DECOLOURISATION OF PULP PLANT EFFLUENT

Thesis submitted in part fulfillment of the requirements for the degree of
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BY

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CERTIFICATE

This is to certify that the thesis entitled **"DECOLOURISATION OF PULP PLANT EFFLUENT"** submitted in part fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in ENVIRONMENTAL SCIENCES** to the **TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE** is a record a ***bonafide*** research work carried out by **Ms.D. ANANDAVALLI** under my supervision and guidance and that no part of this thesis has been submitted for the award of any degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

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Environmental pollution has become a global concern. The continuous disposal of untreated pulp plant effluent on land and water causes aesthetic pollution and affects the soil-water ecosystem. A number of methods of colour removal such as ultrafiltration, lime coagulation, adsorption on activated carbon etc., have been developed. As several operational difficulties are encountered in these methods, these are not being adopted by industries.

In this contest, a detailed investigation was carried out to evolve possible, effluent specific decolourisation technologies, which could be adopted in field situations. The native ligninolytic fungal microflora were isolated from effluent enriched soil. Their bioremediation efficiency with special reference to decolourisation was studied and was compared with the known standard cultures. The possibility of using moringa seeds (*Moringa oleifera*) for removing colour of the effluent was also investigated. With an aim

of evaluating the soil as an adsorbent, the coloured effluent was treated using columns packed with red and sandy soil. The changes in soils physical, chemical and biological properties were evaluated after the passage of effluent to assess the impact.

The results revealed that the microbial dynamics of soil did not change under the treated effluent irrigation. The enzymatic assay of the soil before and after effluent irrigation revealed that the dehydrogenase, phosphatase and urease activities did not significantly change, with the effluent irrigation. In addition, an increased enzymatic activity was evidenced in most of the blocks under effluent irrigation.

There were 12 ligninolytic fungal cultures which were isolated, among whom the fungal cultures C5, C8, C11 and C12 were identified as efficient ligninolytic strains. The culture C8 registered the highest colour reduction of 59.9 per cent in the presence of 0.3 per cent glucose. The fungus *Fusarium* sp., in the presence of 0.1 per cent diammonium sulphate, registered the highest colour removal of 53.6 per cent, during 16 days of incubation with the effluent.

The decolourisation potential of the immobilised fungal strains packed in column under aerated effluent with 0.3 per cent glucose ranged from 86.8 to 87.8 per cent during 12 hours of retention time.

Moringa seeds (*Moringa oleifera*) at different doses of application significantly reduced the colour in all the treatments. A maximum colour reduction of 94.7 per cent was achieved with 40g of moringa seeds / 150 ml effluent. A decrease in the pH of the effluent with simultaneous increase in EC was evidenced under moringa seed treatment.

The evaluation of the physical, chemical and biological properties of the various segments of the packed soil column revealed that there had been varied impact under the two types of soils investigated.

In this present investigation, the bioremediation efficiency of ligninolytic fungal cultures, the treatment with plant product *viz.* moringa seeds and passage through living soil columns have been established to be good options for pulp plant effluent. The above could be exploited effectively for decolourising and recycling of the pulp plant effluent which would result in least environmental impact on the soil-water ecosystem.

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INTRODUCTION

I. INTRODUCTION

Rainfall is the main source of ground water recharge. In addition, to some extent, river courses and irrigation tanks also help in recharge of groundwater. Constant and heavy inflow of coloured industrial effluents into water body is suspected to raise its colour concentration, as well as the level of other pollutants, thereby turning the colour of the water to dark brown. When such secondary sources are polluted, the ground water recharged by them also gets polluted.

Effluent from pulping units, apart from causing aesthetic pollution, also affect the soil and water ecosystem. Presence of toxic organic chlorine substituted phenolic compounds, chlorinated lignins acetones, dioxins, furans, chlorobenzenes and non-chlorinated organic compounds cause toxicity (Gergov *et al.*, 1988; Boominathan and Reddy, 1992).

In recent years, discharge of treated pulp plant effluents on land is increasingly being recognized not only as an alternate method of effluent disposal but also as a source, of water and nutrients to crops (Kannan *et al.*, 1990). Possible environmental problems associated with the application of effluent to soils are contamination of surface and ground water, plant uptake and toxicity to soil biota (Kookana and Rogers, 1995). In addition, leaching of chromophores (lignin and its derivatives) to underground water has also given rise to concerns about land application (Kannan and Oblisami, 1990). The change in colour of the ground water is not only an aesthetic problem but is a visual indication of contamination. Presence of these colour imparting compounds in water bodies, inhibits photosynthesis and increases the biological (BOD) and chemical oxygen demands (COD), which further leads to insufficient availability of oxygen to sustain aquatic life (Choudhury *et al.*, 1998).

Lignin derived molecules are present in pulp effluent in a fully dissolved state and therefore escape through most of the physical and chemical treatment processes. Colour removal technologies such as coagulation / precipitation or ultrafiltration are considered expensive. In addition settled sludges always pose the problem of disposal and these are effective for removing high molecular weight compounds only (Bryant and Barkley, 1991; Bajpai and Bajpai, 1994).

Conventional biological treatment methods are less efficient and scarcely effective for decolourisation (Manzanares *et al.*, 1995; Choudhury *et al.*, 1998). Therefore reducing the colour of pulp plant effluent using cost-effective technology remains to be explored. Considering the paucity of information regarding effective decolourisation of pulp plant effluent and its use as a irrigation source in agricultural land, the present study has been conducted.

