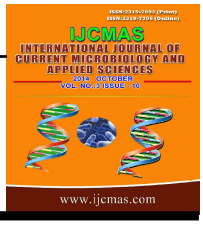
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 686-692**
http://www.ijcmas.com



Original Research Article

Impact of Brewery industry Effluents on soil enzyme activities

Pattlola Aishwarya*, Lavudi Saida, K.Venkateswar Reddy and P.Ranjit

Center for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad-500085, T.S., India

**Corresponding author*

Keywords

Brewery industry effluents, Physico-chemical, Biological, Soil enzymes

A B S T R A C T

Release of industrial effluents causes indicative changes in nutrient cycling and organic matter processing. In view of importance of soil enzymes in biochemical functioning of natural resource - soil system for recycling of nutrients, impact of industrial effluents on enzyme activities in soil such as cellulase, amylase and invertase were examined in this study. In this direction, soil samples were collected from Charminar Breweries Ltd, Sivampet Village, Pulkal Mandal, Medak District, of Telangana State, India. The experimental results indicated that, most of the physicochemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen, phosphorus, and potassium contents, microbial population and selected 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 effected at 28 d. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

Introduction

Soil is a major part of natural environment, along air and water, and in vital to the existence of life on the planet. Soil is the main stay of agriculture and horticulture, forming as it does the medium in which growth and ultimately the yield of food producing crops occurs. Soil is increasingly being recognized as playing a fundamental role in the quality and distribution of our water supply.

There is a direct impact of pollutants on minerals, organic matter and microbial community of soil (Lowry *et al*., 1951). The discharge of industrial effluents especially without treatment may have profound influence on physico-chemical and biological properties of soil related to soil fertility. A wealth of information on occurrence of changes in properties of soils due to discharge of effluents from other industries is available such as cotton ginning

mill (Nagaraju *et al*.,2007), sugar industry (Megharaj *et al*.,1999), paper mill (Nelson and Sommers, 1996), dairy industry (Nelson, 1944), and dairy wastewater (David Shyam Babu, 2010). Thus determination of enzyme activity and microbial biomass, chemical soil parameters seems to be the best approach for evaluating the state of microbial activity. Brewery industry can achieve an effluent discharge of 3–5 m3/m3 of sold beer (exclusive of cooling waters).Untreated effluents typically contain sus-pended solids in the range 10–60 milligrams per liter (mg/l), biochemical oxygen demand (BOD) in the range 1,000–1,500 mg/l, chemical oxygen demand (COD) in the range 1,800–3,000 mg/l, and nitrogen in the range 30–100 mg/l. Phosphorus can also be present at concentrations of the Order of 10–30 mg/l.
In reality, the soil enzymes occupy a vital role in catalyzing reactions associated with organic matter decomposition and nutrient cycling (Poonkothai and Parvatham, 2005).

In the present study, an attempt has, therefore been made to find out the impact of effluents of brewery industry on soil physical [pH, EC, water holding capacity], chemical [organic matter, total nitrogen, phosphorus and potassium], biological [bacterial and fungal populations] properties and selected soil enzyme activities.

Materials and Methods

Collection of soil samples

Soil samples were collected from the surrounding areas [1/4 km] of Charminar Breweries Ltd, Sivampet Village, Pulkal Mandal, Medak District of Telagana State, India. Soil sample without effluent discharges served as control was collected from adjacent site [1 km away] of industry. Soil samples both with and without effluents were used for determination of Physico-chemical, biological and enzyme activities. These two soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to < 2 mm sieves for determination of soil texture.

Physico-chemical properties of soil

The physical, chemical and biological properties of test and control soils were determined by the following standard procedures. The soil particles like sand, silt and clay contents were analyzed with the use of different sieves by the method of Alexander (Alexander, 1961). Whereas water holding capacity, organic carbon, total nitrogen, and soluble phosphorous of soil samples were determined by the methods of a Johnson & Ulrich (Jackson, 1971), Walkley-black (Narasimha *et al*., 2011), and Microkjeldal (Jaffer mohiddin et al., 2011) and Kurrevich and Shcherbakova (Kaushik et al., 2005), respectively. Electric conductivity and pH were determined by Elico conductivity meter and pH meters, respectively.

Biological parameters

Micro flora such as bacterial and fungal populations of both soil samples were enumerated by serial dilution technique. One gram of each soil sample was serially diluted and 0.1 ml was spread with a sterile spreader on nutrient agar medium and Czapeck-Dox agar medium for the isolation of bacteria and fungi respectively.

