, such as the Amazon, for thousands of years , resisting the rapid rates of mineralization common to organic matter in these environments and producing a distinct black colour. Such Biochar is typically older than any other form of carbon in soils **(Pessenda et al., 2001).** Despite this high level of resistance, we know that Biochar will ultimately be mineralized to CO_2; otherwise, soil organic matter would be dominated by Biochar accumulated over geological time scales **(Goldberg etal., 1985).** Nutrients are retained in the soil and remain available to plants, mainly by adsorption to minerals and organic matter. While we are usually unable to change the mineralogy of a given soil, we can change the amount of soil organic matter. Typically, the ability of soils to retain cations in an exchangeable form available to plants (cation exchange capacity [CEC]) increases in proportion to the amount of soil organic matter, and this holds for Biochar as well. However, Biochar has an even greater ability than other soil organic matter to adsorb cations per unit carbon **(Sombroek et al*.,* 2003**), due to its greater surface area, greater negative surface charge, and greater charge density **(Liang et al., 2006).** In contrast to other organic matter in soil, Biochar also appears to be able to strongly adsorb phosphate, even though it is an anion. Any bio-energy production will lead to a maximum removal of biomass from land. This highly extractive procedure potentially leads to widespread soil degradation, with negative effects on soil productivity, habitats, and off-site pollution. Pyrolysis, coupled with an organic matter return through Biochar applications, addresses this dilemma, because about half of the original carbon can be returned. In addition, the Biochar is extremely effective in restoring soil fertility. Several overviews have presented evidence for the improvement of soil productivity by Biochar. The extraordinary persistence of Biochar makes it possible to extend its application beyond the area from which the biomass was obtained to generate the bio-energy. Once applied to a certain location, additions do not need to be repeated annually, as exemplified by the persistently high fertility of Amazonian Dark Earths over several hundred to thousands of years, as well as by remnants of historic charcoal production **(Glaser *et al.,* 2002, Lehmann and Rondon, 2006).** This allows the application to areas which were not harvested for bio-energy production, but which would benefit from improved soil fertility or reduced pollution by agro-chemicals.

Materials and methods

Enzymatic assay in potted soil Acid and Alkaline Phosphates was performed by the author (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977)**.** 1 gram of soil (<2 mm) was taken in a 50 ml Erlenmeyer flask and added 0.2 ml of toluene, 4 ml of MUB (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline

phosphatase), then 1 ml of p-nitrophenyl phosphate solution was made in the same buffer, and swirled the flask for a few seconds for mix the contents. Stopper flask was removed, and it was placed in an incubator at 37^{o}C. After an 1hour, the stopper was removed and added 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH solution, swirled the flask for a few seconds, then the soil suspension was filtered through a Whatman No. 2 V folded filter paper. Yellow color intensity of the filtrate with a Klett-Summerson was measured using photoelectric colorimeter. P-nitrophenol content of the filtrate by reference was calculated and a calibration graph was plotted from the results obtained with standards containing 0, 10, 20, 30, 40, and 50µg of p-nitrophenol, respectively. A graph was prepared in a diluted 1 ml of the standard p-nitrophenol solution in a 100 ml volumetric flask and the solution was mixed thoroughly. Then pipette out 0, 1, 2, 3, 4 and 5 ml aliquots of this diluted standard solution in 50 ml Erlenmeyer flasks and adjusted to the volume of 5 ml by addition of water, and proceeded as described for p-nitrophenol analysis of the incubated soil sample (i.e., add 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M of NaOH, mix and filter the resultant suspension). Color intensity of the filtrate was exceeded that of 50µg of the p-nitrophenol standard, an aliquot of the filtrate was diluted with water until the colorimeter reading falls within the limits of the calibration graph. Controls were performed with each soil analysis to allow for color not derived from p-nitrophenol released by phosphatase activity. Controls were performed by the following procedure described for assay of phosphatase activity, an addition of 1 ml of PNP solution after the additions of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH (i.e., immediately before filtration of the soil suspension).

