Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):445-449

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of pulp and paper mill effluents on soil enzyme activities

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ABSTRACT

Release of pulp and paper mill effluents on to the agricultural lands causes an indicative change 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. The soil enzyme activities such as Cellulase, Amylase, Protease, Lipase and Urease were examined. The experimental results indicated that, the selected Soil enzyme activities were significantly higher in the test sample than in the control. Additionally, activities were increased with increasing the incubation period up to 21 d over 0 d, however, activities were adversely affected at 28 d. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

Keywords: Pulp and Paper mill effluents, Cellulase, Amylase, Protease, Lipase and Urease activities.

INTRODUCTION

Soil is one of the most vital natural resources. 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 [1, 2, 3] strongly indicates that, they cause marked changes in physico chemical, biological and enzymatic properties.

The paper industry is one of the largest industries in India, consuming large amount of water[4] 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 hemicellulose) and lignin is a complex, highly cross- linked hydroxylated and methoxylated phenyl propane polymer. About 300m^3 of waste water is generated per tone of pulp manufacture [5].

Soil enzymes activities have been suggested as suitable indicators of soil quality because:(a) they are a measure of the soil microbial activity and therefore they are strictly related to the nutrient cycles and transformations; (b) They rapidly may respond to the changes caused by both natural and anthropogenic factors; (c) They are easy to measure[6]. Soil enzyme activities may be considered early and sensitive indicators to measure the degree of soil degradation in both natural and agro ecosystems, being thus well studied to measure the impact of pollution on the quality of soil.

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 cellulase, amylase, protease, lipase and urease in the test and control samples with different incubation period.

EXPERIMENTAL SECTION

Soil samples were collected from pulp and paper mill effluent polluted area in the city of Rajahmundry, Eastgodavari 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 air-dried, passed through a 2mm (milli metres) sieve and stored at 4^0C.

Enzyme assays: Five grams of soil samples contaminated with/without effluents of pulp and paper mill effluents were transferred to test tubes. Soil samples were maintained at 60% water holding capacity at room temperature in the laboratory (28 ± 4^0c). Triplicate soil samples of each waste water treated and controls were withdrawn at periodic intervals to determine the soil enzyme activities as detailed earlier by Tu., (1982) [7]. The method employed for the assay of cellulase, amylase, protease, lipase and urease were essentially the same developed by Pancholly and Rice (1973)[8],Cole(1977) [9], Speir and Ross(1975) [10],Chandan and Sahani(1964) [11] and Zantua and Bremner (1975)[12] 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 either 1% CMC (Cellulase), 2% Starch (Amylase), 2% Casein (Protease) were added to soil samples and flasks were plugged with cotton and held for 30 min (cellulase), 48 hrs (Amylase), 24 hrs (Protease) at 30^0C. After incubation, soil extracts were passed through Whatmann filter paper, then glucose (Cellulase &Amylase) and tyrosine (protease) contents in the filtrate were determined by the method of Nelson – Somagyi [13] Lowry [14], respectively. For Lipase 5mlof 1M citrate phosphate buffer (pH 8.0) and 2ml of Triacetene was added to enzyme sample. Incubate the reaction mixture at 37^0C for 2hrs. After incubation terminates the reaction by adding 25 ml of absolute alcohol. Then titrate the contents for released free fatty acids with 0.05 N NaoH using phenolphthalein indicator. Appearance of pink color is the end point. For urease the method comprises release of ammonia up on incubation of soil with 4ml of Sodium phosphate buffer (pH 7.0), 1ml of 1M urea solution incubated for 30 min and 10ml of 2M Kcl was added and kept at 4^0C for 15 min and Centrifuged, then 0.5 ml of Nesslers reagent followed by 3.5 ml of distilled water were added and the color was read at 495 nm, in the digital spectrometer.

RESULT AND DISCUSSION

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete enzymes such as Cellulase, Amylase, Protease, Urease, Phosphotases and Pectinases are extracellular. Those microbial secreted enzymes constitute an important part of soil matrix as extracellular enzymes[(15]. There is a considerable interest in the study of enzyme activities of soils [16] such activities may reflect the potential capacity of soil to form certain biological transformations of importance of soil fertility [17].

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 d. The cellulase activity was decreased after 21 d of incubation. For instance, the cellulase activity in test soil with substrate was increased from 46 µg GE g^{-1} 30 min^{-1} to 80 µg GE g^{-1} 30 min^{-1} at 21 d. Later it was decreased to 24 µg GE g^{-1} 30 min^{-1} at 28 d 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[18] in soils polluted with effluents of sugarcane industry stimulated the soil cellulase activity (fig.1).

