DISCUSSION
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Captalinganj distillery has been manufacturing industrial alcohol, using molasses as the raw material. The waste water of the distillery is commonly called the "spentwash" or raw effluent. This effluent is further treated in a bioreactor for methane production, which gives double benefits in the form of fuel as well as sharp reduction in pollutants. It is a very safe and economic practice. Further, the effluent of this unit is again treated in oxidation ponds with aerators (flow diagram, Appendix VII). This step requires huge electricity consumption and cost to cut only a small amount of pollutants. It is well documented in the literature and also observed in the present investigation that the large number of inorganic nutrients are present in the distillery effluents with a very high BOD and COD values. However, these pollutants are sharply reduced after bioreactor treatment. Therefore, bioreactor effluent (BE) can be directly used for field application for its safe utilization. In view of the above facts an investigation was undertaken to assess the overall impact of BE on physico-chemical and microbial properties of the soil. However, most of the work have been done on the physico-chemical analysis of the effluent and soil during the course of irrigation in the form of spentwash. Further, a limited work has been done so far on the pollution load arising from different stages of distillery effluents with respect to pollution level, use as fertirigtion and their impact on soil microorganisms.

The present investigation, therefore, envisages two major aims

1. Physico-chemical characteristics of distillery effluents of three stages viz. spentwash, bioreactor effluent and secondary treated effluent (STE).

2. Comparative study of the secondary treated effluent and different concentration of BE on soil microbial properties, the fate of phytopathogenic fungi, nitrogen fixing and biological control agents have been investigated. The role of cellulase and amylase enzyme producing microorganisms have also been evaluated. Unfortunately very little work has been done in India on the
above problems, particularly in respect of distillery waste water. The results obtained in the present study are therefore, discussed and compared with the limited data available in the literature on these aspects.

**Physico-chemical characteristics of the effluents viz. spentwash, bioreactor effluent and secondary treated effluents.**

The colour of the spentwash from Captainganj distillery was found to be dark brown on visual observation which turned blackish and light brown in BE and STE (Table 1). Report of Verma et al., (1974) and Sahai et al., (1983) also recorded the same colour of spentwash in case of Sardarnagar and Saharanpur distilleries. The waste water from distilleries owes its colour due to the presence of humus present in molasses (Jorgensen, 1979). Ohmomo et al., (1998) have stated that dark brown colour of the distillery effluent is due to melanoidin, a colour compound present in molasses. It is a recalcitrant compound and can be degraded by limited number of soil microorganisms. In the present investigation, BE did not show dark brown colour in bioreactor effluent, it indicate that melanoidin decolorizing microorganisms are present in the bioreactor. Moreover, blackish colour of the bioreactor effluent is due to high activity of microorganisms resulting in generation of microbial bio mass and degradative product.

The ISI have laid down the standards that industries must remove as much colour as possible before discharge because the colour reduces the penetration of light into the water thereby affecting the photosynthesis in turn the primary productivity of the aquatic ecosystem. Secondly, the substances in water bodies make complexes with metal ions. Such organometallic compounds have direct inhibitory effect on the aquatic biota. The spent wash has molasses based odour, which is due to unfermented constitutions of residual sugars and other organic compounds in the form of essence present in it. However, a odorless effluent was found in bioreactor due to utilization of unfermented constituents.
The mean pH of the spentwash and BE of Captainganj distillery was found to be 4.2 and 7.6, respectively, which was within the limit of 5.5 – 9.0 recommended by ISI for surface water discharge and on-land irrigation. Jorgenson (1979) has also reported that waste water from molasses based rum distilleries is slightly acidic. The pH of the raw waste from molasses based rum distilleries also ranged from 4 to 5 (Hiatt, 1972 and shea et al., 1974). The pH of spent wash from Saraya distillery, Sardarnagar was also found to be 4.6 (Sahai et al., 1983). The high acidic nature of the spent wash arises due to the use of sulphuric acid for adjusting the pH of the molasses before fermentation. The pH of bioreactor effluent was found to be in the range of 7.3 - 7.7, as methanogenic bacteria utilize the organic acids for methane production (Atlas, 1994).