Estimation of Humic acid fraction (HA)

40 gram of acid washed (0.1N HCL) soil sample was taken in a polyethylene centrifuge bottle and 200 ml of 0.5N NaOH was added. The mixture was shaken for 12hours on a mechanical shaker and centrifuged at 3000 rpm for 10 minutes. Dark coloured supernatant liquid was filtered and the pH of the filtrate had adjusted to 1.0 with conc. HCL. Further, 200 ml of 0.5N NaOH was added into the residual soil, shaken, centrifuged and filtered **(Aguilera *et al.,* 1997).** The residue was dispersed in 200 ml distilled water, centrifuged and the supernatant liquid added into the previous extracts and the pH had adjusted to 1.0 with concentrated HCL and the humic acid was allowed to settle down. The supernatant liquid in the acidified extract is fulvic acid and it was siphoned off. The suspension was transferred to a polyethylene bottle and the HA was centrifuged off at 3000 rpm for 10 minutes. HA was re-dissolved in 0.5N NaOH and re-precipitated with conc. HCL. This purification was repeated several times and the supernatant liquid in each case transferred to the original acid filtrate. HA was washed with distilled water until free of chloride. HA was extracted and dried in a rotary evaporator and ground to a fine powder. This was weighed and reported as a percentage of organic matter.

Estimation of Dehydrogenase Assay

Thoroughly mixed 20 gram of air dried soil (<2mm) and 0.2 gram of $CaCO_3$, and 6g of this mixture in each of three test tubes were taken. 1 ml of 3% aqueous solution of TTC and 2.5 ml of distilled water each tube was added. This amount of liquid would be sufficient that a small amount of three liquid appears at the surface of the soil after mixing. Contents of each tube were mixed with a glass rod. The tubes were stopper and incubated at 37^{o}C. After 24 hours, the stopper was removed and added 10 ml of methanol, and stoppers the tube and shakes it for 1 minute. Un-stopper the tube, and filter the suspension through a glass funnel plugged with absorbent cotton in a 100 ml volumetric flask. The tubes were washed with methanol and quantitatively transferred the soil to the funnel, and then added additional methanol (in 10 ml portions) to the funnel until the reddish color disappeared from the cotton plug. Filtrate was diluted in 100 ml volume with methanol. The intensity of the reddish color was measured by using a spectrophotometer at a wavelength of 485nm and a 1cm cuvette with methanol as a blank. The amount of TPF was produced by reference to a calibration graph prepared from TPF standards and calculated. The graph was prepared with dilute 10 ml of TPF standard solution to 100 ml with methanol (100µg of TPF ml-^{1}). Pipette out 5, 10, 15 and 20 ml aliquots of this solution into 100 ml volumetric flasks (500, 1000, 1500, and 2000µg of TPF 100 ml^{-1}), make up the volumes with methanol, and it was mixed thoroughly. The intensity of the red colour of TPF as described for the samples and it was measured. The absorbance reading against the amount of TPF in the 100 ml standard solution was plotted. The concentration of dehydrogenase in the sample was obtained from the standard graph and drawn by using Tri Phenyl Formazan (TPF) as standard and expressed as µg of TPF released per g soil on dry weight basis **(Casida et al., 1964)**

Estimation of soil microbial biomass carbon (SMBC) was performed by this author (Jenkinson and Powlson, 1976). Microbial biomass carbon of soil sample was determined by the fumigation-incubation technique (FIT). 10 gram of soil was weighed in 100 ml beaker and placed in a 250 ml air tight plastic container ,which about 5 ml of water was added. 100 ml of ethanol free chloroform was prepared, immediately before fumigation by passing 100 ml of chloroform through a glass column containing 75g of basic aluminium oxide. The fumigation was carried out with ethanol free chloroform for 20 hours at 25°C. After fumigation, chloroform was removed by repeated

evacuations. After the removal of chloroform, the beaker holding the soil was returned to the air tight container together with a scintillation vial holding 5 ml of 0.5N NaOH. Soil samples were inoculated with a pinch of fresh soil in the respective treatments and the soil was incubated for a further period of 10 days at 25°C. Evolved CO_2 was determined by titrating the alkaline traps with 0.5N HCL after precipitation of CO_3^2 -with 50% barium chloride and using phenolphthalein as an indicator.
Soil Microbial biomass C was calculated using the formula: SMBC (µg/g) = (F_c-UF_c) / K_c
Where, F_c - CO_2 from fumigated soil; UF_c - CO_2 from un-fumigated soil; K_c - 0.45 (**Jenkinson and Ladd, 1981**).