Objectives

1. To assess the impact of pulp-plant effluent on soil biological system
2. To study the basic characteristics of the viscose effluent with special reference to the colour
3. To find out the soil tolerance limit for chromophore adsorption
4. To screen the native fungal flora from effluent irrigated soil
5. To study the effect of the standard cultures and isolated cultures on effluent colour reduction and
6. To assess the possibility of using moringa seeds in wastewater treatment with special reference to colour removal.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

2.1. Introduction

Environmental pollution has become a global concern. An awareness of environmental problems and potential hazards caused by industrial wastewaters has prompted many countries to limit the discharge of certain toxic effluents. The toxic pollutants include acids, alkalies, oils, fats, floating, dissolved organic matter and colouring agents. Industries which are contributing to such pollutants are pulp and paper plant, distilleries, dye, iron and steel, tannery, etc., (Saravanan *et al.*, 1998). Among these, effluent from pulp plant is one of the major environmental bug bear in India, contributing to soil and water pollution. The problem of colour removal from pulp plant wastes has been a subject of study during the last few decades. A number of colour removal methods have been developed such as ultrafiltration, lime coagulation, adsorption on activated carbon and polymeric adsorbents etc. Since it encountered several disadvantages such as high cost, large sludge volume, development of toxic secondary pollutants, operational difficulties etc., (Milstein *et al.*, 1987), they lost their popularity among the industries especially in developing countries like India. Considering these aspects, the need for colour removal, different methods used in past and present and their efficiencies in colour removal reuse of treated effluent on land and the fate and behaviour of toxic compounds in soil are reviewed hereunder.

2.2. Decolourisation of pulp plant effluent

2.2.1. Origin of colour in the pulp plant effluent

Pulp is extracted from different cellulosic materials like wood of *Eucalyptus*, Bamboo etc., while making pulp (Kraft method) the chipped cellulosic raw materials are digested with sodium sulphate, sodium hydroxide and sodium sulphide. The process thus loosens the cellulosic fibres and dissolves the lignin, resin and other cellulosic materials. The fibre is separated from the spent liquor (Black liquor) washed, dewatered and bleached.

Of the total pollution load of the pulp industry, the spent liquor constitutes 10 to 15 per cent only, but amounts for 80 per cent of the colour, 30 per cent of the biological oxygen demand (BOD) and 60 per cent of the chemical oxygen demand (COD). The main contributors to colour of the liquor are polymeric chlorinated and heavily oxidised degradation fragments of lignin (Marwaha *et al.*, 1998). Since the colour of the effluent is mainly due to lignin and its derivatives, the characteristics of the effluent vary from industries to industries based on the raw materials used and method of pulping engaged (Gergove *et al.*, 1988; Johnson *et al.*, 1993; Kookana and Rogers, 1995; Satyendra and Modi, 1999).

In Kraft process, lignin is converted into thio- and alkali- lignin, whereas in sulphite process it is converted into ligno-sulphonates. But, in general the effluent exhibits high chemical oxygen demand (COD), high suspended solids and dark brown colour (Subrahmanyam and Mohan Rao, 1973; Upadhyaya and Singh, 1991).

Kannan and Oblisami (1990) and Upadhyaya and Singh (1991) have reported that the brown colour of the pulp plant effluent is mainly due to lignin – based compounds derived from the blow-heat condensate, pulp-decker washing, chlorine and alkali bleach waste and foul evaporator condensate. Whereas the double bond conjugated with aromatic ring, quinone methides and quinone groups are the main contributor of the brown colour to the effluent have been reported by Sankaran and Van-Ludwig (1971).

In addition, Klibanov *et al.* (1983) and Davis and Burns (1992) stated that the brownish discolouration of receiving stream is mainly due to the constant discharge of phenolic and resinous compounds from pulp plants.

2.2.2. Need for colour removal

The untreated and partially treated viscose factory effluent, by its brown colour, causes aesthetic problem and contaminates the surroundings by their toxic components (Belsare and Prasad, 1988; Sreenivasulu *et al.*, 1999). It makes the down stream water unfit for domestic and irrigation purposes (Bajpai and Bajpai, 1994). Due to the absorbance of sunlight by their chlorolignin compounds, the natural process of photosynthesis gets inhibited in streams (Bajpai and Bajpai, 1994). In addition, due to the oxygen starvation, the receiving body is unable to sustain aquatic life (Vandenbusch and Sell, 1992; Choudhury *et al.*, 1998).

There are several reports of the presence of chromophoric substances such as lignin and its derivatives in pulp plant effluents (Roald, 1977; Stuthridge *et al.*, 1990; Bajpai and Bajpai, 1994). The component of pulp plant bleaching effluent mainly responsible for the mutagenicity is produced by chlorolignins (Nazar and Rapson, 1980).

In addition, toxic compounds like tetrachloro-o-benzoquinone, trichloroguaicol, tetra chloroguaicol, mono and dichloro dihydroabietic acid, epoxy stearic and dichloro stearic acid have also been reported in the pulp plant effluents (Das, 1969; Leach and Thakore, 1975). Harmful effects of pulp plant bleaching effluents on plant and soil biological property and on the fishes, copepods and other aquatic forms have been reported by many workers (Nampoothery *et al.*, 1976; Roald, 1977).

2.2.3. Decolourisation studies: the past and present status

The various microorganisms employed so far in the field of decolourisation are given in table 2.1. The requirement of nutrients for optimum culture condition and also the per cent colour removal by various fungi has been reviewed in detail below.

2.2.3.1. Role of co-substrates

2.2.3.1.1. Carbon

Lignin alone cannot serve as the sole substrate for the fungal system (Eaton *et al.*, 1982). Addition of carbon sources *viz.*, glucose, sucrose, cellulose etc., are essential for colour removal especially for ligninolytic fungi.