The mean alkalinity of the spentwash was found to be 841.6 mg/l. This value is however, much above the permissible limit of 15 mg/l as per ISI standards for inland, surface water discharge and on land irrigation. The present investigation shows several folds higher alkalinity in bioreactor effluent and STE as compared to spentwash. Hatt et al., (1972) have reported that spent wash from rum distilleries has an alkalinity (as CaCO₃) of above 1000 mg/l. Alkalinity in the waste water arises due to the combined residual effects of carbonates, bicarbonates and hydroxides, which are used in a distillery for adjustment of pH of the fermentation broth and washing of fermentor etc. Therefore, the acidity and alkalinity concentrations in waste water can be critical factors for the aquatic life.

The solid components in the industrial waste water are generally classified into three main categories viz. (i) dissolved solids (DS), includes solid materials which can pass through 0.4 µm filter paper. It includes both dissolved and colloidal solids (ii) suspended solids (SS), solids that are about 1 µ in diameter and are retained during filtration (iii) total solids (TS), which is the sum of DS + SS. As seen in the results (Table 1a), the mean concentration
of DS, SS and TS in the spent wash, BE and STE were 81733, 29500 and 631 mg/l; 5933, 2766 and 168 mg/l and 87060, 32266 and 800 mg/l, respectively. The decrease in these values in BE was due to settling/sedimentation of the heavier particles during storage of spent wash in the large settling tanks and secondly the large flocculated organic matters are degraded in the bioreactor. Further the bioreactor biomass is separated through clarifier before discharge as effluent, similar phenomenon does occur in the STE.

As against these values in spentwash ISI has set the maximum limit of dissolved solids, suspended solids and total solids in the waste water to be 2100 mg/l, 100 mg/l and 2200 mg/l respectively. The DS and SS in spent wash of Captainganj distillery were considerably higher than the ISI limits Shea et al., (1974) have reported that raw waste (before treatment) from molasses based rum distilleries has total suspended solid in the range of 30,000 to 10,000 mg/l, whereas level of TDS ranged from 70000 to 85000 mg/l. It is extremely important to reduce these higher values of TDS, TSS and DS from the effluent. However, these higher values of TDS, TSS and DS can be reduced in bioreactor.

In the present investigation two cations viz. potassium and sodium were estimated in the spentwash, bioreactor and secondary treated effluents. In bio reactor and STE, a sharp reduction was observed as compared to spent wash (Table 1b). The ISI upper limit for K⁺ and Na⁺ in the industrial wastes is 200 and 60 mg/l, respectively. Potassium is an essential element for most life forms however, Na is considered as essential for only a few plant species, but essential for most animal life. Thus the bioreactor effluent can be used after dilution to achieve the recommended ISI norms for safe utilization, but other parameters like BOD, COD and its impact on beneficial microorganisms must be evaluated.
The spentwash, BE and STE were also analyzed for two important anions species viz. Cl⁻ and SO₄²⁻ (Table 1b). The results indicate a sharp reduction in these contents in BE and STE in comparison with spent wash and satisfy the ISI recommendation. Verma et al., 1977 have also reported higher values of 745 and 560 mg/l for Cl⁻ and SO₄²⁻ in the waste water from Mohan Maliks Distillery, Ghaziabad, U.P. The higher concentration of these cations and anions along with phosphate, nitrate and trace elements can result in excessive algal growth (eutrophication) in receiving water bodies and when this growth ceases it can exert an oxygen demand which, may cause killing of water animals.

The dissolved oxygen (DO) in the spentwash and BE was found to be zero, while it was extremely low (1.03 mg/l) in STE (Table 1a), which exert anaerobic condition in the ponds or river. It has been reported that when the dissolved oxygen level drops below 5 mg/l, desirable species of fishes are adversely affected. Below an oxygen level of 2 mg/l, fishes disappear and environment shifts towards anaerobic life (Middle brooks, 1979). Considering the above, it is essential that spentwash be made free of organic matter to recover from the oxygen depletion state. Since BE also shows zero level of DO, due to anaerobic life as well as utilization of oxygen during decomposition of organic matter in bioreactor. Inspite of a nil DO, the low Organic load favours the further utilization of this effluent for the safe disposal in agriculture land.

**BOD₅ and COD of the effluents**

The mean BOD₅ and COD values of the spentwash, BE and STE from Captainganj distillery were found to be 46666, 4316 and 405 and 104130, 34666 and 3200 mg/l, respectively. The COD was found to be higher than BOD (Table 1a). The reduction in BOD was 90.7% in BE as compared to spent wash and further a sharp reduction of 90.6 %, in STE was observed when compared to BE. Similar findings have also been reported by most of the
workers (Verma et al., 1977, Devrajan et al., 1994 Chhonkar et al., 2000). The ISI has set an upper limit of 30 and 100 mg/l for discharge into inland surface water and on -land irrigation, respectively. The BOD of Captainganj distillery is considerably higher than the recommended upper limits. Likewise the COD value of the BE and STE is also considerably higher than ISI recommended upper limit of 250 mg/l for industrial effluent discharge into inland surface water. It is thus clear that spent wash needs further elimination through proper treatment devices before discharge and the distillery has set up a bioreactor and oxidation pond with aerators to cut down the pollutants of the effluent.