Estimation of Total Organic Carbon (TOC)
Total organic carbon (TOC) was measured, based on the Walkley-Black chromic acid wet oxidation method. Oxidisable matter in the soil was oxidized by 1N $K_2Cr_2O_7$ solution. The reaction was assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume of the dichromate. The remaining dichromate was titrated with ferrous sulphate. The titer was inversely related to the amount of C present in the soil sample. 0.5 gram sample to 250 ml conical flask was measure accurately and added 10 ml 1N $K_2Cr_2O_7$ and swirled the flask gently upto disperse the soil in the solution. 20 ml of concentrated H_2SO_4 was measured and directing the stream into the suspension. Immediately swirled the flask until the soil and the reagent mixed. It was set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without sample) had run in the same way to standardize the $FeSO_4$ solution. It was cooled (20–30 minutes), diluted to 200 ml with de-ionised water and proceeded with the $FeSO_4$ titration using 10 ml of Phosphoric acid and 1 ml of Diphenylamine indicator solution. The solution was titrated with 0.5N $FeSO_4$ and the end point is approached, the solution took a dull greenish color at the beginning and then changed to a dark green color.

Calculations

$$\text{Organic Carbon (\%)} = \frac{0.003g \times N \times 10\ ml \times (1 T/S) \times 100}{W}$$

Where:
N = Normality of $K_2Cr_2O_7$ solution
T = Volume of $FeSO_4$ used in sample titration (ml)
S = Volume of $FeSO_4$ used in blank titration (ml)
W = Sample weight (g)

Results

Soil enzymes analysis in tomato plant pot soil

Phosphatase analysis:

The procedures described for assay of phosphomonoesterase activities are based on colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphate activity) sodium p-nitrophenyl phosphate solution and toluene. The colorimetric procedure used for estimation of p-nitrophenol is based on the fact that alkaline solutions of this phenol have a yellow color (acid solutions of p-nitrophenol and acid and alkaline solutions of p-nitrophenyl phosphate are colorless). The $CaCl_2$-NaOH treatment described for extraction of p-nitrophenol after incubation for assay of acid and alkaline phosphatases serves (i) to stop phosphatase activity, (ii) to develop the yellow colour used to estimate this phenol, and (iii) to give quantitative recovery of p-nitrophenol from soils. Results were given in Tables 1 and 2 and Figures 1 and 2.
In an incubation experiment, the soil acid phosphatase activity and alkaline phosphatase was increased in Biochar alone (T3) treatment. Where as in pot culture experiment the acid phosphatase activity was increased in T6 and alkaline phosphatase was increased in T2 and T6 than other treatments on 45th day of soil analysis.

Table.1-. Analysis of acid phosphatase activity on potted soil

Treatments	**0th day**	**22nd day**	**45th day**
T1 Control (Soil)	2.124	2.142	**2.132**
T2 (Soil+FYM 2g)	2.150	**2.150**	2.152
T3 (Soil+Biochar 2g)	2.144	**2.151**	2.152
T4 (Soil+Biochar 4g)	2.140	**2.177**	2.158
T5 (Soil+FYM 2g+Biochar 2g)	2.170	2.175	**2.164**
T6 (Soil+FYM 2g+Biochar 4g)	2.164	2.183	**2.166**

Table.2. Analysis of alkaline phosphatase activity on potted soil

Treatments	0^{th} day	22^{nd} day	45^{th} day
T1 Control (Soil)	2.153	2.164	**2.166**
T2 (Soil+FYM 2g)	2.137	**2.198**	2.171
T3 (Soil+Biochar 2g)	2.133	**2.171**	2.165
T4 (Soil+Biochar 4g)	2.141	**2.176**	2.166
T5 (Soil+FYM 2g+Biochar 2g)	2.139	2.166	**2.169**
T6 (Soil+FYM 2g+Biochar 4g)	2.126	2.173	**2.175**