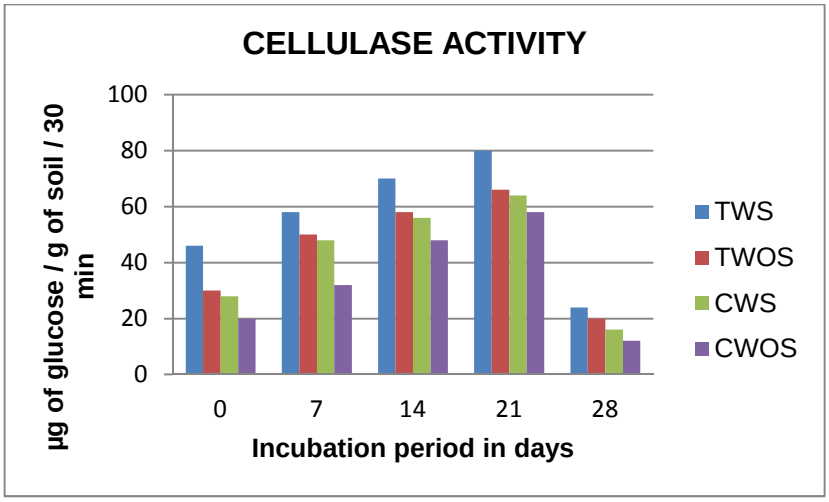

Fig.1: Cellulase activity in test/control soil (with/without substrate) after 30 min incubation as influenced by pulp and paper mill effluents

The amylase activity was measured in terms of release of glucose from starch. There was an increase in activity up to 21d incubation, there after activities were adversely affected. For instance, amylase activity in polluted soil with substrate increased from 60 µg GE g^{-1} 48 hrs^{-1} 0 d to 84 µg GE g^{-1} 48 hrs^{-1} on 21 d and later declined at 52 µg GE g^{-1} 48 h^{-1} at 28 d.Comparision 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 nonpolluted soils. Narasimha et al., (Cotton ginning mill) [19] and Kannan and oblisami (pulp and paper mill) [20], made a similar observations soils polluted with effluents stimulated the soil amylase activity (fig.2).

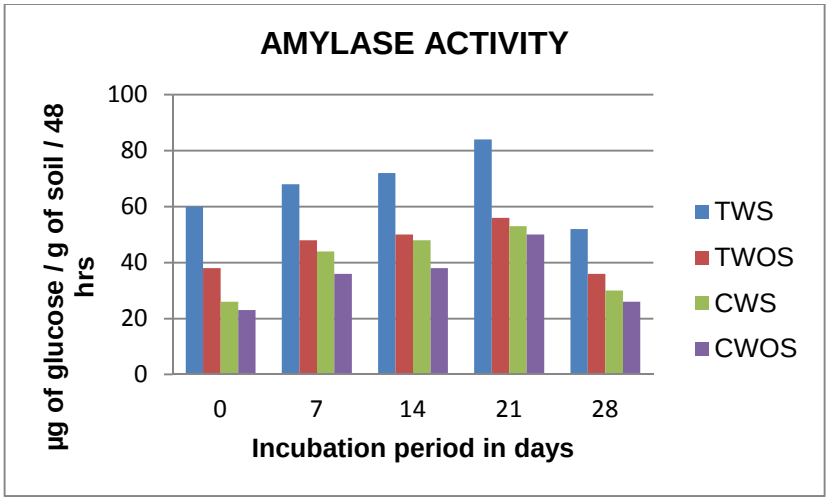

Fig.2: Amylase activity in test/control soil (with/without substrate) after 48 hrs incubation as influenced by pulp and paper mill effluents

The protease activity was measured in terms of release of tyrosine from casein. The activity of protease, as evidenced by the accumulation of tyrosine from casein was considerably greater in the soils polluted with effluents at all incubations over control. Furthermore, both the samples showed increased activity up to 21d of interval and then the activity was declined at further Incubation. For instance, test sample with substrate exhibited 84 µg TE g^{-1} 24 hrs^{-1} at 0 d incubation later it was increased 148 µg TE g^{-1} 24 hrs^{-1}. However the increased protease activity in polluted soil over control may be due to availability of substrate and or Casein degrading micro flora in polluted soil. Similar results were reported by Reddi pradeep and Narasimha et. al [21] leather industry effluents increased the soil protease activity (fig.3).

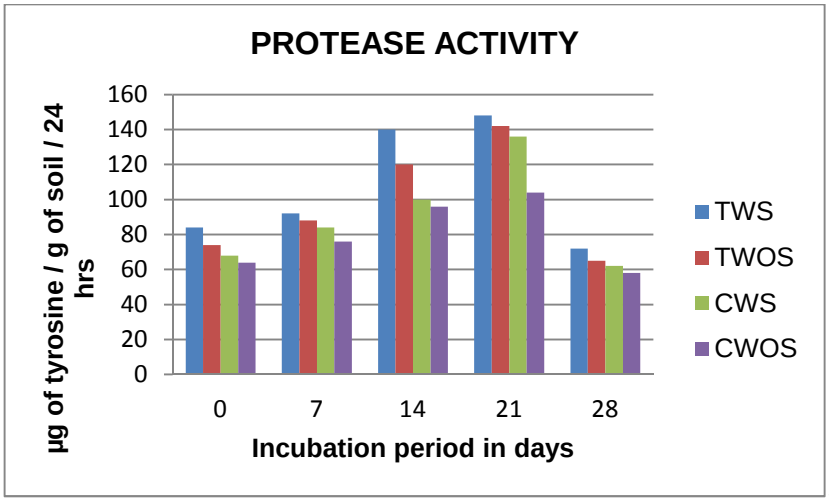

Fig.3: Protease activity in test/control soil (with/without substrate) after 24 hrs incubation as influenced by pulp and paper mill effluents

