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Advances in Applied Science Research, 2012, 3 (3):1772-1775

ISSN: 0976-8610
CODEN (USA): AASRFC

Soil pH, microbial population, nitrate reductase and alkaline phosphatase activities of different environment of Dibrugarh district, Assam

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ABSTRACT

The present paper reported the soil pH, microbial population, nitrate reductase and alkaline phosphatase activities of five different types of soil environments of Dibrugarh district. Soil pH was ranging between 5.40-7.02. Microbial population was found to be more in tea garden soil (4.6 x 10^5) and less in rice field soil (3.4 x $10^{5)}$. Rice field soil showed more nitrate reductase activity (11.09 µg/ml/hr), while botanical garden soil showed higher alkaline phosphatase activity (26.40 µg/ml/hr). Isolation, characterization and identification of microbes from soil with higher alkaline phosphatase activity and their proper culture and implementation as a source of bio fertilizer will open a new area in organic cultivation.

Key words: Soil pH, microbes, nitrate reductase and alkaline phosphatise.

INTRODUCTION

Soil is a favourable habitat for microorganisms; spatially fertile soil is inhabited by tremendous number of microorganisms. All kinds of organic matters deposited on soil can be decomposed by soil microorganisms, releasing different kinds of enzymes,responsible for various oxidation-reduction reactions to release the nutrients. Enzyme activity of soil, playing an important role in nutrient cycling, is the result of enzyme producing activity of soil microbes present in it. Nitrate is used by many microorganisms as a source of terminal electron acceptor under anaerobic conditions and converts it to ammonia through a series of reactions. In the assimilatory process nitrate is converted to nitrite in presence of enzyme nitrate reductase, the process called nitrate reduction, and then to dinitrogen gas sequentially [1]. The enzyme nitrate reductase is a membrane bound protein and identical for both the assimilatory as well as dissimilarly nitrate reductase processes. The enzyme has been extensively studied in different nitrate reducing soil bacteria such as the ammonifier *Escherichia coli* and the denitrifiers *Paracoccus denitrificans, Pseudomonas stuzeri, Pseudomonas denitrificans*, and others [2,3,4,5and 6]. Alkaline phosphatase is a hydrolysing enzyme responsible for removal of phosphate group from different types of organic molecules like proteins, nucleic acids and alkaloids. The process of removal the phosphate group is calledas dephosphorylation. As this enzyme is active at alkaline pH,hence, called as alkaline phosphatise [7] . This group of enzymes catalyse the phosphomonoester linkages, split the substrate molecules by direct addition of a water molecule.Although the actual purpose of the enzyme is still not fully understood, but it is assumed that that the function of this enzyme is to generate free phosphate groups in alkaline and phosphate starving conditions [8].Several types of phosphatases, like acid and alkaline phosphatase, can accelerate the rate of organic phosphate solubilisation in soil and may occur from the microbial inhabitants of ordinary as well as rhizospheric soil [9].

Soil itself has no any enzyme activity for solubilization as well as mobilization of minerals. But the huge number of microorganisms present in soil makes it possible to recycle the nutrients from both organic and inorganic substances. Nitrate reductase and alkaline phosphatase activity of soil is the product of microbial secretion of these enzymes to its nearest soil particles. Activity of soil nitrate reductase is useful for maintaining the nitrogen ratio in the

atmosphere as well as removal of hazardous nitrate compounds of soil. Acid as well as alkaline phosphatases are responsible for quick regeneration of organic phosphates and making them easily available for plants.

MATERIALS AND METHODS

Locations and sampling:
Soil samples were collected from five different environments of Dibrugarh district of upper Assam including 1) botanical garden of Dibrugarh University, 2) tea garden, 3) rice field, 4) oil drilling area and 5) Vegetable farm soil. Soil samples were collected randomly from 5 cm. depth in sterile poly bags and immediately stored at 4^0C for further analysis. Five subsamples were collected from each area and analysed separately in triplicates. The mean value of these three subsamples comprised the value of a sample.

Microbial population count:
Soil samples were subjected to serial dilution and spreaded on nutrient agar plates, incubated for 48 hrs to grow the microbial colonies properly. Colony forming units (cfu) were counted by using a colony counter.

