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INFLUENCE OF PAPER INDUSTRY EFFLUENTS ON SOIL CELLULASE AND AMYLASE ACTIVITIES

LAVUDI SAIDA * AND KONDA KINDI VENKATESWAR REDDY

Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad 500 085, Telangana, India

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Abstract–Release of pulp and paper industry effluents on to the agricultural lands causes an indicative charge in nutrient cycling and organic matter processing. In the present study, pulp and paper mill effluent discharged soil (test) and undischarged soil (control) were collected from the surrounding areas of pulp and paper mill industry. The soil enzyme activities such as Cellulase and Amylase were examined. The experimental results indicated that, the selected soil enzyme activities were significantly higher in the test soil sample than the control. Additionally, activities were increased with increasing the incubation period up to 21 day over 0 day, however, activities were adversely affected at 28 day. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

INTRODUCTION

Soil is an important system of terrestrial ecosystem. It produces food for teeming millions and supplies raw materials for a large number of industries on which the world economy is sustained. In fact, on the other hand, progress of civilization and rapid industrialization brought with it danger of soil pollution. A perusal of the literature on the discharge of effluents on the soil (Monanmani et al., 1990; Kannan and Oblisami, 1990a; Narasimha et al., 1999) strongly indicates that, they cause marked changes in physico-chemical, biological and soil enzyme activities.

The paper industry is one of the largest industries in India, consuming large amount of water (Trivedy and Raj, 1992) nearly 75-95% of the water was discharged by the industries as effluent from paper and pulp mill contains several toxic and non-biodegradable organic materials, which include, sulphur compounds, pulping chemicals, organic acids, chlorinated lignin's resin acids, phenolics, unsaturated fatty acids and terpenes. Wood consists of polysaccharides (a mixture of cellulose and hemicelluloses) and lignin is a complex, highly cross- linked hydroxylated and methoxylated phenyl propane polymer. About 300m^3 of waste water is generated per tonne of pulp manufacture (Zhang and Chuang, 1998).

Soil enzymes are a group of enzymes whose usual inhabitants are the soil and continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility, and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh et al., 1991). They are important in catalyzing several vital reactions necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture (Dick et al., 1994).

An attempt has, therefore, been made to determine the effects of pulp and paper mill effluents on soil enzyme activities. The specific objectives of the study are quantifying the activities of cellulose and amylase in the test and control samples with different incubation period.

MATERIALS AND METHODS

Soil Sample Collection

Soil samples were collected from pulp and paper mill effluent polluted area in the city of Rajahmundry, East Godavari district of Andhra Pradesh, India. Soil samples without effluent discharges served as control was collected from adjacent site (1km away) of pulp and paper mill. Soil samples both with and without effluents were used for determination of soil enzyme activities. Prior to testing, the soils were prepared for analysis as per APHA 2012 guidelines.

Assay of Soil enzymes

Five grams of soil samples contaminated with/ without effluents of pulp and paper mill effluents were transferred to test tubes. Soil samples were maintained 60% water holding capacity at room temperature in the laboratory (28 ± 4 °C). Triplicate soil samples of each waste water treated and controls were withdrawn at periodic intervals like 0,7,14,21 and 28 days to determine the soil enzyme activities. The method employed for the assay of cellulase and amylase developed by Pancholly and Rice (1973) and Cole (1977) respectively.

The soil samples were transferred to 250 mL of Erlenmeyer flasks and 1 mL of toluene was added. After 15 minutes, 10 mL of acetate phosphate buffer (pH 5.9) containing 1% CMC for (Cellulase), 6 mL of 2% Starch (Amylase) in 0.2M acetate buffer (pH 5.5) (Speir and Ross, 1975) were added to soil samples and flasks were plugged with cotton and held for 30 min (cellulase) and 48 hrs (Amylase), at 30^0C. After incubation, the suspension was filtered by Whatman No. 1 filter paper, and the amount of reducing sugar content in the filtrate was determined by the Nelson-Somagyi method (1944) using an Elicodigital spectrophotometer at 495 nm.

Statistical analysis

The activities of the Cellulase and Amylase were calculated on the basis of soil weight (over dried). Data were analysed using one way ANOVA and the differences contrasted using Duncan's Multiple Range Test (DMRT) (Lowry *et al.*, 1954; Megha Raj *et al.*, 1999; Jaffermohiddin *et al.*, 2011). All statistical analysis was performed at ($p<0.05$) using the SPSS statistical software package.