Both BOD and COD are the measure of organic matter in the waste water. BOD values give the pollution strength of waste water in terms of oxygen required to oxidize or convert organic matter to a non-putrescible product. It measures the O₂ consumed by living system (microorganisms), which utilize the organic matter in the waste water. The COD is an alternative to the BOD. It measures the quantity of oxygen required to oxidize the organic materials that can be oxidized chemically and it is therefore, higher than BOD, as has been observed in the present study (Table 1a)

Distilleries are forced to invest huge energy and cost in secondary treatment to further reduce the pollution to achieve the norm of BOD₅ (30 mg/l), which so far is not achieved anywhere in the world. The norm of BOD (100 mg/l) for disposal of treated effluent on land is also not yet achieved in Indian conditions. Almost all the distilleries have secondary treatment plant, but the power requirement is so high that the same is beyond the scope of distillery to bear this burden.

In a workshop on “Pollution control in distilleries” jointly organised by FICCI and AIDA on 17th Oct, 1987, Commounder Narendra Singh, Advisor, Ministry of Energy, Department of non-conventional energy sources, doubted the wisdom of spending huge amount for secondary treatment without any
success in achieving the norms. He also emphasized that distillery effluent should be used as ferti-irrigation after bioreactor treatment when the BOD of the effluent is brought down to about 5000 mg/l.

**Effect of distillery effluents on soil microorganisms**

In the present investigation, an attempt was made to see that how different stages of distillery effluent affects soil microbial population. The ecology of microbes in distillery effluent treated soil may also solve many problems of pollution by effective utilization as manure. Such studies are extremely useful in the evaluation of long term effects of industrial waste water on soil, particularly with reference to changes in the fertility of agriculture land. The results obtained in the present study on the effect of Captainganj distillery effluent from different units viz. spentwash, bioreactor effluent and secondary treated effluent (STE) on microbial population are briefly discussed in the light of literature available.

The results of the present investigation clearly indicate that 30 and 40% bioreactor effluent significantly increased the population of beneficial microorganisms as compared to untreated soil. These findings clearly satisfy the statement of captain Mahendra Singh, advisor, Govt. of India, Ministry of Energy, 1987, not to spend much cost and energy in STE treatment for those industrial effluents which do not have higher toxic values for soil microorganisms rather provide growth substrate for soil microorganism. A higher population of fungi in same treatments was observed in BE treated soil in both years and seasons. Inspite of high pH (7.6) of BE, detrimental for the growth of fungi, there was increase in the population of fungi upto 40% BE treatment. The BE pH is suitable for the growth of bacteria and actinomycetes, hence bacterial population increased significantly in 30 and 40% BE treated soil in both the years and seasons.
Recent concern about environmental quality and recognition of the role of the soil has created a new awareness and appreciation of soil microorganisms. The majority of bacteria, actinomycetes and fungi are saprophytes as organic matter decomposers. These organisms bring about the hydrolysis and oxidation of organic compounds through their enzymes resulting in production of simple chemical compounds. The microorganisms which are of immense importance in the maintenance of soil fertility, are greatly influenced by the soil type, physico-chemical properties, depth of the soil and nutrient status (Ramaswami, 1956, Gupta et al., 1980). The findings of present investigation also show a significant change in the population of bacteria, fungi and actinomycetes in the BE and STE treated soils (Table 3 - 6). A detailed study was made to see the effect of different concentration of BE effluent on the soil microorganisms in general, and specific microorganisms such as the thermotolerant/thermophilic cellulolytic and amylolytic microorganisms etc., nitrogen fixing, biological control agents and phosphate-solubulizing microorganisms in particular.