Table. 2.1. Microorganisms employed for decolourisation of pulpplant – effluents and waste waters containing phenolics:

	Organism		Reference
I.	**Bacteria**		
	Bacillus cereus	-	Bourbonnais and Paice, 1987
	Nocardia	-	Marchand, 1978
	Pseudomonas aeruginosa	-	Bourbonnais and Paice, 1987
	Pseudomonas ovalis	-	Sundman and Selin, 1970; Kawakami and Kanda, 1976
	Pseudomonas putida	-	Marchand, 1978
	Corallina	-	Marchand, 1978
	Cyanobacteria	-	Bharti *et al.*, 1992
II.	**Fungi**		
	Aspergillus niger	-	Kannan, 1990
	Aspergillus spp.	-	Tono *et al.*, 1968; Archibald *et al.*, 1990
	Lentinus edodes	-	Vinciguerra *et al.*, 1995
	Penicillium spp.	-	Tono *et al.*, 1968; Archibald *et al.*, 1990
	Phanerochaete chrysosporium	-	Eaton *et al.*, 1980; Eaton *et al.*, 1982; Huynj *et al.*, 1985; Prouty, 1990; Sayadi and Ellouz, 1992
	Polyporus versicolor	-	Marton *et al.*, 1969
	Schizophyllum commune	-	Ramaswami, 1987; Belsare and Prasad, 1988
	Tinctoporia borbonica	-	Fukuzumi, 1980
	Trametes versicolor	-	Livernoche *et al.*, 1983; Royer *et al.*, 1985; Bajpai *et al.*, 1993; Martin and Manzanares, 1994; Mehna *et al.*, 1995
	Trichoderma spp.	-	Martin and Manzanares, 1994
	Bjerkandera sp.	-	Moreira *et al.*, 1998
	Heterobasidion annosum, Pleurotus ostreatus	-	Choudhury *et al.*, 1998
III.	**Algae**		
	Microcystis sp.	-	Lee *et al.* 1978.

As carbon source glucose, sucrose, cellulose, primary, secondary sludges from effluent treatment plants, brewery and sugar refinery wastes have been tried by various workers and achieved good colour reduction (Eaton *et al.*, 1982, Livernoche *et al.*, 1983; Huynj *et al.*, 1985; Royer *et al.*, 1985; Prasad and Joyce, 1991; Ramaswamy, 1987; Belsare and Prasad, 1988; Archibald *et al.*, 1990, Royer *et al.*, 1991).

The problem of colour removal from pulp plant wastes has been a subject of study during the last few decades. A number of colour removal methods have been developed, such as ultrafiltration, lime coagulation, rapid land infiltration, adsorption on activated carbon and polymeric adsorbents etc., and these physicochemical methods have operational difficulties and are rather expensive, whereas the conventional biological methods such as activated sludge processes and aerated lagoons are effective in reducing the pollution load due to other parameters except colour. It is mainly due to the low nutrient content and to the toxicity caused by the presence of phenolic compounds (Royer *et al.*, 1985).

Bajpai and Bajpai (1994) have reported that the type and the amount of carbon sources for the effective colour removal vary from organism to organism. By using the fungi *Aspergillus* spp. and *Penicillium* spp. 70 – 80 per cent of the colour has been removed in seven days (Tono *et al.*, 1968; Kannan, 1990). In regard, they used glucose as the carbon source. Similarly, Morton *et al.* (1969) had achieved seventy per cent colour reduction in pine Kraft black liquor in 3 days with *Polyporus versicolor*, in the presence of simple sugar, glucose. Prasad and Joyce (1991) also reported 60 per cent colour reduction in the E_1 stage (extraction stage) effluent using *Trichoderma* sp. with glucose as carbon.

It is reported that the glucose addition @ 1 per cent (w/v) triggered the decolourisation potential of *Phanerocheate chrysosporium* (Satyendra and Modi, 1999).

Using mycelial pellets of *Trametes versicolor*, 93 per cent colour reduction was obtained within 3 days in shake flask cultures. And the fungus exhibited the following order of co-substrate efficiency glucose > malt extract > pulp = sucrose > carboxymethyl cellulose > micro crystalline cellulose (Bajpai *et al.*, 1993; Martin and Manzanarer, 1994, Mehna *et al.*, 1995; Modi *et al.*, 1998).

Eriksson *et al.* (1986) have reported that the supplementation of a critical low concentration of glucose in the effluent to induce the activity of sugar oxidising enzymes, which oxidise the sugar-producing hydrogen peroxide, required for lignin degradation.

About 15 strains of white rot fungi have been screened by Livernoche *et al.* (1983) and he has achieved 60 per cent colour reduction (in bleached Kraft effluent) using *Coriolus versicolor* within 6 days in the presence of sucrose.

About 80 per cent colour reduction was achieved in pulp plant effluent in 7 days (Ramaswamy, 1987; Belsare and Prasad, 1988). The decolourisation efficiency of the fungi (*Schizophyllum commune*) has been reported in following order of different carbon sources: Sucrose (60 per cent) > Glucose (48 per cent) > Cellulose (35 per cent) > Pulp (20 per cent) (Belsare and Prasad, 1988).

Archibald *et al.* (1990) have attempted the use of inexpensive sugar from refinery or brewery wastes as substrate for decolourisation, by *Coriolus versicolor*. Kadam and Drew (1986) stated that recalcitrant carbohydrates such as micro crystalline cellulose is the second best carbon source, while other workers reported simple monosaccharides particularly glucose as the second best carbon source.

