Annals of Plant Sciences
ISSN: 2287-688X
Original Research Article

Application of Distillery Effluent Irrigation to Agriculture Soil and Profiling of Biochemical Activity

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Received for publication: July 02, 2013; **Accepted**: August 28, 2013.

Abstract: Anaerobically treated effluent produced from sugarcane-molasses based distillery is a rich source of organic compounds, complex minerals and nutrient ions. Disposal of treated distillery effluent to adjacent water bodies is a common practice, though its application to agriculture soil has received a substantial response in recent time. Modi distillery, located at Modinagar an area of western Uttar Pradesh in north India, being a major alcohol-processing unit with 26 KLPD production capacity discharges enormous effluent volume. In this report, application of distillery effluent released from Modi distillery was analyzed on agriculture soil. Physio-chemical analysis of distillery effluent exhibited higher levels of dissolved organic and inorganic entities. Application of distillery effluent was measured in time-bound concentration-dependent strategies. Field experiments were designed under factorial scheme using randomized pot method. Further analysis revealed significant increase in physical, intrinsic soil properties and a higher soil microbial population in effluent treated soil in comparison to the untreated control. Existence of oxidized solids and produced acids lowered effluent pH and increased COD and BOD levels resulted in soil toxicity. Towards the management of such polluted compounds, biochemical and enzymatic activities of *Bacillus cereus* (JN700160), *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* strains those were derived from distillery effluent and identified earlier through molecular characterization, were analyzed. Conclusively, present study represented an account of physio-chemical properties of distillery effluent irrigated agriculture soil that further complimented with profiling of biochemical activity of effluent degrading bacteria towards the improvisation of ferti-irrigation strategies in contemporary agriculture practice.

Keywords: Distillery Effluent, Physio-Chemical Analysis, Agriculture, Soil Profile, Irrigation.

Introduction

Central Pollution Control Board has marked sugarcane distilleries as one of the 17 most polluting industries in India. With 319 alcohol producing distilleries, India holds second largest network of molasses based distilleries in Asia with an installed capacity (IC) producing 3.29 billion liter of alcohol (Subramanian *et al.,* 2005). Uttar Pradesh and Maharashtra as being two of most sugarcane–growing states retain highest IC with more than 40% of the total IC followed by Madhya Pradesh (14.2%) and Tamil Nadu (9.7%). It has been estimated that molasses based distilleries generate 8-15 lit of waste water for every liter of produced alcohol (Uppal, 2004).

Modi distillery is located at Modinagar, a sugarcane produced area of western Uttar Pradesh. At present, distillery has acquired a capacity of 26 KLPD productions (UP Excise Dept. -4843KL). Modi distillery produces enormous amount of anaerobically treated effluent, which regularly discharged into small drainage streams leading to Kali East, a recipient river located at a 0.05 KM distance from distillery (UPPCB, 2010). Such discharged post-methylation effluent comprises substantial quantity of organic matter and inorganic nutrients and recognized as a rich source of minerals and ions (Joshi *et al.* 1996). Application of such anaerobically treated effluent may provide a source of major nutrients *viz.* K^+, P^+, Ca^+, SO_4^{2-}, NO_3^{2-}, Cu^{2+}, Mn and Zn; irrigation of which to agriculture soil is recognized to increase the crop yield of the crops (Pathak *et al.,* 1999).

Approach of distillery effluent to agriculture soil as irrigation source is a promising alternative towards its secure and sustainable disposal. Exploration of such approaches may reduce the requirement of fertilizers that may increase share in farmer's savings. Although, advantages of distillery effluent on agricultural crop productivity are known, very less data is available on the effect of effluent application for irrigation of agriculture soil. Such practice of effluent in irrigation may enhance the hydraulic conductivity and reduce bulk density of the soil. Therefore, present study explores the scope of potential advantages or limitation of effluent irrigation to the agriculture soil. In the course of observation, effort has been made to evaluate the effect of varying