A similar trend of increase/decrease in the microbial population in most of the treatments was recorded, in both the years. This trend proves that the native soil microorganisms are affected by the effluents. Alexander (1974) and Griffen (1972) have reported imbalances and disturbance of microbial ecosystem in polluted soils. The importance of both the microflora and micro fauna in the treatment of waste water is well documented (Curds and Hawkes, 1975). These organisms may not only utilize the organic matter as a food source, but also reduce the quantity of organic matter to form a less noxious and more stable substances, defined as sludge. It is clearly indicated in the table that BE and STE treated soil have significantly ($P = 0.05$) increased the soil microbial population in 20, 30 and 40% BE treated soils.

In bioreactor effluent a diversified biota of facultative and strict anaerobes have been reported (Crowther and Harkness, 1975). However,
anaerobes are completely inactivated when BE is applied in the field. Therefore, soil microorganisms get easily metabolizable carbon and nitrogen source from microbial biomass generated during methanogenesis that led to increase in number of soil bacteria, fungi and actinomycetes.

In STE, actively growing aerobic microflora of aquatic origin (Hawkes, 1963), when applied to soil, there is competition between these microorganisms and native soil microorganisms, where the organic matter is also less as compared to BE. Hence, the population of microorganisms did not increase in the same magnitude as observed in BE treated soil.

In addition, the bioreactor sludge, serves as a fertilizer and soil conditioner, because of its beneficial chemical and physical properties. These properties include a high organic content, including humus (Mitchell et al., 1977), and a capacity for increasing soil aggregate stability (Epstein, 1975). However, sludge may contain pathogens (Doran et al., 1977, Strauch, 1977), and heavy metals, such as Cd, Cu and Zn which may be toxic to plant and animals. Chaney et al., 1977).

**Effect of effluents on phytopathogenic fungi and biological control agents**

In the present investigation a significant ($P = 0.05$) reduction in the population of pathogenic fungi has been observed (Table 19). The phytopathogenic fungi viz. *Rhizoctonia solani*, *Fusarium oxysporum* *Pythium ultimum* and *Sclerotium rolfsii* were greatly reduced in the soil treated with 40% BE. This could be due to the fast growth of other saprophytic microorganisms, that were able to produce higher amount of extracellular cellulase, amylase, proteins, ligninase and other enzymes required for the decomposition of organic matter in the soil. The microbial biomass is also higher in BE, however, the lipase, protease and other enzyme producing microorganisms might have increased and might have antagonised the
pathogenic microorganisms. It is clearly proved from an experiment in the present investigation that *Trichoderma harzianum*, a potential biocontrol agent against *Rhizoctonia solani* was not affected to 40% BE treated soil and also suppressed disease significantly (*p* = 0.05) in the soil experiments. (Table 20)

The survival of the pathogens depends on a number of factors such as concentration in the effluent, application rate, soil types and soil conditions, including temperature and moisture. These factors determine the rate of pathogen in activation (Gudding and Kroyztd, 1975; Morrison and Martin, 1977). The role of the biota affecting these changes has not been fully ascertained. Although there is some information on the microflora and their enzyme production in natural ecosystem (Miller, 1974, Varanka et al., 1976). The higher microbial population and the suppressions of pathogenic fungi in BE treated soil shows the dominance of saprophytic fungi in terrestrial ecosystem. Most of the fungi isolated from treated soil showed thermotolerant and produced cellulase and amylase enzymes at higher temperatures (Fig 1& 2). The higher microbial population in the effluent treated soil indicate the higher decomposition of organic matter in the soil, which play an important role in soil fertility. In moderate quantities, these materials provide texture that is favorable for plant growth. Furthermore, many organic compounds form complexes with mineral nutrients (chelation) that enhance uptake by plants. Mineralization of carbonaceous and organic nitrogenous compounds of detritus and humus is the most important process in returning the essential nutrients to soil and maintaining its fertility (Garret,1981). Heterotrophic microorganisms including bacteria, fungi and actinomycetes play an important role in the sequential process of detritus formation, humification and mineralization. Darmwal and Gaur, (1988) reported an associative effect of cellulytic fungi and Azosprillum lipoferum on yield and nitrogen uptake by wheat both, yield and nitrogen uptake were increased significantly. In the present study, it was attempted to investigate the fate of enzyme producing microorganisms in the effluent treated soils. A higher number of thermotolerant / thermophilic
cellulase and amylase producing microorganism with higher production of enzymes was observed at concentration of 20 30 and 40 % BE and STE while at high concentration of BE (50%), a reduction in populations was reported (Table 11, Fig.1&2). The major reason for significant reduction in microbial population at the higher concentration of effluent in the soil may be attributed to lac of oxygen in the soil.