2.2.3.1.2. Nitrogen

Unlike carbon, the addition of nitrogen source in the effluent for decolourisation was not found to be critical. Variable results were noted when the effect of nitrogen on decolourisation of pulp plant effluents was studied has been reported by different workers. Kannan (1990) observed an enhanced rate of decolourisation in the presence of carbon and nitrogen supplements. But he had not mentioned the role of nitrogen specifically on decolourisation of the effluent.

The rate of decolourisation by *Trichoderma* sp. was found to be stimulated by the addition of glucose but not with nitrogen was reported by Prasad and Joyce (1991). The efficiency of colour removal by *Schizophillum commune* (Belsare and Prasad, 1988) and *Trametes versicolor* (Modi *et al.*, 1998) in glucose supplemented effluent was reported to further improved by addition of ammonium chloride and ammonium nitrate respectively.

In several white-rot fungi, a positive correlation has been reported between colour reduction and the nitrogen limitation (Keyser *et al.*, 1978; Jeffries *et al.*, 1981; Crawford and Crawford, 1984; Dodson *et al.*, 1987; Martin and Mazanares, 1994). While Chang *et al.* (1983) found a significant increase in the efficiency of colour removal by *P. chrysosporium* upon supplementation of a small quantity of nitrogen. Whereas neither a decrease nor any increase in the rate of colour reduction was observed in the presence of nitrogenous substances by *Trametes versicolor*. The reduction in efficiency of colour removal in the effluent when supplemented with inorganic nitrogen, has been reported by Martin and Mazanares (1994).

2.2.3.1.3. Micronutrients

In addition to carbon and nitrogen sources, the decolourisation process has also been influenced by sulfur, trace metals and oxygen concentration in effluent. Whereas it is strongly repressed by glutamate and other amino acids was reported by Eaton *et al.* (1980); Bajpai and Bajpai, (1994).

Net consumption of chromophores by *Coriolus versicolor* was enhanced in the presence of Mg^{2+} ions. These ions are known activators of many oxidases that play a role in delignification of effluents by ligninases (Royer *et al.*, 1985).

Kirk *et al.* (1986) reported a 1.7 fold increase in ligninolytic enzyme activity following an incorporation of a sixfold excess of a trace metal solution containing Mn, Mg, Fe, Co, Ca, Zn, Cu, Mo and Al in the culture medium of *P. chrysosporium*. Synergistic effect between nitrogen and mineral micronutrients in the presence of Tween-80, has been reported by Vasudevan (1984).

2.2.3.1.4. Hydrogen ion concentration (pH)

The optimum pH for colour removal by the fungus varies with the initial culture condition. For example, the decolourisation process appears to be less sensitive to decrease in effluent pH; if the fungus is first allowed to grow in optimum pH range at 4.3 to 4.8, then decolourisation is not adversely gets affected even at pH 3.0, contrary to that if direct decolourisation is attempted, it is seriously retarded at pH below 4.0 or above 5.0, due to poor fungal growth (Eaton *et al.*, 1980). Whereas the pH range from 6 to 8 was reported as optimum pH by Tono *et al.* (1968). But, predominantly pH range from 4.0 to 5.5 was reported as optimum pH for efficient colour removal by many workers (Royer *et al.*, 1985; Belsare and Prasad, 1988; Prasad and Joyce, 1991; Royer *et al.*, 1991; Bajpai *et al.*, 1993; and Mehna *et al.*, 1995).

2.2.3.1.5. Temperature

The temperature range between 25 and 30°C has been reported as optimum condition for colour removal for most of the fungi (Tono *et al.*, 1968; Eaton *et al.*, 1980; Royer *et al.*, 1985; Belsare and Prasad, 1988; Bajpai *et al.*, 1993 and Mehna *et al.*, 1995). Whereas the temperature range of 35 – 40°C was reported as optimum condition for colour removal by white rot fungi, *Phanerocheate chrysosporium* (Satyendra and Modi, 1999).

2.2.3.1.6. Culture condition

Most of the decolourisation works have been carried out in batch experiment both under shake and still culture. In still culture, as there is an availability of long contact period between mycelium and lignin, efficient colour reduction can be achieved (Eaton *et al.*, 1982). Agitation is generally used to increase the rate of gas exchange between atmosphere and culture medium. Kirk *et al.* (1978) reported that an initial period without agitation is needed to avoid severe inhibition of lignin degradation; as the agitation results in pellets formation and strongly suppresses ligninolytic activity.

2.2.3.1.7. Air / oxygen tension

The oxygen partial pressure has a defined profound effect on the rate and extent of lignin degradation but not on the growth of the organism was reported by Kirk *et al.* (1978); Bar-lev and Kirk; (1981). Further, lignin is degraded much faster in the presence of oxygen than air and that ligninolysis is not observed in sub atmospheric (5 per cent) partial pressure of oxygen (Kirk *et al.*, 1978). In a 100 per cent oxygen atmosphere, the lignin degradation was 2 to 3 fold higher compared with that in air (21 per cent oxygen). Yang *et al.* (1980) observed that the cultures of *P. chrysosporium* reduced lignin levels in pulp when maintained under 100 per cent oxygen instead of air. Molecular oxygen is expected to induce the ligninolytic enzymes in *P. chrysosporium* were reported by Reid and Seifert (1980).

2.2.3.2. Recent development in colour removal processes

2.2.3.2.1. Mycor process (Table 2.2)

The immobilisation of microbial cells on solid supports is an important biotechnological technique; this novel technique has been employed in various biotechnological applications. Bioreactors using immobilised microbial cells have several advantages over traditional fermentation techniques. The problems encountered with MyCoR-RBC (rotating biological contact disk) technique are short fungal life times of

5 to 7 days, wastages of biomass, and requirement of high oxygen tension. Therefore, an alternative treatment process using aerated reactor was developed (Prouty, 1990). However, certain internal problems related to foaming, settling rate of fungal solids, precise oxygen transfer requirement of the fungus to calculate exact aeration rate need to be studied before the aerated reactor system recommended for commercial decolourisation.