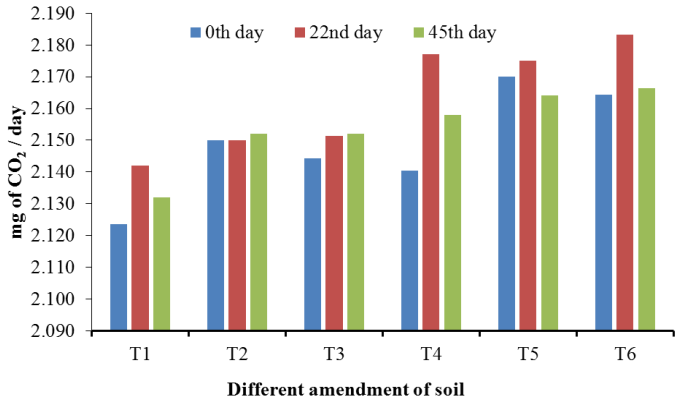

Figure.1. Analysis of acid phosphatase activity on potted soil

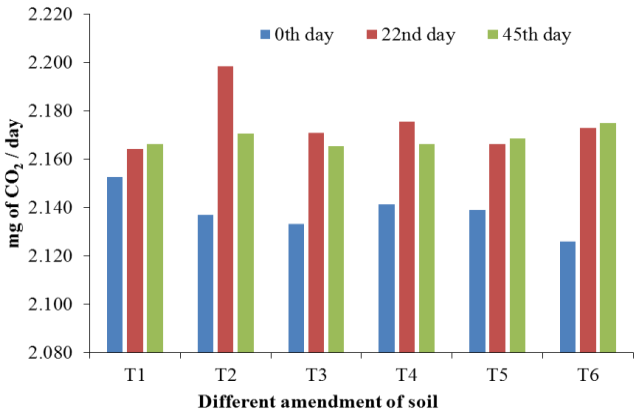

Figure.2. Analysis of alkaline phosphatase activity on potted soil

Dehydrogenase Assay:

Thoroughly mixed 20 gram of air dried soil (<2mm) and 0.2 gram of $CaCO_3$, and 6g of this mixture in each of three test tubes were taken. 1 ml of 3% aqueous solution of TTC and 2.5 ml of distilled water each tube was added. This amount of liquid would be sufficient that a small amount of three liquid appears at the surface of the soil after mixing. Contents of each tube were mixed with a glass rod. The tubes were stopper and incubated at 37^{o}C. After 24 hours, the stopper was removed and added 10 ml of methanol, and stoppers the tube and shakes it for 1 minute. Un-stopper the tube, and filter the suspension through a glass funnel plugged with absorbent cotton in a 100 ml volumetric flask. The tubes were washed with methanol and quantitatively transferred the soil to the funnel, and then added additional methanol (in 10 ml portions) to the funnel until the reddish color disappeared from the cotton plug. Filtrate was diluted in 100 ml volume with methanol. The intensity of the reddish color was measured by using a spectrophotometer at a wavelength of 485nm and a 1cm cuvette with methanol as a blank. The amount of TPF was produced by reference to a calibration graph prepared from TPF

standards and calculated Results were given in Table 3 and Figure 3. The dehydrogenase activity was high in treatment T2 (T2=0.0580µg) on potted soil than other treatments was less.

Table.3. Analysis of Dehydrogenase activity on potted soil

Treatments	0th day	22nd day	45th day
T1 Control (Soil)	0.0463	0.0437	0.0367
T2 (Soil+FYM 2g)	0.0403	0.0517	0.0580
T3 (Soil+Biochar 2g)	0.0500	0.0427	0.0107
T4 (Soil+Biochar 4g)	0.0520	0.0323	0.0213
T5 (Soil+FYM 2g+Biochar 2g)	0.0597	0.0410	0.0387
T6 (Soil+FYM 2g+Biochar 4g)	0.0620	0.0413	0.0447

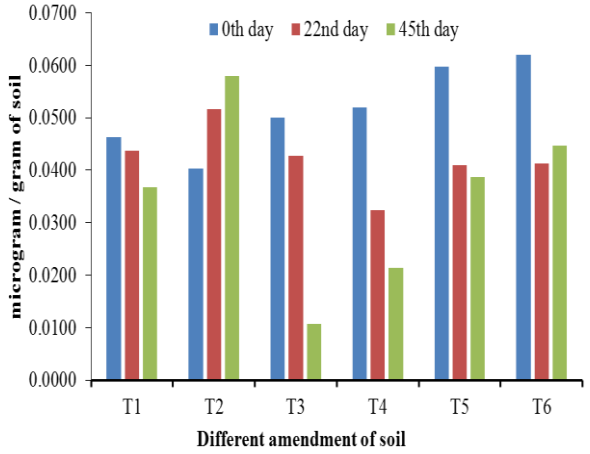

Figure.3. Analysis of Dehydrogenase activity on potted soil

Humic acid fraction (HA):

40 gram of acid washed (0.1N HCL) soil sample was taken in a polyethylene centrifuge bottle and 200 ml of 0.5N NaOH was added. The mixture was shaken for 12hours on a mechanical shaker and centrifuged at 3000 rpm for 10 minutes. Dark coloured supernatant liquid was filtered and the pH of the filtrate had adjusted to 1.0 with conc. HCL. Additional 200ml of 0.5N NaOH was added to the residual soil, shaken, centrifuged and filtered. The residue was dispersed in 200 ml distilled water, centrifuged and the supernatant liquid was added to the previous extracts and the pH had adjusted to 1.0 with conc. HCL and the humic acid was allowed to settle down.