Now a day's Microbial lipases are more preferred for commercial applications due to their multifold properties, easy extraction procedures and unlimited supply. In our research also soil microorganisms secrete lipases. The Lipase activity was measured in terms of release of free fatty acids from triacetene. Lipase activity was increases with increasing the incubation period later it was declined at 28 d. Lipases which are stable and work at alkaline conditions which are usually the suitable wash conditions for enzymated - detergent powders and liquids, have also been found, and these hold good potential for use in the detergent industry [22] (fig.4).

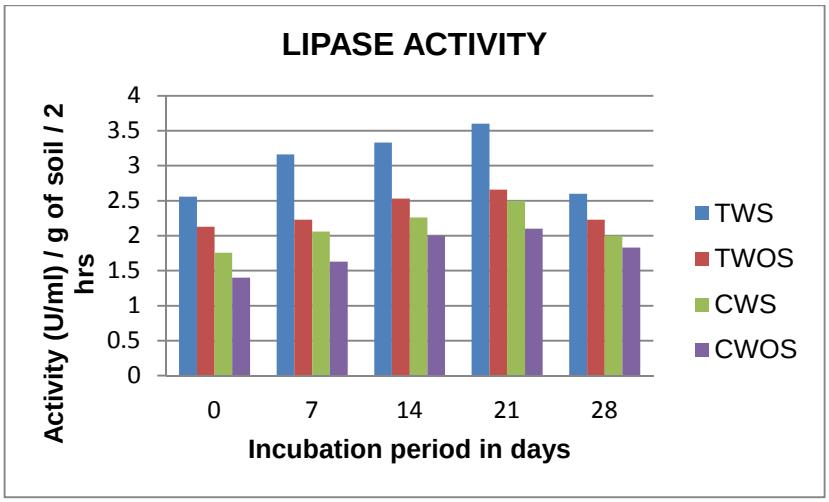

Fig.4: Lipase activity in test/control soil (with/without substrate) after 2 hrs incubation as influenced by pulp and paper mill effluents

Urea is an organic chemical complex used mainly as nitrogenous fertilizer in agriculture. Conversion of this nitrogen to inorganic nitrogen-ammonia and carbon dioxide takes place due to activity of urease enzyme, secreted by certain microorganisms and is responsible for supply of nitrogenous demand to growing crops. Assay of urease activity in soil samples involves quantification of ammonia released up on hydrolysis of urea. Urease activities in soils with/without effluents discharges were measured. Urease activity also increased up to 21d of incubation and later declined. For instance the urease activity in test soil with substrate at 0 d was 146 µg g^{-1} NH_4^+ -N g^{-1} 30 min^{-1} to 182 µg g^{-1} NH_4^+ -N g^{-1} 30 min^{-1} at 21 d and later decreased to 130 µg g^{-1} NH_4^+ -N g^{-1} 30 min^{-1} at 28 d similar results were noticed by Narasimha et. al. that urease activity was increased in soil contaminated with cotton ginning mill effluents.[3]. (Fig.5)

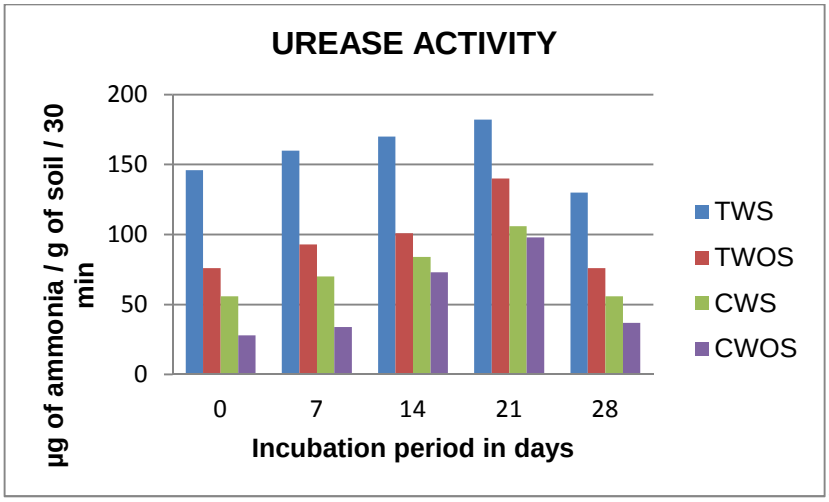

Fig.5: Urease activity in test/control soil (with/without substrate) after 30 min incubation as influenced by pulp and paper mill effluents

CONCLUSION

The present study clearly indicates that the disposal of effluents from pulp and paper mill alters the soil enzyme activities such as Cellulase, Amylase, Protease, Lipase and Urease 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&species).

Acknowledgements
Authors are thankful to the staff of A.P. Paper Mill Rajmundhry A.P. India for their timely help and encouraging us throughout this study.

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