2.2.3.2.2. Decolourization of pulp plant effluent using enzymes (Table 2.2)

The decolourisation potential of certain enzymes can be exploited for treating phenolic pulp plant effluents. These enzymes oxidise phenolics to aryl-oxy radicals, which polymerise spontaneously to form insoluble complexes, by making their removal easier by simple precipitation, filtration or centrifugation (Alberti and Klibanov, 1981).

Even though entrapment of these enzymes in insoluble polymers (L-tyrosine) by co-polymerisation enhanced the decolourisation efficiency, about 20-30 per cent greater performance was observed by batch culture of *Coriolus versicolor* over entrapped laccase (Davis and Burns, 1990).

However, enzymic decolourisation of effluents has certain merits over fungal treatment in that (1) oxidative treatment of effluent employing fungus requires glucose as co-substrate and adjustment of pH to 4.5, which will add to the cost of the process and (2) dilution of added effluent is necessary before fungal treatment in contrast to enzymic treatment in which the enzyme to substrate ratio determines the extent of decolourisation and is independent of dilution (Klibanov *et al.*, 1983).

Table 2.2. **Decolourisation of pulp plant effluent using mycelial biomass and ligninolytic enzymes**

I. Mycor process (Mycelial colour removal)

S.No.	Particulars	Per cent colour removal	Reference
1.	White rot fungus *Phanerocheate chrysosporium* immobilised on rotating biological discs (RBC). Suspended cellulose was used as carbon source	60	Eaton *et al.*, 1981.
2.	The fungus *Coriolus versicolor* immobilised in calcium alginate beads. Sucrose was used as carbon source.	60-70	Royer *et al.*, 1983; 1985.
3.	The fungus *Coriolus versicolor* immobilised in the beads of calcium alginate gel. Sucrose was used as carbon source.	80	Livernoche *et al.*, 1983.
4.	The fungus *Phanerochaete chrysosporium* immobilised in ropes of cotton, jute and wheat straw.	71-97	Satyendra and Modi, 1990

II. Enzymatic decolourisation

S. No.	Particulars	Per cent colour removal	Reference
1.	Laccase	2.60	Forss *et al.*, 1987.
2.	Immobilized phenol oxidase, laccase and horseradish peroxidase along with *C. versicolor* in alginate beads.	70.80	Davis and Burns, 1990.
3.	Peroxidase	50 – 60	Hakulinen, 1988.
4.	Laccase and Mn-dependent peroxidase	65	Kadhim *et al.*, 1999.

2.2.3.2.3. Wetland treatment of pulp plant wastewater

Constructed wetlands are catching attention in the last two decades. These are one of the brighter new water treatment, offer low maintenance, require little energy are quite stable and provide very good secondary or tertiary treatment.

Constructed wetlands have been installed in many countries to treat a wide variety of effluents from industry and agriculture (Thut, 1989; Wolverton, 1982). In USA wetland ponds, each 1430 m^3 in area have been utilized to treat wastewater from a pulpplant. The ponds were operated at a depth of 46 cm and detention times were 2 and 10 days. Out of ten ponds, six of the ponds were planted with Cattails (*Typha lattifolia*) and three with bulrush (*Scirpus acutus*). One of the pond was filled with large stone. After the period of 10 days, 5-10 per cent reduction in colour was achieved. Whereas, at 2 days detention time, 2-8 per cent reduction was achieved (Moore *et al.*, 1994). In addition, the author reported that cattail ponds recorded maximum colour reduction over 10 days retention time followed by bulrush.

The comparison of decolourisation by different organisms shows that white rot fungi are suitable for efficient degradation of the recalcitrant chromophoric material in bleach plant effluents. However, the requirements for high oxygen tension and a growth substrate, constraint the practical implementation of fungal decolourisation. Therefore, under present situation (to meet the industrial demand) an alternative, low cost biological treatment methods involving efficient microbial consortia are needed to be developed.

2.3. Land treatment

Discharge of pulp plant effluents on land is considered not only as an alternate method of effluent disposal but also as a resource of water and nutrients to crops

(Kannan *et al.,* 1990). This method relies on soil processes to ameliorate potentially toxic components (Marchand, 1971; Johnson and Ryder, 1988). Effluent from pulp plants is a rich source of organic matter, nitrogen, phosphorus, calcium, magnesium and trace elements (Kannan and Oblisami, 1990) and consequently, the application of pulp effluents on land is becoming a common practice.

But, due to the presence of toxic organic carbons (TOCs) in the effluent and the possible environmental problems associated with the application of chlorinated lignin derived compounds to soils; contamination of ground and surface waters, plant uptake and phytotoxicity, entry into the human food chain and toxicity to soil biota has given rise to concerns with regard to the land application (Kannan and Oblisami, 1990). Therefore, it is essential to know the fate of (sorption, desorption and degradation) these compounds in soil together with their impact on soil biological system, prior to land treatment. Due to the lack of information regarding pulp plant effluent disposal to land, many of the studies reviewed here refer to sources of TOCs from other industrial activities rather than pulp plant effluent.

2.3.1. Reuse of pulp plant effluent in Agricultural land

In India, it was reported that crops such as rice, wheat, sugarcane, groundnut, onion and tree species like casuarina, eucalyptus and neem are irrigated with treated pulp plant effluent (Subrahmanyam *et al.*, 1984).