The supernatant liquid in the acidified extract is fulvic acid and it was siphoned off. The suspension was transferred to a polyethylene bottle and the HA was centrifuged off at 3000 rpm for 10 minutes. HA was re-dissolved in 0.5N NaOH and re-precipitated with conc. HCL. This purification was repeated several times and the supernatant liquid in each case was transferred to the original acid filtrate. HA was washed with distilled water until free of chloride. The HA extracted was dried in a rotary evaporator and ground to a fine powder. This was weighed and reported as percentage of organic matter. Results were given in Table 4 and Figure 4. Analysis of humic acid on potted soil was carried out for upto 45 days in different amendments such as Biochar and FYM at different ratio. The result shows in treatment T3 and T5 the humic acid content is high.

Table.4. Analysis of Humic acid on potted soil

Treatments	0th day	45th day
T1 Control (Soil)	0.0798	0.0658
T2 (Soil+FYM 2g)	0.0607	0.0789
T3 (Soil+Biochar 2g)	0.1304	0.1258
T4 (Soil+Biochar 4g)	0.0611	0.0887
T5 (Soil+FYM 2g+Biochar 2g)	0.0800	0.1398
T6 (Soil+FYM 2g+Biochar 4g)	0.0680	0.1011

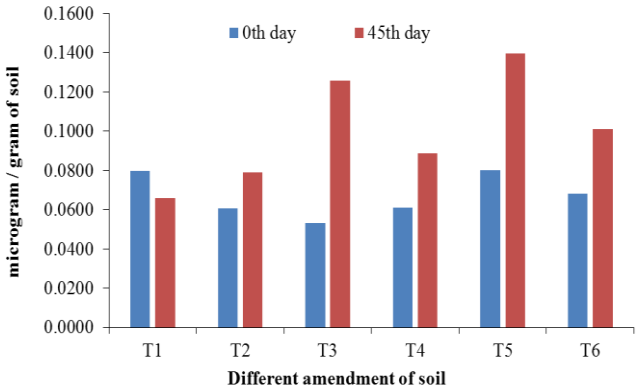

Figure.4. Analysis of humic acid on potted soil

Estimation of soil microbial biomass carbon (SMBC):

Microbial biomass carbon of soil samples was determined by the fumigation-incubation technique (FIT). 10g soil was weighed into 100 ml beaker and placed in a 250 ml air tight plastic container into which about 5 ml of water was added. 100 ml of ethanol-free chloroform was prepared, immediately before fumigation by passing 100 ml of chloroform through a glass column containing 75 grams of basic aluminium oxide. The fumigation was carried out with ethanol free chloroform for 20 hours at 25°C. After fumigation, chloroform was removed by repeated evacuations. After the removal of chloroform, the beaker holding the soil was returned to the air tight container together with a scintillation vial holding 5 ml of 0.5N NaOH. Soil samples were inoculated with a pinch of fresh soil of respective treatments and the soil was incubated for a further period of 10 days at 25°C. Evolved CO_2 was determined by titrating the alkaline traps with 0.5N HCl after precipitation of CO_3^{2} -with 50% barium chloride and using phenolphthalein as indicator. Microbial biomass C was calculated using the formula:

SMBC (µg/g) = (F_c-UF_c) / K_c

Where, F_c-CO_2 from fumigated soil; UF_c - CO_2 from unfumigated soil; K_c-0.45 (Jenkinson and Ladd, 1981). Results were given in Table 5 and fig 5. Analysis of soil microbial biomass carbon (SMBC) on potted soil was carried out for upto 45 days in different amendments such as Biochar and FYM at different ratio. The result shows in treatment T6 SMBC content is very high.