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effluent concentration on soil physiology and texture. Although, application of effluent to the soil having certain advantages, it suffers from specific environmental issue and technical limitations (Cruz *et al.* 1991); since distillery effluent besides retaining organic content and nutrients, also enriched of heavy metals, colored components, complex inorganic salts and phenolic substitutes. Such compounds may alter soil physico-chemical soil composition and catalytic processes of the microbial enzymes present in soil. Most of such catalytic activity is derived from bacterial agents, wherein enzymes play a crucial role in degradation and decomposition of organic matter and nutrient cycling in such small ecosystems (Johansson *et al.,* 2000). Dick and Tabatabai (1992) suggested that profiling of soil bacterial enzymatic activities may reflect status of soil biological activity. Increasing number of evidences over time has demonstrated remediation application of hydrolases, lyases and oxidoreductases family enzymes (van Wyk, 1999; Singh, 2002; Gianfreda and Rao, 2004; Rodríguez Couto and Toca Herrera, 2006). Earlier, in laboratory practice to isolate and characterize bacterial populations from molasses effluent, three bacterial agents were identified *viz. Bacillus cereus* (JN700160), *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* (derived from M1, MO3 and C2 effluent isolates respectively) based on morphological and molecular characterization and their potential application in the decolonization of distillery effluent was analyzed (Chaudhary *et al.,* 2013). Consistent to the fact associated with effluent application to soil, field experiments were conducted to investigate the effect of varying effluent concentration on soil that further leads to profiling of catalytic activity of three bacterial agents.

Materials and Methods

Preparation of experimental blocks

The field study was performed in experimental soil blocks at Krishna Kunj (28°49'33"N 77°34'38"E), located at a distance of 3.7km and 2.7km from Modi distillery and Deptt. of Botany, MM [PG] College Modinagar, respectively. Towards investigating the irrigation effect of distillery effluent on soil properties, field blocks i.e. 50cm in diameter were formed as per entirely randomized design scheme. The field practice was replicated four times and marked for varying effluent concentrations *viz*. 0 (Lab tap water (LTW), was taken as internal control), 10, 20, 60 and 100%. Detailed analysis of process and parameters of distillery effluent was performed by the author with the help of research staff at Dept. of Botany, MM (PG) Modinagar.

Effluent sample collection and analysis

Anaerobically treated effluent samples were collected from Modi distillery, Modinagar. Effluent samples were analyzed for different physico-chemical parameters *viz.* TS (total solids), TDS (total dissolved solids), TSS (total suspended solids), EC (Electric conductivity), turbidity and pH, BOD (Biological oxygen demand), COD (Chemical oxygen demand), Cl^-, hardness, Na^+, K^+, $Ca2^+$, $Mg2^+$, HCO_3-,NO_3^{2-}, PO_4^{3-} and SO_4^{2-} following standard methods (APHA, 2005).

Pot preparation, filling, effluent irrigation, sampling and analysis

The soil used in experimental block was collected from agriculture field at of 0-25 cm depth. Each pot (50x50cm) was filled with 5kg collected soil. The 500L distillery effluent (DE) was introduced weekly on 10, 20, 60 and 100% concentration at the specific dilution of 10, 20, 60, 100 ml/kg soil. The soil of each pot was kept moist with effluent concentrations in the course of irrigation. The soil properties were analyzed before and after irrigation with effluent (three irrigation time points). Various physico-chemical parameters were analyzed using standard methods (Buurman *et al.,* 1996) to evaluate the levels of moisture content and EC, (Bouyoucos, 1962) for soil texture, (Carter, 1993) for bulk density, and WHC (water holding capacity). The pH of soil was analyzed with the help of glass electrode pH meter, while concentration of Cl^-, Na^+, K^+, $Ca2^+$, $Mg2^+$, HCO_3^-,NO_3^{2-}, PO_4^{3-} and SO_4^{2-} was determined using standard methods (APHA, 2005). Johnson and Ulrich (1960) method was employed to determine water holding capacity of effluent treated soil.

Biochemical characterization

Distillery effluent derived and molecular characterized bacterial agents *viz. Bacillus cereus* (JN700160), *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* were provided by microbial culture facility, Dept. of Botany, MM (PG) College Modinagar. Biochemical characterization led enzyme profiling was performed as per standard procedure (James, 1983).

Statistical analysis

All experimental sets were performed at least in triplicate (four sets for effluent irrigation model system). Data was analyzed with one way analysis of variance (ANOVA) method to determine the values of experimental difference in irrigation test undergoing different effluent concentration treatment, standard error, linear for soil microbial growth analysis were also calculated with the help of statistical tool (Sigma plot, version 11.0).