Although a number of investigators have found changes in the physical and chemical properties of soil after sludge application (Epsteen et al., 1976, Trout et al 1976), but very limited information is available regarding the impact of BE on Survival of saprophytic beneficial soil microorganisms in soil. In a study, fungal biocontrol agent, *Trichoderma harzianum* significantly decreased the population of a phytopathogenic *Rhizoctonia solani* and disease incidence in soil. Further, the treatment of BE from 20 - 40% did not damage the population of *T. harzianum*, while *R solani* population and disease reduced significantly in those treatments (Table 20). Similarly, phosphate solubulizing fungus, *Aspergillus niger* was also not affected in BE treated soils, while population was significantly increased in the treatments (Table 21).

From the analysis of the species diversity of various fungi in effluent treated soil, it emerged that various species of *Aspergillus* and other saprophytic fungal species were predominated by suppressing the phytopathogenic populations (Table 15-16). Effluent disposed on land affects the Physico-chemical characteristics of soil. These physico-chemical factors influence the qualitative and quantitative changes in microorganisms in the soil. Bacteria, fungi and actinomycets are generally found to be more in soil rich in organic matter (Alexeunder, 1978).

Organic matter and pH are two important factors that affect the soil microbial population. Organic carbon content of the soil shows highly significant and positive correlation with the bacterial and fungal population,
which is understandable, because of heterotrophic nature of these microorganisms. Organic matter is a natural substrate for saprophytic microorganisms and also provides nutrients to plants indirectly through the activity of decomposition. Soil fungi mostly use organic residue easily but their numbers vary in soil, depending upon weather the species has a dormant or reproductive phase in the soil environment. *Aspergillus, Trichoderma, Rhizopus, Humicola, Chaetomium* are some of the fungal genera, that can utilize and decompose the cellulase, hemicellulose, lignin starch and humic acid like substrates of organic matter. Fungal population show abundance in soil having organic carbon range of 0.5 to 1.0%, but the bacterial population increases in the soil containing organic carbon greater than 1.0 % (Gupta & Tripathi, 1988). In the present study, maximum bacterial population was due to higher leaves of carbon. On the other hand fungal and actinomycetes population also increased.

Nitrogen, phosphorous, potassium and sulphur are other elements of organic matter which can be utilized and accumulated by microorganisms in soil. Population of bacteria increased significantly with increased level of nitrogen and phosphorus, however, fungi was least affected with these factors (Badiyala et al., 1990). Bacteria capable of oxidizing inorganic sulphur compounds could be either aerobic or anaerobic ie., *Thiobacillus* and *Beggiatoa*. *Aspergillus* and *Pencillium* have also been reported to be sulphur oxidizers microflora may also release soluble inorganic phosphate (H₂PO₄) into soil through solubulization and decomposition of phosphate rich organic compounds. Many fungi ie., *Aspergillus, Pencillium* and bacteria, *Bacillus* and *Pseudomonas* are potential solubilizers, of bound phosphates. (Gaur and Algawadi, 1989 and Darmwal, et al., 1989). Calcium is another soil factors that can influence the microbial population. A significant and positive correlation of bacterial numbers and Calcium was reported by Gupta et al., (1980) but fungal population was not influenced significantly by Calcium content of the soil.
It was observed that organic matter rich effluent improved the nutrient status as well as physical and chemical conditions of soils in the vicinity of distillery industry for favorable growth of microorganisms.

**Effect of effluents on seed germination**

The seed germination was not affected upto 40% BE treatment, while further increase in concentration (50%) showed a slight suppression in germination. These findings are consistent with the observation of other investigators who have worked on effluents of distillery and pulp and paper mill on seed germination of a number of crop species including paddy, finger millet (Jowar), bajra and other crop species (Sahai *et al.*, 1983, Somasekhar *et al.*, 1984, Dixit, 1989, Choudhary *et al.*, 1987 and Sahai 1986). Somasekher *et al.* (1984) also observed that undiluted pulp and paper mill and other industrial effluents inhibited crop growth, but dilution showed promotion in root and shoot growth. The reduction in growth was attributed to the toxic effects of effluents such as the presence of heavy metals, excess/deficit levels of micro nutrients, decomposition products as well as soil porocity and aeration. In the present study high concentration of BE inhibited the germination which is consistant with the observations made by Raza and Vijayakumari (1989), while working on effect of liquor factory effluent on germination and growth of paddy seedlings.