Nutrient agar plates were incubated at 37º C for 24 h, where as Czapeck-Dox plates were at room temperature for 7 d. After incubation period, colonies formed on the surface of the medium were counted by colony counter (Nagaraju *et al*., 2007).

Enzyme assays

Five grams of soil samples contaminated with/without effluents of brewery industry effluents were transferred to test tubes. Soil samples were maintained at 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 to determine the soil enzyme activities as detailed earlier by Tu (Sparling *et al*., 2001). The method employed for the assay of cellulase, amylase and invertase were essentially the same developed by Pancholy and Rice (Nilima and Madhuri, 2005), Cole (Cole, 1977) and Tu (1982) respectively. The soil samples were transferred to 250 mL of Erlenmeyer flasks and one mL of toluene was added. After 15 min, 6 mL of 0.2 M acetate phosphate buffer [pH 5.5] containing either 1% CMC [cellulase] , 2% starch [amylase], and 0.2M Citrate phosphate buffer [pH 5.5] containing 18Mm Sucrose were added to soil samples and flasks were plugged with cotton and held for 30 min [cellulase] ,48 h [amylase], 6 h [invertase] at 30 °C. After incubation, soil extracts were passed through whatman filter paper, then glucose [cellulase, amylase, and invertase] were determined by the method of Nelson-Somagyi (Narasimha *et al*., 1999).

Results and Discussion

Soil samples of both with and without effluents discharge were analyzed for their physico chemical properties and their results were represented in Table 1. Soil samples with brewery industry effluent underwent changes in all measured parameters of physical and chemical properties in comparison to control. There was a noticeable change in the pH of the test soil over control. However, soil textures in terms of percentage of clay, silt, sand were 50, 25, 25 in test and 42, 22, 36 in the control soils, respectively. Higher water holding capacity was observed in test soil than control values were found to be 3.0 and 1.5 mL g−1, respectively. The electrical conductivity of both test and control soils were 1.98 and 0.59 μMhos cm−1, respectively. Increased water holding capacity and electrical conductivity in contaminated soil may be due to the accumulation of organic waste such as amino acid residues, acids and alkalis in the Brewery industry effluents.

The results were in conformity with the studies of Sparling *et al.* (2000), Narasimha *et al.* (2011), Poonkothai and Parvatham (2005), and Xiao *et al.* (2005) had increased electrical conductivity in soil contaminated by the effluents of dairy, cotton ginning, automobile, and black liquor for straw pulping industries, respectively. The parameters like organic matter percentage, total nitrogen, phosphorus, and potassium were higher in test soil than the control soil. The values of above properties of test sample were 5.6 %, 0.36 g kg−1, 550 kg ha−1, 1100 kg ha−1, and control soil were 3.0 %, 0.22 g kg−1, 400 kg ha−1, 800 kg ha−1, respectively (Table 1). Higher organic matter of the polluted soil may be due to the discharge of waste water in organic nature. Also, increased Organic matter enhanced soil enzyme activity. Narasimha *et al.* (2011) and Kaushik *et al.* (2005) made similar reports on the discharge of effluents from cotton ginning and distillery industries, respectively. Thus, soil is a potent system of terrestrial ecosystem, and direct discharge of industrial effluents especially that without treatment may have profound influence on physico-chemical and biological Properties of soil related to soil fertility. Similarly, discharge of effluents from various industries like sugar industry, dairy factory and petrochemical industry influenced the physico-chemical properties of soil.

Table.1 Physico-chemical characteristics of soil as effected by brewery industry effluents

Character	Control[a]	Test[b]
Color	Black	Thick Black
Odor	Normal	Unpleasant
pH (1:1.25 soil-water slurry)	6.51	8.4
Texture: Clay (%) Slit (%) Sand (%)	 42 22 36	 50 25 25
Electrical conductivity(µmhos/cm)	0.59	1.98
60% Water-holding capacity(mL g−1)	1.5	3
Organic matter (%)	3.0	5.6
Total nitrogen (g kg−1 soil)	0.22	0.36
Available phosphorus (P2O5)in (kg/ha)	400	550
Available potassium(K2O) in (kg/ha)	800	1100

a = Soil without brewery industry effluents; b = Soil polluted with brewery industry effluents

Table.2 Biological characteristics of soil samples as effected by brewery industry effluents

Micro flora*	Control[a]	Test[b]
Bacteria	25×10^{6}	40×10^{6}
Fungi	18×10^{5}	25×10^{5}

*Microbial population in terms of Colony forming units per g of soil

a = Soil without brewery industry effluents; b = Soil polluted with brewery industry effluents