Results

The effluent samples of sugarcane molasses based distillery were collected from Modi distillery, located at Modi Nagar (Coordinates-28°49'39"N 77°34'6"E), in sugarcane producing area of western Uttar Pradesh, province comes under north Indian Territory. Modinagar is located in north-east to New Delhi at a distance of 46.2km in Ghaziabad district (Fig.1A). Modi distillery that is located in the mid of city and has acquired a capacity of 26 KLPD alcohol production (Fig. 1B; UP Excise Dept. - 4843KL).

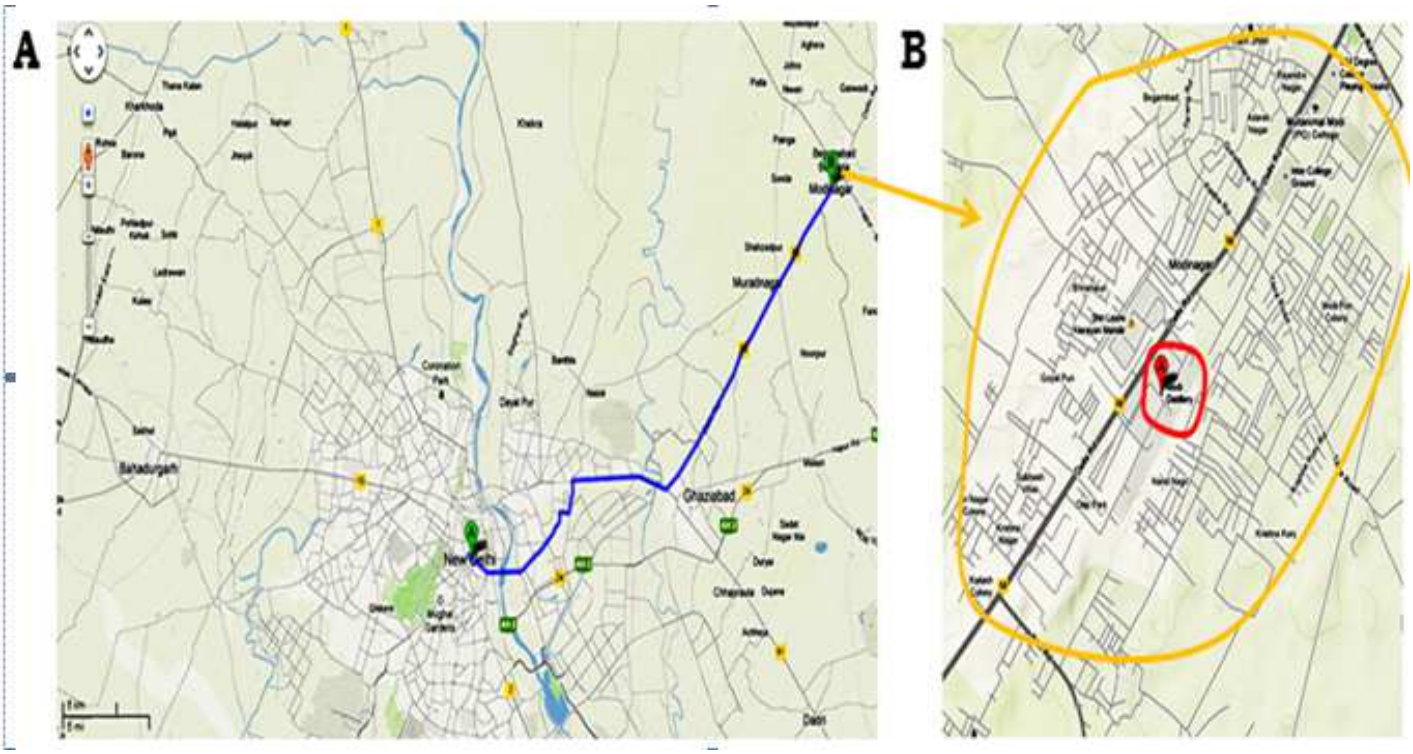

Fig. 1: Schematic map demarking the sample site. A. Map showing location of Modinagar and its distance from New Delhi (distance marked by blue line). B. Map demonstrating Modi distillery in the mid of Modinagar on NH58 (yellow line showing periphery of the city, while red circle denoting position of Modi distillery).