Irrigation of wheat, oat, corn, alfalfa and beans with fully treated secondary effluent (from a pulp plant in California) gave yield better than the average has been reported by Narum *et al.* (1979). The increased growth and nutrient composition in pasture followed by spray irrigation with pulp plant effluent was reported by Johnson and Ryder (1988).

Similarly, Abasheyeva *et al.* (1993) reported that application of treated pulp plant effluent to pasture grasses for years did not affect yield and nutrient content of the pasture. In addition he reported that the long term irrigation of pasture grass and lucerne for eight years with pulp plant effluent did not develop any toxicity problem. However, irrigation with untreated pulp effluent has been reported to adversely affect yields of potatoes and field beets over a three year period (Sev and Papazov, 1971).

2.3.2. Fate and behaviour of toxic organic compounds in soil after land treatment

The toxic organic compounds in the effluent include a large number of individual chemicals (Table 2.3) which have been categorised as alkanes / alkenes, chlorinated acids, chlorophenols, chlorobenzenes, dioxins, furans, chloroligno compounds, terpenes, sterols and non chlorinated organic compounds (Kookana and Rogers, 1995). The sorption and other related properties of these compounds are given in Table 2.4.

A variety of physicochemical mechanisms / forces, such as van der Waals, H-bonding, dipole-dipole interactions, ion exchange, covalent bonding, ligand exchange, cation bridging and water bridging can be responsible for sorption of organic compounds in soils (Senesi and Chen, 1989). Several of these mechanisms or forces may operate in tandem causing the sorption of a compound to the soil surfaces (Koskinen and Harper, 1990). Chemical characteristics, such as polarity, ionic nature, functional groups and solubility, determine the nature of bonding mechanisms as well as the extent and strength of sorption.

Considering the potential toxicity, bio availability, mobility and ground water contamination, the most important groups in pulp plant effluents are chlorinated alkanes / alkenes, chlorophenols and chloroligno compounds.

Table 2.3. **Important organic compounds found in pulp plant effluent, following bleaching with hypochlorite, chlorine dioxide, and secondary biological treatment**

Compound	Conventional Cl bleach process g t^{-1} pulp	50-60% ClO$_2$ substitution g t^{-1} pulp	Concentration in aerobically (activated sludge / aerated lagoon) treated effluents (g L^{-1})
Chlorinated phenols	1.4 – 4.8[b]	0.4[b]	0.1[b]
2,4-dichlorophenol	0.3 – 0.5[a]	0.4[a]	-
2,4,5-trichlorophenol	0.8 – 1.1[a]	0.6 – 0.7[a]	-
Tetrachlorophenol	2.1 – 2.2[a]	0.4[a]	-
Pentachlorophenol	1.0 – 1.3[a]	1.4[a]	-
Chloroguaiacols	5.6 – 17.3[b]	0.8[b]	0.2[b]
4,5-dichloroguaiacol	1.6 – 3.3[a]	7.6 – 9.0[a]	-
3,4,5-trichloroguaiacol	10.1 – 10.7[a]	6.8 – 7.9[a]	-
4,5,6-trichloroguaiacol	0.8 – 1.2[a]	1.2[a]	-
Tetrachloroguiaiacol	3.7 – 4.9[a]	1.1 – 1.3[a]	-
Chlorocatechols	5.9 – 34.0[b]	0.5[b]	0.1[b]
4,5-dichlorocatechol	2.6 – 2.7[a]	3.1 – 3.9[a]	-
3,4,5-dichlorocatechol	16.1 – 18.9[a]	12.0 – 16.4[a]	-
Tetrachlorocatechol	5.2 – 8.4[a]	1.3 – 1.7[a]	-
Chloroalkanoic acids, Chloroalkanes, Chloroalkenes			
1,2-dichloroethane	0.3[c]	-	-
1,1,2,2-tetrachloroethane	2.0[c]	-	-
Pentachloroethane	0.1[c]	-	-
1,2-dibromo-3-chloropropane	0.2[c]	-	-
1,4-dichlorobenzene	0.1[c]	-	-
Chloroform	9.8 – 32.6[b]	1.8[b]	0.7[b]
Chlorovanillins	0.4 – 2.6[b]	0.2[b]	0.1[b]
Chloroacetones	06 – 71.1[b]	0.0[b]	0.0[b]
Dioxins			-
TCDF	0.000318[c]	0.000021[c]	0.41[c]
TCDD	0.000024[c]	0.00000815[c]	
Chlorinated terpenese	70-600[d]	-	-
Chloro – lignins	3360[bf]	-	-
Non-chlorinated organics			
Toluene	0.4[c]	-	-
m,p-Xylene	0.9[c]	-	
Naphthalene	0.8[c]	-	
Dibutylphthalate	3.0[c]	-	

(Source : Kookana and Rogers, 1995)

[a] Axegard (1986); [b] Gergov *et al.* (1988); [c] Carlberg *et al.* (1986); [d] Stuthridge *et al.* (1990); [e] Johnson *et al.* (1993); [f] O'Connor and Voss (1992); [g] Chlorolignin estimates as 80% of AOX (O'Connor an dVoss, 1992), using AOX figure of 4.2 kg t^{-1} for hypochlorite bleached birch pulp (Gergov *et al.*, 1988).