Table.5. Analysis of soil microbial biomass carbon on potted soil

Treatments	0th day	45th day
T1 Control (Soil)	0.888889	0.222222
T2 (Soil+FYM 2g)	0.666667	3.333333
T3 (Soil+Biochar 2g)	5.555556	1.77778
T4 (Soil+Biochar 4g)	3.555556	5.11111
T5 (Soil+FYM 2g+Biochar 2g)	6	9.111111
T6 (Soil+FYM 2g+Biochar 4g)	5.777778	11.11111

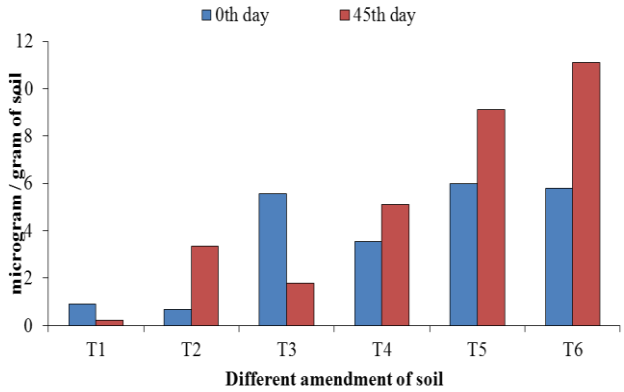

Figure.5. Analysis of soil microbial biomass carbon on potted soil

Estimation of total organic carbon in Soil:

Total organic carbon (TOC) was measured, based on the Walkley-Black chromic acid wet oxidation method. Oxidisable matter in the soil was oxidized by 1N $K_2Cr_2O_7$ solution. The reaction was assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume of the dichromate. The remaining dichromate was titrated with ferrous sulphate. The titer was inversely related to the amount of C present in the soil sample. 0.5 gram sample to 250 ml conical flask was measure accurately and added 10 ml 1N $K_2Cr_2O_7$ and swirled the flask gently up to disperse the soil in the solution. 20 ml of concentrated H_2SO_4 was measured and directing the stream into the suspension. Immediately swirl the flask until the soil and the reagent was mixed. It was set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without sample) had run in the same way to standardize the $FeSO_4$ solution. It was cooled (20–30 minutes), diluted to 200 ml with de-ionised water and proceeded with the $FeSO_4$ titration using 10 ml of Phosphoric acid and 1 ml of Diphenylamine indicator solution. The solution was titrated with 0.5N $FeSO_4$ and the end point is approached, the solution took a dull greenish color at the beginning and then changed to a dark green color. Results were given in Table 6 and Figure 6. Analysis of soil total organic carbon (TOC) on potted soil was carried out for upto 45 days in different amendments such as Biochar and FYM at different ratio. The result shows in treatment T6 TOC % content is high.

Table.6. Analysis of soil total organic carbon on potted soil

Treatments	0^{th} day	45^{th} day
T1 Control (Soil)	2.2	3.3
T2 (Soil+FYM 2g)	1.76	5.72
T3 (Soil+Biochar 2g)	3.52	6.6
T4 (Soil+Biochar 4g)	8.36	12.1
T5 (Soil+FYM 2g+Biochar 2g)	12.76	13.2
T6 (Soil+FYM 2g+Biochar 4g)	16.06	17.38

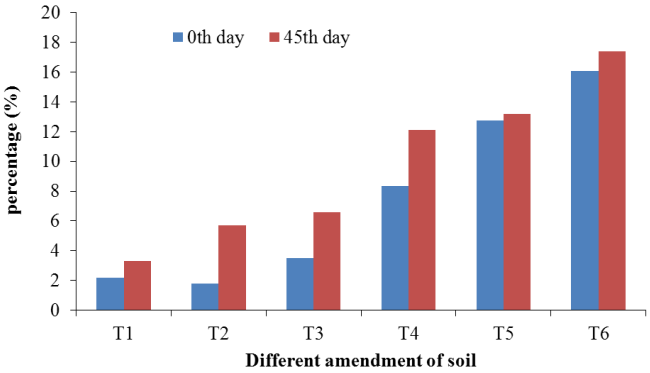

Figure.6. Analysis of total soil organic carbon on potted soil

Discussion

Effect of Biochar in phosphatase enzyme activity:

In an incubation experiment, the soil acid phosphatase activity and alkaline phosphatase increased in Biochar unique treatment. In pot culture experiment the acid phosphatase level was higher in Biochar and FYM mixture with 1:2 ratio and alkaline phosphatase was higher after the addition of FYM alone, due to above addition overall phosphatase activities increased with an increase of crop residue content. Soil total Phosohorus was correlated negatively with phosphodiesterase activity and positively with other phosphatase activities. Organic P had a positive correlation with the acid phosphomonoesterase activity, but a negative correlation with phosphodiesterase activity. Available P had no significant correlation with phosphatase activities. **Wang, *et al.,* (2011)** had suggested that no-till and residue input could increase soil P contents and enhance the activities of phosphatase.