Estimation of enzyme activity:
Estimation of nitrate reductase was done by following the standard protocol for nitrite estimation in solutions. 50 ml of peptone water media amended with 1% KNO_3 were poured into 150 ml conical flasks and theninoculated with 5 grams of different soil samples in triplicate. All the flasks were incubated at 30^0C for three hours. After incubation, 10 ml of soil suspensions from each flask were centrifuged separately at 5000 rpm for 10 mins. and 1 ml of supernatants from each samples were treated with 1 ml of Sulphanylamide. After waiting for 20 mins. ,1 ml of N (naphthyl) EtheleneDiamineDihydrochloride (NEDD) were added to each samples and left for development of a pink colour. Intensity of the pink colour was measured at 540 nm wave length by taking an uninoculated media (with sulphanilamide and NEDD) as blank by UV-VIS spectrophotometer. For alkaline phosphatase using p-nitrophenyl phosphate as the chief source of phosphate [10] in minimal salt medium prepared in 0.01M tris-HCl buffer with pH 9.Five grams of soil samples (in triplicate) were inoculated to 50 ml of MS-nitrophenyl mediumin 150 ml conical flasks and then incubated at 30^0C for three hours. Development of yellow colour indicates break down of p- nitrophenyl phosphate into p-nitriphenol and phosphate due to the action of alkaline phosphatase on p-nitrophenyl phosphate. 10 ml of soil suspensions from each flask were centrifuged at 5000 rpm for 10 mins. 1 ml of supernatants from each samples were added with 2 ml of 0.1 N NaOH to stop the activity of the enzyme. Intensity of yellow colour was estimated at 420 nm by UV-VIS spectrophotometer (Model: ST-UV-752(N) UV-VIS,LAB FAC, China)

Soil pH:
After preparation of solution, pH was measured with the help of digital pH meter (Model: HK-3C Table Model PH Meter Manufacturer, Trading Company, China)

Statistical approach:
Mean and standard deviation of enzyme activities were calculated by using standard formula.

RESULTS

Soil of oil drilling sites and tea gardens were acidic (pH 5.40-5.70) in nature, whereas soils other sampling sites were basic (pH 6.90-7.20) in nature.

Range of microbial population was found between 2.7 x 10^5 and 4.6 x 10^5 in the following trends Tea garden > Dibrugarh University vegetable farm > Botanical garden > Rice field >oil drilling sites.

Nitrate reductase activity was found in the following trends Rice field > Tea garden University vegetable farm > Botanical garden >oil drilling site with maximum in rice field soil (11.09 µg/ml/hr) and minimum in oil drilling site (3.55 µg/ml/hr).

Alkaline phosphatase activity was found to be maximum in the botanical garden soil (26.40 µg/ml/hr) and minimal in the soil of Oil drilling site (11.05 µg/ml/hr) and showed the following trends Botanical garden > Rice field > Vegetable farm>Tea garden> University vegetable farm > oil drilling site.

Table1: Results of microbial population and enzyme activities of soil samples

Sl. Nos.	Soil environment	pH	Microbial population/gm of soil	Nitrate reductase activity (µg/ml/hr)	Alkaline phosphatase activity (µg/ml/hr)
1	Tea garden	5.70	4.6×10^5	9.02 ±.041	16.20 ±.031
2	Botanical garden	7.02	3.6×10^5	6.50 ±.012	26.40 ±.029
3	Rice field	6.22	3.4×10^5	11.09 ±.022	24.20 ±.015
4	Veg. Farm	6.90	4.2×10^5	7.22 ±.031	22.5 ±.026
5	Oil drilling area	5.40	2.7×10^5	3.55 ±.024	11.05 ±.030