Table 2.4. Properties of TOCs (Toxic organic compounds) in different soils

Compound	Water solubility mg L^{-1} (25°C)	PK_a*	Log K_{ow}	Sorption in soil (log K_{oc})
Chloroalkanes / alkenes				
1,1,1-trichloroethane	4400-5500[a]		2.49[c]	2.26[c]
Trichloroethylene (TCE)	1000-1100[a]		2.29[c]	2.03[c]
Tetrachloroethylene, Perchloroethylene (PCE)	140-150[a]		2.60[c]	2.56[c]
Chlorophenols and Chloroguaiacols				
2-chlorophenol	28,000[c]	8.55[l]	2.15[c]	3.69, fine sediments 3.6, coarse sediments[c] 1.71 clay loam[c]
4-chlorophenol	27,000[c]	9.43[l]	2.39[c]	1.85, clay loam soil[c] Negligible, sandy aquifer[c] 1.69[b]
2,4-dichlorophenol		7.85[j]	3.23[j]	2.25[b] 2.42[j] 2.68[b]
2,4,6-trichlorophenol		6.15[j]	3.72[j]	2.92[j]
2,3,4,5-tetrachlorophenol		6.35[j]	4.87[j]	3.03[b] 4.12[j]
Pentachlorophenol		4.75[j]	5.24[j]	4.59[j]
4,5,6-trichloroquaiacol tetrachloroguaiacol				4.15[b] 2.80[h] 2.85[h]
Veratroles				3.02[h]
Chlorobenzenes				
Chlorobenzene			2.71[d]	2.41[h] 2.59[h]
1,4-dichlorobenzene	87[c]		3.38[b]	2.86 2.44 silt loam 2.59 fine sand 2.84 in five soils
1,2,4-trichlorobenzene	16.3 (23°C)[l]		4.05[d]	3.37[d]
1,2,4,5-terachlorobenzene			4.72[d]	3.93[d]
Dibromochloropropane	1230[o]		2.43[o]	6.50[h]
2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD)	7.9 x 10^{-6}[g]	-	6.15[o]	6.60[k] (6 soils)
Dibutyl phthalate	11.2[c]	-	4.72[c]	3.14, New York soil
Naphthalene			3.30[c]	2.94[c] (17 soils)
o –xylene			3.13[o]	2.34[o] 2.25[h]
Toluene	550 (20°C)[n]		2.69[d]	2.0[m] at low initial conc. 2.4[m] at high initial conc.
Vinyl chloride	2763[c]	-	1.38[c]	0.4 in standard soil 1.74, calculated from solubility[c]

Source : Kookana and Rogers, 1995)

*pK_a = pH at which 50% of a TOC is dissolved; k = sorption coefficient; K_{ow} = Octanol

[a] Urano and Murata (1985); Lee *et al.* (1991); [c] Howard (1989); [d] Cited from Schwarzenbach and Westall (1981); [e] Cited from Grathwohl (1990); [f] Ware (1988b); [g] Adams and Blaine (1985); [h] Meylan *et al.* (1992); [i] Chiou *et al.* (1986); [j] Schellenberg *et al.* (1984); [k] Walters and Guiseppi – Elie (1988); [l] Dean (1987); [m] Jim and O'Connor (1990); [n] Windholz (1983); [o] Szabo *et al.* (1992); [p] Garbarini and Lion (1986).

2.3.2.1. Chlorinated phenols (CPs)

CPs have received the most attention with regard to their presence in pulp plant effluents. The concentration in untreated effluent ranges from 0.3 to 1.4 g t^{-1} of pulp. Whereas, quaicols and catechols have been found at much higher levels, ranging from 1 to 20 g t^{-1} (Axegard, 1986). Concern has been expressed due to their toxicity and potential for bioaccumulation (Brownlee *et al.*, 1993).

2.3.2.1.1. Sorption and mobility

Sorption of CP_s is dependent on the degree of chlorine substitution and resultant hydrophobicity. Lagas (1988) noted an increasing CP sorption coefficient with increasing chlorine substitution. Sorption of CP_s in soils is also affected by pH. In the common soil pH range, most CP_s will totally or partially ionise, affecting their solubility, sorption, transport and bioavailability. The PK_a (pH at which 50 per cent of a TOC is dissociated) of CP_s varies from 4.7 to 9.4 and decreases with an increase in Cl substitution (Lagas, 1988).

2.3.2.1.2. Role of ion – pairing mechanisms

Molecular ion pairing mechanisms, when organic ions paired with inorganic counter ions, get transferred to the organic phase (Maklnen *et al.*, 1993), may also be important in CP sorption. Rao *et al.* (1990) and Lee *et al.* (1991) found that ionic strength increased the sorption of CP_s logarithmically, emphasizing the importance of ion pairing mechanisms.

2.3.2.1.3. Competition for sorption by other CP_s

The presence of other TOC_s in solution and the competition between different organic compounds might be significant in multi-sorbate mixtures, such as those occuring in effluent disposal systems or waste disposal sites. Rao *et al.* (1990). Therefore, the sorption of individual TOC_s should not be considered in isolation.

2.3.2.1.4. Release of bound CP_s

In addition, they demonstrated, release behaviour of radioactive TOCs and catechol bound to humus as a result of microbial activity; only 12.4 per cent was released during an incubation period of 13 week. Dec and Bollag (1988) have reported that once TOCs are bound to OM, very little is subsequently released.

2.3.2.1.5. Colloids and organic macromolecules affecting sorption and mobility

Organic matter (OM) is the most important sorbent for the CPs present in molecular form. However, iron oxides and to a lesser extent, the aluminium oxides, have also been reported to sorb CP_s (Kung and McBride, 1991).

Bengtsson *et al.* (1993) studied the relative mobility of PCP (Penta Chloro phenol) in the presence of colloids and macromolecules in the solution. He reported that in the presence of colloidal bacteria or macromolecules the relative mobility of PCP can be increased by a factor 2.

2.3.2.1.6. Studies on mobility of CPs in soils

Helling (1971) studied the movement of 2,4-dichlorophenol on thin-layer chromatography plates, packed with five different soils. The author reported that this compound showed an intermediate mobility compared to some herbicides, *viz*., atrazine and diuron.