**Effect of effluent on nitrogen fixing bacteria and plant growth promoters**

In present investigation, the plant growth promoting rhizobacteria e.g. *Azotobacter* and *Rhizobium* population were estimated in effluent treated soil. A positive correlation was observed in the population with both the bacteria in almost all the treatments except 50% BE treatment. The population of *Azotobacter* was higher as compared to *Rhizobium* population in all the treatments, shows the competent behavior of the bacterium. The possible
reasons for increased population of *Azotobacter* and *Rhizobium* spp. in the treated soils are

(i) Increased nutrient status of the effluent treated soils.

(ii) Shorter generation time and rapid growth of this PGPR in soil.

*Azotobacter*, which is free living nitrogen fixer, has exceptionally high respiratory rates than other aerobic bacteria. *Azotobacter* species also produce resting cells known as cysts that are quite resistant to desiccation but not to heat (Atlas, 1994).

In this study, a significant (*p = 0.05*) increase in the growth of wheat plants and yield was reported in soil amended with *Azotobacter* as compared to soil alone (Table 23-24). It seems that *Azotobacter* is contributing nutrient or other growth promoting substances to the wheat plant. This strain of *Azotobacter* could be of value for crop seeds planted into STE and 40% BE treated soils, both as protectant of plant root against soil pathogens (Garret, 1981) and as a selective soil recolonizer in BE treated soil along with other beneficial microorganisms to create the favorable effect of the treatment. Further work is needed to ascertain the above preliminary observations. Several workers, Baker, (1988) and Nair et al., (1990) have also reported an increased plant growth response in the presence of PGPR in the soil, where the pathogenic population was suppressed. These factors directly or indirectly were favorable for plant growth and yield of the crops (Katan, 1987). Microbial shift alone can not be responsible for increased growth response but this phenomenon can be attributed to a combination of biotic and abiotic mechanisms which may vary in different types of soils. (Ganliyal and Katan, 1991). Thus, the involvement of other factors related to increased growth response such as mycorrizae association, phytotoxic decomposition products and plant growth promoting substances should not be excluded (Broadbent, et al., 1977, Baker, 1988) and their role should be assessed with great accuracy. Besides these factors other biological and physical aspects of soil and crop ecology must be examined in ardor to determine the basis for increased plant growth response in effluent treated soil with suitable dilution.
SUMMARY

Captaienganj distillery is manufacturing industrial alcohol using molasses as the raw material. The waste water of distillery is commonly called "spentwash". This effluent is further treated in a bioreactor for methane generation which gives double benefits in the form of fuel as well as sharp reduction of pollutants. It is a very safe and widely used economic practice. Further, the effluent of this unit is again treated in oxidation ponds with aerators. This step requires huge electricity consumption and cost to cut only a small amount of pollutants. It is well documented in the literature and also observed in the present investigation that the large number of inorganic nutrients are present in the distillery effluents with a very high BOD and COD values, therefore, bioreactor effluent (BE) can be directly used for field application. In view of these facts, an investigation was made to assess the overall impact of BE on physico-chemical and microbial properties of the soil. Findings of the present investigation are summarized in this text.

1. Physico-chemical characteristics of the effluents and experimental soil.

The colour of spentwash was dark brown which changed to blackish in bioreactor and light brown in secondary treated effluents (STE). The temperature of spentwash was 82°C and subsequently reduced in bioreactor effluent (38°C) and further reduced to 26°C in secondary treated effluent. The pH of the spentwash was highly acidic (4.2) and increased to 7.6 and 7.5 in BE and STE, respectively. The alkalinity of bioreactor effluent was more than five times higher than spentwash which increased further in secondary treated effluent as compared to bioreactor effluent.

The total solids and total dissolved solids were reduced by 63% and 64% in bioreactor effluent as compared to spentwash. The total suspended
solids (TSS) was 5933 mg/l in spentwash which sharply decreased in STE. The percent reduction in TSS was 53% in BE as compared to spentwash, while 94% reduction was observed in secondary treated effluents. The spentwash and bioreactor effluent did not contain dissolved oxygen (DO), whereas STE contained 1.03mg/l.