Values are mean ±SD of five replicas

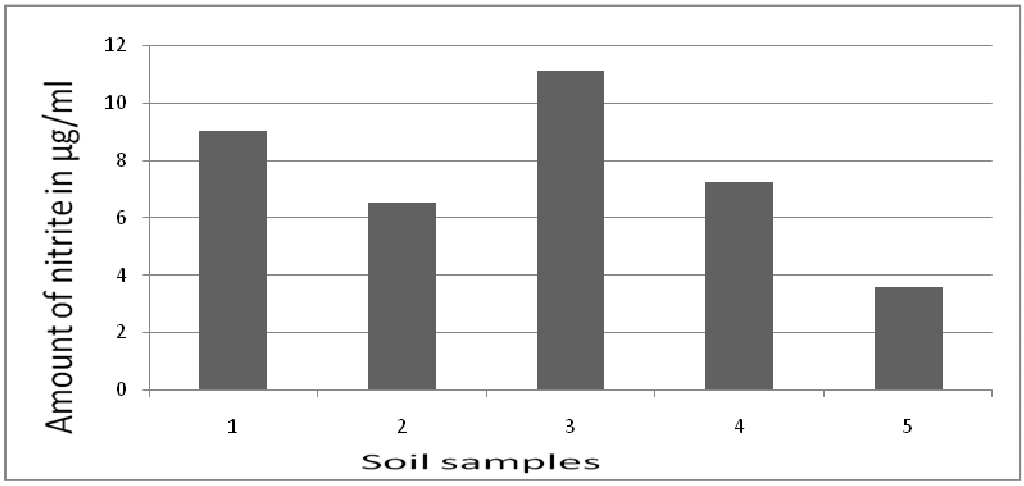

Fig. 1: Trends in nitrate reductase activity of soil samples

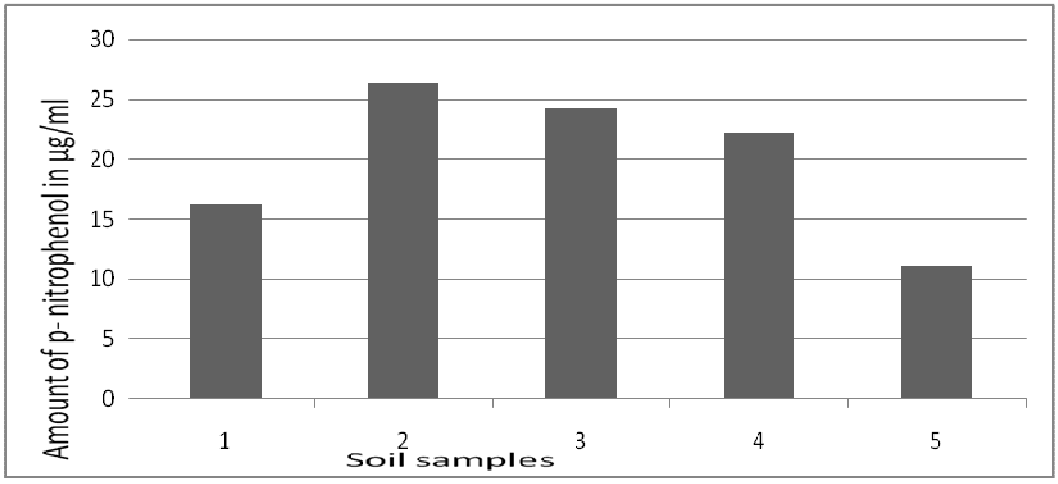

Fig. 2: Trends in alkaline phosphatase activity of soil samples

DISCUSSION

Oil contaminated (drilling site) soil are slightly more acidic in nature may be due to formation of toxic acids in the spilled oils. This finding is in agreement with the earlier findings of [11 and 12]. Usually microbial utilization of hydrocarbon led to formation of organic acids [13]. Thus, the acid probably produced by the microorganisms implicated for reduction in pH levels in crude oil contaminated soil.

Depending on soil conditions like nutrient availability, pH, aeration, temperature, moisture etc. microbial population in different soils varies. Tea garden soils are often supplemented with different kinds of fertilizers making most of the essential elements easily absorbable and enhance the easy and rapid multiplication of common microbial flora of soil. Enzyme activity of soil is the product of the total enzyme activity of the microbial flora of soil. Soil enzyme activity highly depends on population and kinds of microbes colonize in an area. As rice field soils are highly irrigated and remain submerged for a long period, therefore anaerobic microbial population may be higher in rice field in comparison to others and this may be the cause of higher nitrate reductase activity of rice field soil [14].

Alkaline phosphatase is a major enzyme responsible for making organic phosphates easily available for plants and thereby helps in plant growth. Activity of phosphatases depends on the microbial flora as well as the amount of available phosphates in a particular area. Increase of available phosphates by chemical fertilizers decreases phosphatase activity. As botanical garden soils are not supplied with any chemical fertilizers and have a high litter deposition, hence phosphatase is the main enzyme for making phosphates available for plants and that may be the cause of higher alkaline phosphatase activity of botanical garden soil. Rice is generally cultivated in basic or low acidic pH soils and rice straws are left in the field, hence for decomposition and recycling of straw organic phosphates, microbes must secrete a remarkable amount of phosphatase. Oil drilling areas are generally affected by oil spills and microbial population is lesser than other areas. Hence these kinds of soils have lesser enzyme activities in comparison to others.

CONCLUSION

Proper evaluation of microbes with higher nitrate reductase activity may be a good source of bioremediation for nitrate compounds and may help to reduce the loss of nitrogenous fertilizers. Isolation, characterization and identification of microbes from soil with higher alkaline phosphatase activity and their proper culture and implementation as a source of bio fertilizer will open a new area in organic cultivation.

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