Towards evaluating effect of anaerobically treated distillery effluent on agriculture soil, irrigation of varied effluent concentration (i.e. 10, 20, 60 and 100%) on pot soil samples carried out in comparison to lab tap water (LTW) control (Table 1). In measured analysis, physical characteristics of soil underwent substantial variations in comparison to the control. On gradually increasing concentration of colored effluent, levels of opacity, turbidity and electric conductivity (EC) of the soil were increased; though it lowered soil pH up to 4.7 on absolute effluent irrigation. Treatment of anaerobically treated effluent increased COD and BOD levels of the soil significantly up to 9430mgL^{-1} and 4120mgL^{-1} that found higher than the Indian Standards (I.S.) recommendations for irrigation water. Levels of TS and TDS were found under the recommended level, though levels of TSS were found higher (upto 494mgL^{-1}). Distillery effluent treatment increased hardness (up to 2329 mgL^{-1}) of soil. Irrigated soil was enriched of crucial minerals *viz.* Na^{+}, K^{+} and Ca^{+}, while level of Cl^{-} ion also increased. Increased levels of $NO3^{2-}$, $PO4^{-2}$, $SO4^{2-}$, $CO3^{-2}$ and $HCO3^{-}$ enriched inorganic components of the soil; those has been considered as essentially important factors for the soil

fertility. Thus, irrigation with anaerobically treated distillery effluent at varying concentration gradually increased ratio of minerals and ions in the soil.

In order to investigate intrinsic properties of soil in context of fertility, moisture content of the soil increased on effluent treatment (differential factor- 1.328); while increased water holding capacity reflected accumulation of organic wastes and ions in the distillery effluents (Table 2). Such irrigation practice gradually decreased bulk density of soil. Towards evaluating effect of distillery effluent on soil texture, consistent observations revealed the lighter textured loam profile. Altogether, distillery effluent treatment cumulatively increased vital properties of soil, suggesting increase in soil quality and fertility.

Physio-chemical analysis of distillery effluent treated soil further leads to investigate soil microbial content on varying effluent concentration. On three consequent irrigation, distillery effluent treatment significantly enriched soil microbial population. Irrigation with absolute distillery effluent leads maximum microbial growth (highest number of colony forming units per gram of soil), though it slightly declines on further irrigation (at third irrigation) (Fig. 2; log CFUs per gm of soil). Less diluted effluent concentration harbors greater number of microbial growth. The soil irrigated with laboratory tap water exhibited relatively constant growth at each subsequent irrigation points. The soil with 20% and 60% of the effluent concentration retains higher microbial population at first time irrigation that increased on second irrigation point, though further growth seems static on third point of irrigation (Fig. 2).

Table.1: Table showing analysis of physio-chemical properties of distillery effluent treated agriculture soil at different concentrations. LTW was taken as internal control. Table also showing Indian Standard (I.S.) recommendations for irrigation water

Characteristics	Effluent concentration (%)					I.S. recommendation (Irrigation water)
	LTW (Ctrl)	10	20	60	100	
Color	-	Light pale	Light brown	Brown	Dark brown	Colourless
Turbidity [NTU]	4.12±0.65	11.2±1.2	15.30±1.9	45.21±5.2	71.32±3.4	10
pH	7.6±0.31	7.4±0.21	7.1±0.54	6.3±0.7	4.7±0.8	5.5-9
EC (dS^{-1})	1.31±0.19	3.8±0.65	6.3±0.58	13.4±1.73	18.9±2.1	-
COD(mg L^{-1})	5.21±2.3	943±34	1985±78	5230±89	9430±142	250
BOD(mg L^{-1})	4.32±0.45	365±12.3	932±21	2312±23	4120±43	100
TS(mg L^{-1})	254.34±8.87	289±9.89	398±11.67	1298±22.32	1734±13.23	2100
TSS(mg L^{-1})	12.78±1.98	87.44±3.45	97.78±6.7	223±8.93	494±12.6	200
TDS(mg L^{-1})	201.87±3.22	232.32±8.65	432.43±7.76	1132±13.54	1754±74	1900
Hardness (mg L^{-1})	20.23±2.12	2.76.45±4.55	543.45±7.54	1643±9.43	3239±12.67	600
Na^{+} (mg L^{-1})	6.78±0.98	16.56±3.2	46.74±4.33	165.67±18.56	323.78±22.88	-
K^{+} (mg L^{-1})	5.78±0.72	45.66±3.54	133.87±8.73	323.13.22	545.54±37.4	-
Ca^{+} (mg L^{-1})	34.30±3.76	264.23±9.45	543.53±21.35	1290.56±23.43	2345.56±23.43	20
Cl^{-} (mg L^{-1})	14.32.34±4.32	189.34±9.54	423.86±21.45	987.44±32.34	2156.43±34.54	500
$NO3^{2-}$ (mg L^{-1})	23.67±5.33	184.43±7.53	532.54±9.54	895.46±43.23	1780.67±74.33	100
SO_4^{2-} (mg L^{-1})	12.33±1.32	166.33±9.45	321.32±5.33	756.76±7.44	1386±34.2	1000
HCO_3- (mg L^{-1})	183.54±2.31	189.75±3.55	212.67±6.32	242.32±8.98	312.44±9.43	-