Effect of Biochar in dehydrogenase activity:

The dehydrogenase activity was higher in a sample treated with FYM alone and dehydrogenase amount was lesser in treatment of 2g of Biochar alone. Dehydrogenase is considered an indicator of overall microbial activity because it occurs intracellularly in all living microbial cells. The use of dehydrogenase activity as an index of

overall microbial activity has been suggested by many authors **(Alef *et al*., 1995 Casida *et al*., 1964; Lenhard, 1956; Nannipieri *et al*., 1990)**. It can be assumed that the quality of the soil corresponds to the soil microbial activity. Therefore, one can expect a relationship between the activity of dehydrogenases and the physico-chemical properties of soils, which determine their quality, such as pH, sorption capacity, degree of base saturation *etc.* The measurement of this enzyme was used to confirm that the stress applied was strong enough to kill living microbes and denature their intracellular enzymes, but not necessary affect stabilized extracellular enzymes. The soils had generally the higher dehydrogenase activity than the other positions at all landscape positions. In all positions, except for shoulder, dehydrogenase activity was greater macro aggregates of <1 mm than in the other macro aggregate size **(Kussainova *et al.,* 2013).**

Effect of Biochar in soil microbial biomass carbon:
Analysis of soil microbial biomass carbon (SMBC) on potted soil Biochar and FYM mixture with 1:2 ratios showed higher level of SMBC content. The change in MBC reflects the process of microbial growth, death and organic matter degradation. Our results showed that MBC increased with Biochar amendment compared to CK, which suggested that microbial growth could be accelerated by Biochar addition. Reported Biochar effects on soil MBC are quite inconsistent. Several studies found that there was no significant effect of Biochar amendment on soil MBC **(Zavalloni *et al*., 2011)**. Dempster *et al* found that MBC significantly decreased with Biochar addition while MBN was unaltered in a coarse textured soil, and others observed the same positive effects of Biochar addition on microbial biomass as ours **(Dempster *et al*., 2012, Lehmann *et al*., 2011, Liang *et al*., 2010, Kolb *et al*., 2008)** Moreover, a positive linear relationship between microbial biomass and Biochar concentration was also observed in a highly weathered soil (Steiner *et al*., 2008). Biochar type was thought as the driving parameter for any effects on soil microbial biomass, community, and activity **(Steinbeiss *et al*., 2009, Lehmann *et al*., 2009)**

Effect of Biochar in total organic carbon and humic acid in potted soil:
Total organic carbon (TOC) % on potted soil, the result showed that in the treatment 1:2 ratio additions of Biochar 4 g and FYM2g, TOC 17.38 % was observed. The cultivation, crop rotation, residue and tillage management, fertilization and monoculture affect soil quality, soil organic matter (SOM) and carbon transformation. SOM is not only a source of carbon but also a sink for carbon sequestration **(Liu *et al.,* 2006).** Carbon sequestration has the potential to offset fossil fuel emissions by 0.4 to 1.2 Giga-tons of Carbon per year or 5 to 15% of the global fossil-fuel emissions (Guruprasad, *et al.,* 2012). The soil organic carbon (SOC) markedly increased with an increase in rate of application of Biochar. And suggest that the Biochar has great potential for carbon sequestration in soil. While marked increase in soil microbial biomass carbon and humic and fulvic acid fraction of SOC was evident during incubation **(Shenbagavalli, *et al.,* 2012).** The humic acid percentage increased to 47% in addition of (Biochar 2g and FYM2g) 1:1 ratio.

Conclusion
The utilization of biochar from *chlorella* improves the physicochemical characteristics of the plant, dehydrogenase activity was increased, FYM mixture with 1:2 ratio showed higher level of soil microbial biomass carbon, humic acid percentage also increased. The application of biochar from micro algae an environmental friendly technology for the implementation of biochar could be used as potential organic fertilizers. The microalgal biochar is cost effective technique. However, practical field studies of the biochar on various soils and crops are necessary to understand the sustainability of biochar as a soil amendment.

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