Table.3 Cellulase activity* in soil after 30 min incubation as influenced by brewery industry effluents

.Incubation in days	Cellulase Activity			
	Test		Control	
	WS	WOS	WS	WOS
0	0.33±0.11	0.30±0.11	0.24±0.08	0.21±0.008
7	0.34±0.08	0.32±0.10	0.27±0.06	0.23±0.08
14	0.39±0.1	0.34±0.1	0.30±0.6	0.25±0.08
21	1.13±0.03	1.0±0.1	0.91±0.07	0.87±0.09
28	0.95±0.05	0.73±0.20	0.69±0.21	0.6±0.22

*mg glucose g−1 30 min−1
Control - Soil without brewery industry effluents
Test – Soil polluted with brewery industry effluents
WS – with substrate; WOS – without substrate
All entries are average mean of triplicate values

Table.4 Amylase activity* in soil after 48 h incubation as influenced by brewery industry effluents

Incubation in days	Amylase Activity			
	Test		Control	
	WS	WOS	WS	WOS
0	0.14±0.07	0.12±0.06	0.09±0.02	0.007±0.022
7	0.65±0.1	0.2±0.1	0.11±0.03	0.10±0.12
14	0.75±0.19	0.36±0.05	0.16±0.05	0.13±0.04
21	0.78±0.18	0.48±0.09	0.34±0.11	0.32±0.1
28	0.55± 0.11	0.40±0.06	0.38±0.06	0.30±0.11

*mg glucose g−1 48 h−1
Refer Table 3 foot note for other details

Table.5 Invertase activity* in soil after 6 h incubation as influenced by brewery industry effluents

Incubation in days	Invertase Activity			
	Test		Control	
	WS	WOS	WS	WOS
0	1.32± 0.39	1.01±0.11	0.58±0.13	0.48±0.12
7	1.73±0.37	1.34±0.37	0.69±0.09	0.55±0.16
14	2.1±0.1	1.4±0.26	0.87±0.56	0.53±0.16
21	3.92±0.30	1.89±0.19	0.98±0.45	0.53±0.31
28	3.10±0.12	1.3±0.19	1.0±0.1	0.01±0.005

*mg glucose g−1 6 h−1
Refer Table 3 foot note for other details

This is due to organic waste that may contribute to maintain or increase the organic matter and nutrient content in the soil. The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete enzymes such as amylase, cellulase, invertases, ureases, proteases, phosphatases, pectinases are extracellular. Those microbial secreted enzymes constitute an important part of soil matrix as extra cellular enzymes. Thus, there is a considerable interest in the study of enzyme activities of soil, because such activities may reflect the potential capacity of a soil to form certain biological transformation of importance to soil fertility. Micro flora of both samples were enumerated and listed in Table 2. Polluted soil caused two fold increases in bacterial and fungal population compared to control soil (Table 2).

The activity of the Cellulase in polluted and non polluted soils was determined and results listed in Table 3. The cellulase activity was measured in terms of release of glucose from CMC. There was an increase in the formation of glucose with increasing soil incubation such as 0, 7, 14, and 21 d. The cellulase activity was decreased after 21

d of incubations. For instance, the cellulase activity in test soil increased from 0.33 mg GE g−1 30 min−1 to 1.13 mg GE g−1 30 min−1 0 to 21 d. Later it was decreased to 0.95 mg GE g−1 30 min−1 at 28 d incubation. Same was reported by Narasimhan et al in soils polluted with effluents of cotton ginning mills stimulated the soil cellulase activity at early d of incubation.

Furthermore, the enzyme amylase plays a crucial role in catalyzing the hydrolysis and solubilising the substrate containing Carbon. The amylase activity was measured in terms of release of glucose from starch and the results were listed in Table 4.

There was an increase in activity up to 21 d incubation, there after activities were adversely affected. For instance, amylase activity in polluted soil increased from 0.14 to 0.78 mg glucose equivalents g−1 48 h−1 from 0 to 21 d. Later it was decreased to 0.55 mg GE g−1 48 h−1 at 28 d (Table 4). Comparison of soil amylase activity in soil samples with/without effluents discharged revealed that the soil polluted with effluents stimulated the amylase activity by ~ 2 fold than control soil. Narasimha et al (2011) made a similar observation in soils polluted with cotton ginning mill effluents stimulated the soil amylase activity. The invertase activity was measured in terms of release of glucose from sucrose and the results were listed in Table 5. The activity of invertase as was considerably greater in the soils polluted with effluents at all incubations over control. Furthermore, both the samples showed increased activity up to 21 d of interval and then the activity was declined at further incubation. For instance, test sample exhibited 1.32 mg of glucose equivalents per gram of soil per 6 h against 0.58 mg g−1 6 h−1 of control at 0 d, later it was increased in both soils up to 21 d and declined at 28 d interval. However, the increased invertase activity in polluted soil over control may be due to availability of substrate and or sucrose degrading Micro flora in polluted soil (Table 5).