Table.2: Measurement of physical properties of distillery effluent treated agriculture soil at different concentrations. Differential factor showing relative difference in the properties of test and control soil.

Observed Soil properties	Soil prior to effluent irrigation	Soil properties after irrigation with distillery effluent					Differential factor
		Effluent concentration (%)					
		LTW (0)	10	20	60	100	
Moisture Content (%)	48.54±1.87	47.56±2.12	50.56±2.33	54.89±2.1	59.67±3.1	64.35±3.2	1.326
Water holding capacity [WHC](%)	53.23±2.13	53.12±3.2	54.87±2.3	57.43±3.12	61.33±2.33	63.29±3.1	1.189
Bulk density (gm cm^{-3})	1.56±0.21	1.52±0.12	1.48±0.14	1.46±0.23	1.43±0.19	1.42±0.32	0.910
Texture	lighter-textured loam	lighter-textured loam	lighter-textured loam	lighter-textured loam	lighter-textured loam	lighter-textured loam	-

However, distillery effluent treatment enhanced intrinsic soil quality and enriched in mineral and ion concentrations; though higher BOD, COD and hardness along with decreasing pH levels suggested need of processing/ biological treatment of distillery effluent. Three bacterial agents *viz. Bacillus cereus* (JN700160), *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* (derived from M1, MO3 and C2 isolates respectively) provided by departmental microbial culture collection facility were taken further for biochemical characterization. Characterization of enzyme profiles and associated *in situ* efficacy holds the potential of their application in the effluent remediation practice. Three of the representative enzyme classes *viz.* hydrolases, lyases and oxidoreductases contributes significantly to degradation of effluent waste through breakdown of amidic, esteric and peptidic bonds. Likewise, carbohydrases, proteases, oxidase and reductase produced by several bacterial agents are suggested to consume insoluble carbohydrates, proteins and plastic materials (van Wyk, 1999; Singh, 2002; Nakamura *et al*., 2001). Moreover, oxidative enzymes are broadly recognized to transform and detoxify variety of pollutant xenobiotic compounds including phenols, polyphenols and PCBs, dyes, PAHs and azodyes.

In order to evaluate biochemical characterization and enzyme profiling several biochemical assays *viz.* citrate, catalase, starch hydrolysis, carbohydrate hydrolysis (Glucose, Lactose and Mannitol) test, urea hydrolysis test, nitrate, oxidase and gelatin hydrolysis test were performed (Figure S1). All three bacterial strains *viz. Bacillus cereus*, *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* found positive for catalase activity, which being an oxidoreductase enzyme catalyses degradation of a number to recalcitrant effluent compounds (Fig.3A). Another key enzyme of oxidoreductase family i.e. nitrate reductase exhibited catalytic activities in C2 isolate of *Pseudomonas grimontii* (Fig.3B). In the hydrolase family, *Pseudomonas grimontii* (C2 isolate) found positive for urease activity (Fig.3C); while *Bacillus cereus* exhibited glucose oxidase, mannitol dehydrogenase catalytic activity towards utilization of glucose and mannitol energy sources (Fig.3D-E). Biochemical analysis revealed citrate (pro-3S)-lyase, catalase, glucose oxidase, mannitol dehydrogenase, gelatinase enzyme activities in *Bacillus cereus* strain (Table 3).

Identification of their enzyme profiles affirmed that lyases, oxidoreductase and hydrolase are the major classes of enzymes modulating substrate utilization and biodegradation of organic compounds. *Bacillus sp.* MH-I6 exhibited citrate (pro-3S)-lyase, catalase, gelatinase enzyme activity, though glucose and mannitol hydrolyzing activity were lacking. *Bacillus sp.* MH-I6 enzyme profile exhibited lyases, oxidoreductase and hydrolase as major classes contributing to the enzymatic activity. *Pseudomonas grimontii* exhibited catalase, urease, nitrate reductase and gelatinase catalytic activities, belongs to oxidoreductase and hydrolase families. Identification of oxidoreductase and hydrolase in *Pseudomonas grimontii;* while lases, oxidoreductases, hydrolase class enzymes in *Bacillus cereus*, *Bacillus sp.* MH-I6 affirmed potential of their application to improve soil

quality on successive irrigation scheme with distillery effluent.