The high BOD and COD of spentwash was reduced sharply by 90.7 and 67%, respectively in bioreactor effluent and further reduced to 90.6% in STE as compared to BE. The inorganic contents like nitrogen phosphorous, potassium and sodium were also decreased in BE treatment. The chloride concentration of spentwash was very high as compared to sodium and sulphate. However, sulphate was greatly reduced as compared to calcium and chloride in bioreactor. A similar trend was also observed in STE. The physico-chemical properties of field soil was also analysed. The percentage of sand, silt and clay was 52, 33 and 15% respectively. Water holding capacity was 45% and pH was found to be 7.0. Nitrogen and organic contents of the soil were 0.1% and 0.6% w/w, respectively, while organic matter was 1.0% w/w.

II. Effect of Effluents on soil Microbial population

In this investigation STE was used without dilution, while BE was used in 10, 20, 30, 40 and 50% dilutions to see the effect on soil microorganisms. In 1997 summer experiments, the population of bacteria fungi and actinomycetes of soil was greatly influenced by the effluents treatment. The population of bacteria was significantly increased in STE and 10, 20, 30 and 40% BE treated soil as compared to control (soil without treatment). Further increase in concentration (50%) decreased the bacterial population. Similar trend was also observed with fungal population. The population of actinomycetes was found to be maximum in 30% BE treated soil. In 1997 winter, also showed the similar trend of increase and decrease in population with slight change from 1997 summer results.
In 1998 summer, the population of bacteria and actinomycetes was greatly influenced in soil treated with different concentration of effluents. The maximum population of bacteria, fungi and actinomycetes was found in 30% BE treated soil. The population of fungi was not much affected in all the treatments. The population of microorganisms in 1998 winter also showed the same trend of increase as found in the previous year.

(a) Population dynamics of thermotolerant/thermophilic microorganisms.

In the summer 1997, the population of thermotolerant/thermophilic microorganisms was maximum in 40% BE treated soil as compared to control. The population of bacteria and fungi did not changed significantly in STE and 10 and 20% BE treated soil. In general, no significant change in the thermotolerant/thermophilic bacterial population was recorded in all the treatments, while fungi and actinomycetes population did not show the same trend of population as observed in bacterial population. In 1997 winter, there was no significant difference observed in the population of thermotolerant/thermophilic bacteria, fungi and actinomycetes in 20, 30 and 40% BE treated soils. In general, population of bacteria, fungi and actinomycetes was lower in winter as compared to summer experiments. In 1998 summer and winter, almost similar trend with slight fluctuation in the population of bacteria, fungi was recorded, while actinomycetes population in winter was higher as compared to the summer experiments.

(b) Population dynamics of cellulolytic and amylolytic microorganisms and enzyme production

In general the population of cellulolytic bacteria (CB), fungi (CF) and actinomycetes (CA) was increased significantly in BE and STE treated soil in both the seasons, as compared to control. The population of CB recorded 1.07 to 1.4 fold and 1.3 to 1.6 fold increase as compared to control in the summer.
and winter of 1997, respectively. In 1998, the maximum population of CB was recorded in 20 and 30% BE treated soil in summer and winter, respectively. The population of CB was significantly (P = 0.05) higher in all the treatments except 50% BE treated soil in summer as compared to control, while in winter, the population increased significantly in all the treatments. With regard to cellulolytic fungi, the population increased significantly (P = 0.05) in 20, 30 and 40% BE treated soil in the summer as compared to control.

In summer and winter of 1997, in general the population of amylolytic (AB), fungi (AF) and actenomycetes (AA) was significantly increased in BE and STE treated soils in the summer as compared to the control, while in winter, AB showed higher population only in 20, 30 and 40% BE and STE treated soils. In 1998 summer, the population of AB was significantly higher in all the treatments except 50% BE and STE treated soils, while AF, increased in all the treatments in both the seasons. The population of AA increased in all the treatments in the summer except 50% BE and STE treated soils.

The extracellular cellulase producing fungi, viz. *Aspergillus niger*, *Trichoderme viride*, *Humicola gresia* and *Penicillium citrinum* isolated from 40% BE treated soil showed maximum cellulase production at 40 - 45°C, whereas least activity at 25°C, while further increase in temperature (50°C) decreased the production of enzyme by all the fungi.

For extracellular amylase production from four saprophytic fungi viz., *Aspergillus niger*, *Humicola gresia*, *Penicillium citrinum*, *Chaetomium globosum*, isolated from 40% BE treated soils, was examined at 25 to 50°C. Production of amylase by all test fungi showed an increasing trends up to 40 and 45°C, and thereafter declined sharply.