The present study clearly indicates that the disposal of effluents from brewery industry alters the physico-chemical, biological properties and activities of enzymes such as cellulase, amylase and invertase were stimulated in soil over control. Nonetheless, prolonged incubation causes adverse effects. Thus, this observation, therefore, greatly warrants a prior treatment of brewery industry effluents before discharging into a water body or on to agricultural land and additional research will be necessary to discriminate the type of these extra cellular enzyme producing microorganisms (genera and species).

Acknowledgement

Authors are thankful to the staff of Charminar Breweries Ltd, Sivampet Village, Pulkal Mandal, Medak District, and Telagana State, India for their timely help and encouraging us throughout this study.

References

Alexander, M. 1961. Introduction to soil microbiology. Wiley Estern Ltd. New Delhi.

Cole, M.A. 1977. Lead inhibition of enzyme synthesis in soil. *Appl. Environ. Microbiol.*, 33: 262–268.

David Shyam Babu, S. 2010. Protease and phospatase activity of soil contaminated with dairy waste water. *Asian J. Microbiol. Biotechnol. Environ. Sci.*, 12(2): 1–5

Jackson, M.L. 1971. Soil Chem. Anal. Prentice Hall India, New Delhi.

Jaffer Mohiddin, G., Srinivasulu, M., Madakka, M., Subramanyam, K.,

Rangaswamy, V. 2011. Influence of selected Insecticides on Enzyme Activities in Groundnut (*Arachis hypogeae* L.) Soils. *Dyn. Soil Dyn.,* 5(1): 65-69.

Kaushik, A., Nisha, R., Jagjeeta, K., Kaushik, C.P. 2005. Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioaendments. *Bioresour. Technol.,* 96: 1860–1866.

Lowry, O.H., Rouse Bough, H.I., Fair, A.L., Randall, R.I. 1951. Protein measurement with the Folin phenol reagent. *J. Biol Chem.,* 193: 265–275.

Megharaj, M., Singleton, I., Kookana, R., Naidu, R. 1999. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. *Soil Biol. Biochem.,* 31: 1549–1553.

Nagaraju, M., Narasimha, G., Rangaswamy, V. 2007. Impact of effluents of sugarcane industry on physicochemical and biological properties. *J. Ind. Pollut. Control.,* 23: 73–76.

Narasimha, G., Babu, G.V.A.K., Rajasekhar Reddy, B. 1999. Physico-chemical and biological properties of soil samples collected from soil contaminated with effluents of cotton ginning industry. *J. Environ. Biol.,* 20: 235–239.

Narasimha, G., Sridevi, A., Venkata Subba Reddy, A., Rajasekhar Reddy, B. 2011. Effects of cotton ginning mill effluents on soil enzymatic activities and nitrogen mineralization in soil. *J. Chem. Pharm. Res.,* 3: 128–137.

Nelson, D.W., Sommers, L.E. 1996. Total carbon, organic and organic matter. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3. Soil Science Society of America Book Series. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin. Pp. 961–1010.

Nelson, N. 1944. A photometric adaptation of Somogyi method for determination of glucose. *J. Biol. Chem.,* 153: 375–380.

Nilima, W., Madhuri, S. 2005. Fungal diversity of paper industry waste and soils irrigated by the effluents. *Nature Environ. Pollut. Technol.,* 4: 44–52.

Poonkothai, M., Parvatham, R. 2005. Bio-physical chemical assessments of automobile waste water. *J. Ind. Pollut. Control.,* 21: 59–62.

Sparling, G.P., Scipper, L.A., Rusasel, R.J.M. 2001. Changes in soil properties after long term irrigation of dairy factory effluents to Newzeland volcanic ash and pumice soils. *Aust. J. Soil Res.,* 39: 505–518.

Tu, C.M. 1982. Influence of pesticides on activities of amylase, invertase and level of adenosine triphosphate inorganic soil. *Chemosphere,* 2: 909–914.

Xiao, C., Fauci, M., Bezdicek, D.F., Mckean, W.T., Pan, W.L. 2005. Soil microbial responses to potassium-based black liquor from straw pulping. *Soil. Sci. Soc. Am. J.,* 70: 72–77.