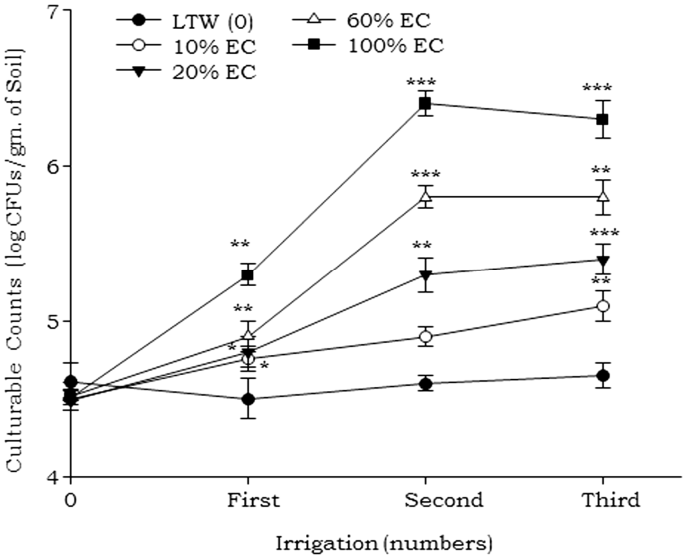

Fig. 2: Graphical representation showing log number of colony forming units (CFUs) per gram of effluent treated soil sample, analyzed on three subsequent irrigation points for varying effluent concentration in comparison to the control. Data shown as means ± SE of triplicate experiments.*p <0.05; **p <0.01; ***p <0.001

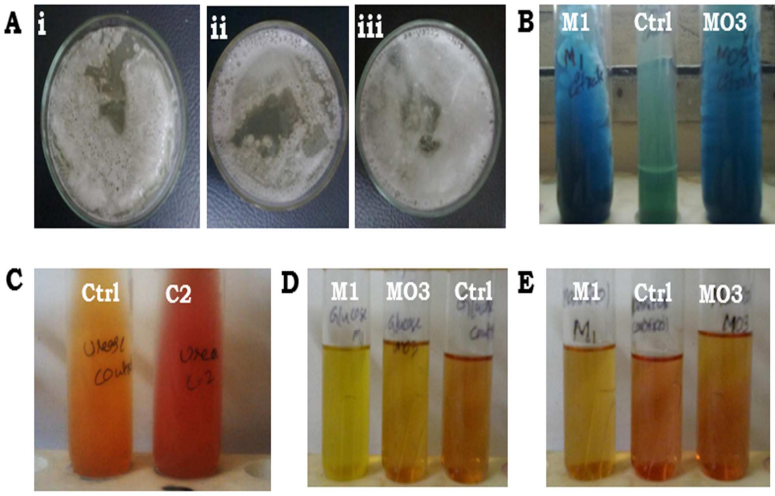

Fig. 3: Biochemical characterization of identified bacterial strains. A. Catalase activity produced by M1, MO3 and C2 isolates, B. M1 and MO3 isolates exhibiting Citrate (pro-3S)-lyase catalytic activity in comparison to the control. C. C2 isolate exhibiting urease catalytic activity in comparison to the control. D-E. M1 isolate exhibiting Glucose Oxidase, Mannitol dehydrogenase catalytic activity in comparison to the MO3 and respective controls.

Table.3: Analysis of enzyme profiling of *Bacillus cereus* (JN700160), *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* bacterial agents listing corresponding enzyme profiles validated through biochemical assays. Table showing enzymes profiles identified in each of bacterial agents and represenative major classes of the enzymes.