RESULTS AND DISCUSSION

The cellulase activity was measured in terms of release of glucose from CMC. There was an increase in the formation of glucose with increasing the soil incubation periods such as 0, 7, 14, and 21 day. The cellulase activity was decreased after 21 day of incubation. For instance, the cellulase activity in test soil with substrate was increased from 120 μg GE g^{-1} 30 min^{-1} to 194 μg GE g^{-1} 30 min^{-1} at 21 day. Later it was decreased to 100 μg GE g^{-1} 30 min^{-1} at 28 day incubation. Comparison of cellulase activity in soil samples with/without effluents discharged revealed that the soil polluted with effluents stimulate the cellulase activity than control. With increasing the soil incubation period, the cellulase activity was also improved in both polluted and non-polluted soils. Same was reported by Nagaraju *et al.* (2007) in soils polluted with effluents of sugarcane industry stimulated the soil cellulase activity (Fig.1).

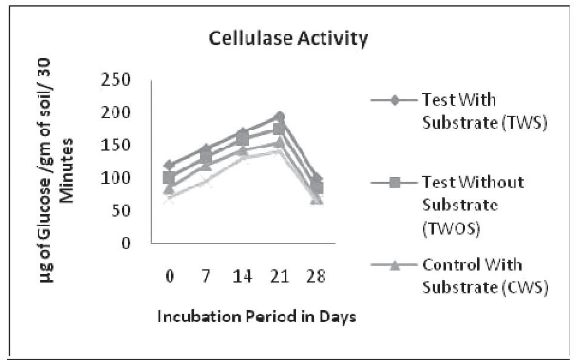

Fig. 1. Cellulase activity in test/control soil (with/without substrate) after 30 min incubation as influenced by pulp and paper mill effluents. The values are the means ± S.D for each incubation periods in each column, are significant $p < 0.05$ according to Duncan's multiple range (DMR) test.

The amylase activity was measured in terms of release of glucose from starch. There was an increase in activity up to 21 day incubation, there after activities were adversely affected. For instance, amylase activity in polluted soil with substrate increased from 100 μg GE g^{-1} 48 hrs^{-1} 0 d to 175 μg GE g^{-1} 48 hrs^{-1} on 21 day and later declined at 90 μg GE g^{-1} 48 h^{-1} at 28 day. Comparison of amylase activity in soil samples with/without effluents discharged revealed that the soil polluted with effluents stimulate the amylase activity than control. With increasing the soil incubation period, the amylase activity was also improved in both polluted and non-polluted soils. Narasimha *et al.*, (2012) (Cotton ginning mill) and Kannan and Oblisami (pulp and paper mill) (Reddy and Narasimha, 2012; Gao *et al.*, 2000), made a similar observations soils polluted with effluents stimulated the soil amylase activity (Fig.2).

CONCLUSION

The present study clearly indicates that the disposal

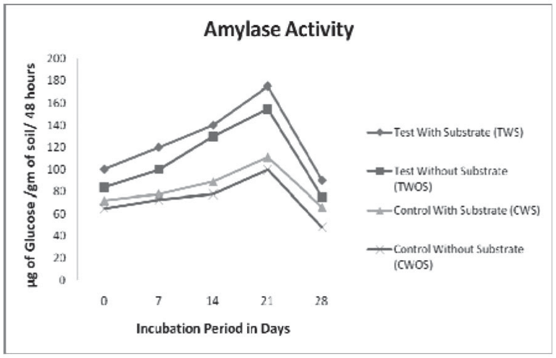

Fig. 2. Amylase activity in test/control soil (with/without substrate) after 48 hrs incubation as influenced by pulp and paper mill effluents. The values are the means ± S.D for each incubation periods in each column, are significant $p < 0.05$ according to Duncan's multiple range (DMR) test.

of effluents from pulp and paper mill alters the soil enzyme activities such as Cellulase and Amylase were stimulated in soil over control. Nonetheless, prolonged incubation causes adverse effects. Thus, this observation, therefore greatly warrants a prior treatment of pulp and paper mill effluents before discharging into water body or on agricultural lands and additional research will be necessary to discriminate these extracellular enzyme producing microorganisms (genera and species).

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