(95)
(c) **Occurrence of fungi in bioreactor effluent treated soil:**

In summer and winter of 1997 and 1998, number of genera and species of saprophytic and phytopathogenic fungi were recorded from soil and 40% BE treated soils. The number of phytopathogenic fungi such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium ultimum*, *Alternaria alternata*, *Macrophomina phaseolina*, *Fusarium Oxysporum*, *F. solani* *F. semitectum* and *Curvularia lunata* were greatly reduced in 40% treated soil in both summer and winter, but no pronounced reduction in the population of saprophytic fungi was observed when compared to non treated soil. Eventhough, the population of some saprophytic fungi viz. *Aspergillus niger*, *A. nidulans*, *A. regulosus*, increased many fold in 40% BE treated soil as compared to control in both the seasons. A similar trend was also observed in 1998 experiments with slights variations.

The thermotolerant/thermophilic fungal species were also recorded in 1997 and 1998, summer and winter in the same soil from where the total population of microorganisms was recorded. Among the thermotolerant/thermophilic fungal species, almost all were saprophytic and their number did not decrease in 40% BE treated soil in both the years and seasons.

**III Effect of effluents on population of phytopathogenic fungi and disease incidence.**

The population of *Fusarium oxysporum* and *Rhizoctonia solani* was greatly reduced in 40% BE treated soil. A minimum reduction of 35 and 25% in the population of *Fusarum* and *Rhizoctonia* was observed respectively in 10% BE treated soil. A marked reduction in wilt disease of chickpea, caused by *F. oxysporum* and damping off of tomato by *R. solani* was observed in 20, 30, 40 and 50% BE and STE treated soils, as compared to untreated soil. The untreated soil showed 60 and 58% diseased plants affected by *F.oxysporum* and *R. solani* respectively. A significant (P = 0.05) reduction in disease was also
reported in the soil treated with STE and 10% BE treatments as compared to control, but maximum reduction in the population and disease was observed in 40% BE treated soil, where a significant decrease in population was noticed.

**IV Effect of effluents on biological control agents**

The effect of effluents on survival and biological control ability of *Trichoderma harzianum* against *Rhizoctonia solani* population and disease incidence in combination with increasing concentration of BE effluent. In general, the population of *T. harzianum* was not affected in all the treatments rather a slight increase in the population was observed. The population of *R. solani* was significantly (*P* = 0.05) reduced in the soil treated with *T. harzianum*. In another combination, *T. harzianum* + STE treated soil also showed a significant reduction in the population of *R. solani* when compared with soil + *T. harzianum* treatment. A marked reduction in disease (50%) was observed in the soil treated with only *T. harzianum* as compared to the soil without any treatment. However, incidence of disease was not recorded in soil treated with 20, 30 and 40% bioreactor effluent.

**V Effect of effluents on population of phosphate solubilizing fungus.**

The various effluents were applied to study their effect on the population of phosphate solubulizing fungus, *Aspergillus niger*. The population of this fungus was not significantly (*P* = 0.05) affected in all the treatments except 50% BE treated soil, where a significant decrease in the population was noticed.

**VI Effect of effluents on seed germination of crop plants.**

Germination of wheat, maize and gram was recorded daily and percent germination was calculated. It was recorded that 10 to 40% concentration of BE and STE did not show any inhibitory effect on germination of all the crop
seeds (96 - 98%). Germination was, however, inhibited with 50% BE concentration in which 78, 71 and 75% germination of wheat, maize and gram were recorded, respectively.

VII  Effect of effluents on plant growth promotion

Effect of effluents on the soil population of *Azotobacter* and *Rhizobium* sp was also studied in 1997 and 1998. The population of *Azotobacter* increased significantly in all the treatments except in STE treated soil in the year 1997, whereas there was a significant increase in all the treatments as compared to control in 1998 experiment. However, there was no significant difference in the population of *Azotobacter* among 30 and 40% BE treated soils in both the years. A similar trend was also observed with the population of *Rhizobium* in the soil in both the years except in 40 and 50% BE treated soil, where the population decreased significantly as compared to other treatments in 1997 experiment.

For plant growth promotion study, wheat crop was selected to see the effect of effluents on growth response of the plant. In this experiment, the plant growth promotion was measured in terms of plant height, plant dry weight, seed number, seed dry weight and root dry weight basis. All the growth parameters were significantly (P = 0.05) increased in 30 and 40% BE treated soils as compared to control and soil treated with *Azotobacter* only. The population of *Azotobacter* recorded from root rhizoplane did not vary significantly in all the treatments except 50% BE treated soil.