Samples	Isolates	Identified bacterial agents	Active enzyme profile	Identified major classes	Performed biochemical tests
MSM-MS-A	M1	Bacillus cereus (JN700160)	citrate (pro-3S)-lyase, Catalase, Glucose oxidase, Mannitol dehydrogenase, Gelatinase	Lyases, Oxidoreductases, Hydrolase	Citrate test, Catalase test, Starch hydrolysis, Carbohydrate hydrolysis (Glucose, Lactose and Mannitol) test, Urea hydrolysis test, Nitrate test, Oxidase test, Gelatin hydrolysis test
MSM-MS-B	MO3	Bacillus sp. MH-16 (JQ068110)	Citrate (pro-3S)-lyase, Catalase, Gelatinase	Lyases, Oxidoreductases, Hydrolase	
MSM-MS-C	C2	Pseudomonas grimontii (JQ282836)	Catalase, Urease, Nitrate reductase, Gelatinase	Oxidoreductase, Hydrolase	

Discussion and Conclusion

The present study demonstrated application of the effluent of the Modi distillery on agriculture soil. Application of distillery effluent increased EC, WHC and moisture content, though bulk density and pH of the soil decreased in effluent concentration dependent manner. Irrigation with effluent leads to increased concentration of the minerals such as Na^+, K^+ and Ca^+, Cl^- and NO_3^{2-}, PO_4^{-2}, SO_4^{2-}, CO_3^{-2} and HCO_3^- ions in the agriculture soil. Application of effluent to the agriculture soil has been suggested to accelerate organic matter decomposition i.e. mineralization of organic complex metals, resulted in greater availability of metals in the soil (Dudley *et al.*, 1986). Presence of minerals, nutrients and trace elements in distillery effluents contributed to the quality

of soil and improvised its composition. Such practice may leads to improved soil fertility and enriched nutrients status at irrigation with lower effluent concentration. Thereby, effluent irrigation enhanced the nutrient status and intrinsic qualities of the soil. Contemporarily, introduction of distillery effluent to degraded soils became a major economical resource towards increase in soil fertility, improved soil structure, texture, water-holding capacity, retention of minerals, ions and nutrients along with increasing bulk density and moisture content (O'Brien *et al*. 2002; Aravena *et al*. 2007; Rato Nunes *et al.* 2008). In addition, long term application of sewage effluent and cotton mill effluents (Narasimha *et al*., 1999) have been shown to increase soil fertility. Increased electrical conductivity and water holding capacity of the effluent treated soil is suggested to be the result of the deposition of organic wastes and minerals (Narasimha *et al*., 1999; Medhi *et al*., 2005; Renukaprasanna *et al*., 2002).

Evaluation of soil microbial population in test soil exhibited a significant increase in numbers, though successive irrigation (third irrigation cycle) appeared to increase soil toxicity. Higher microbial was observed in test soil, reflecting the fact that microbial biomass is proportional to the available organic substrate. This observation is consistent with the earlier finding that suggested a positive correlation between microbial biomass and the availability of organic matter (Nannipieri, 1994). Although, distillery effluent treatment increases soil quality and physio-chemical status, further ensuing irrigation leads to increase soil toxicity and limits microbial growth. Higher COD, BOD levels and presence of heavy metals remained the major limitation in implementation of real time practice. Towards addressing the issue, biochemical enzyme profiling of *Bacillus cereus*, *Bacillus sp.* MH-I6 and *Pseudomonas grimontii* leads to identification of lyases, oxidoreductases, eydrolase class enzymes, which are known to have significant potential in bioremediation applications (Whiteley and Lee, 2006). For instance, bacterial hydrolases derived from *Pseudomonas, Bacillus cereus* and *Nocardia* strains are suggested to transform the pollutants *viz.* parathion and diazinon or carbofuran and carbaryl (Coppella *et al*., 1990; Sutherland et al., 2002; Mulbry, and Eaton, 1991). Likewise, carbohydrases and lyases produced by several bacteria transform insoluble and xenobiotic materials (van Wyk, 1999, Nakamura *et al*., 2001; Singh, 2002). In addition, application of oxidative enzymes widely recognized in numerous process i.e. detoxification of effluent compounds (Torres *et al*., 2003; Durán and Esposito, 2000; Gianfreda and Rao, 2006).

Acknowledgements

We are grateful of Dr. R.C Lal, Principal, MM (PG) College, Modinagar, for constant encouragement and support provided in the present work. Anupama Chaudhary is a research scholar at Department of Botany, Modinagar. Support from Modi distillery staff, Modinagar, is duly acknowledged in providing effluent samples and support in the current investigation. We thank Ms. Richa Yadav for her fruitful suggestions and support.

Conflict of interest:

Author declares no conflict of interest.

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Source of support: Nil
Conflict of interest: None Declared