2.3.2.2. Halogenated alkane / alkenes

The concentration of these commonly occurring compounds in untreated effluent ranges from < 0.1 g t^{-1} of pulp and up to 10 g t^{-1} of pulp as reported by Carlberg *et al.* (1986). However, part of this loading may be removed during biological treatment mainly by adsorption (Jokela *et al.*, 1993). The toxic effects of halogenated alkanes / alkenes on soil biota are poorly understood and require further research.

2.3.2.2.1. Sorption and mobility

Chlorinated alkanes / alkenes are highly soluble, strongly acidic, and have relatively low affinity for soils. They are generally more mobile than chlorine substituted aromatics (Jokela *et al.*, 1993).

The sorption of halogenated alkanes / alkenes by soils is generally low, the process may not be reversible. The irreversibility or partial reversibility of sorbed compounds can have significant bearing on their persistence and transport behaviour in soils (Calberg *et al.*, 1986) and requires further research.

2.3.2.2.2. Vapor phase movement

As chloroalkanes / alkenes are volatile in nature (VOC_s), they can reside in the vapor phase of soil. Migration via gas diffusion may also result in rapid spreading of the contaminant (Farrell and Reinhard, 1994).

2.3.2.2.3. Mobility in field soils

Kookana and Rogers (1995) reported that the transport and fate of 13 organic pollutants had been examined by Wilson and his coworkers in 140 cm long column packed with a sandy soil, low in organic matter (0.078 per cent organic carbon). Numerous field studies have shown that halogenated alkanes / alkenes are freely mobile in the soil environment.

2.3.2.2.4. Contamination of groundwater

Halogenated alkanes / alkenes have been commonly found in ground waters all over the world. Trichloroethylene (TCE), trichloroethane (TCA) and perchloroethylene (PCE) are reported as the common groundwater contaminants in U.S.A. and Netherlands. All these contaminants are mainly from land filling sites (Newsom, 1985). In contrast, Emmett (1993) reported that chloroform, PCE, TCE and other organochlorines reached groundwater at a depth of 2 m within a limestone aquifer in South Australia as a result of the disposal of pulp plant effluents into the unlined drain. Approximately, 0.26 mg l^{-1} of each of chloroform, PCE and TCE was observed in the water of the worst affected wells, located in the vicinity of an effluent drain. The foregoing clearly shows that halogenated alkanes / alkenes present in pulp plant effluent have the potential to contaminate ground waters if the effluent is disposed on a soil with low organic matter content.

2.3.2.3. Chloroligno compounds

Chlorinated lignin compounds are a major component of pulp plant effluent, responsible for colour of the effluent, come from the bleach process (Van Loon *et al.*, 1994). They are an ill-defined group of compounds containing partially degraded chlorinated lignin fractions and up to 30 per cent polysaccharide. Lignocompounds are characterised by high molecular weight (ranging from 1000 to > 20,000 Da). Due to the lack of well defined analytical methods, their structure is still imperfectly understood (Neilson *et al.*, 1991).

It was reported that high molecular weight chlorolignin compounds tend to survive conventional activated sludge / aerobic lagoon effluent treatment with only minimal degradation (Eriksson *et al.*, 1985).

Neilson *et al.* (1991) stated that chlorolignins are able to bind a considerable quantity of TOCs such as chlorocatechols and guaiacols, which may be subsequently released and contaminate the ground water. Eriksson *et al.* (1985) reported that in a sterile aquatic system at pH 7.2, only a limited amount of chlorolignin underwent chemical degradation to chloroguaiacols and catechols.

The persistence of chlorolignin in the environment is mainly due to the slow availability of these compounds to soil biota and not due to the lack of catabolic enzymes was reported by Fitzimons *et al.* (1990).

2.3.2.3.1. Sorption and mobility

No direct measurements of sorption and mobility of chloroligno compounds from pulp effluents are available in the literature. Since there are only limited studies dealing with the disposal of pulp plant effluents to soils, many studies reviewed refer to sources of TOCs from other industrial wastes rather than pulp plant effluent.

The reuse of pulp plant effluent on agricultural land gives rise to pollution hazard due to the presence of potentially toxic organic compounds (TOCs) Though the conventional methods used in industries are effective in reducing pollution load, they failed in removing colour of the effluent mainly due to low nutrient content and the presence of toxic chlorolignin compounds in the effluent. This instigated the development of various colour removal techniques like MyCoR (mycelial colour removal) and enzymatic processes. In addition as the colour of the effluent mainly arises from lignin related compounds, most of the colour removal works have been carried out using white rot fungi. In addition it is necessary to study the sorption behaviour of chromophoric substances by soil system (clay colloids along with organic matter). The impact of pulp plant effluent on soil biota have not been studied especially under realistic effluent disposal conditions. It is paramount that field based investigations of pulp plant effluent with special reference to the impact on crops and soil biota can be carried out before disposal of pulp plant effluent on land is accepted as a reuse option.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

3.1. Study area

South India Viscose Industries Ltd. is situated on the Southern banks of river Bhavani on the fore-shore of lower Bhavani project reservoir at Sirumugai, which is approximately 50 Km from Coimbatore on the foot hills of Nilgiris.

The study area, (Fig. 3.1) where the impact study of effluent irrigation on soil biological properties was carried out, is located at an elevation between +290 m and + 305 m, above mean sea level (MSL) and the area is drained by Bhavani river and Elerumaipallam stream. The Bhavani river flows towards east on the western side of the study area and Elerumaipallam stream flows into the reservoir (Bhavani Sagar) from south to north on the eastern side of the factory. While the Bhavani river is more or less perennial, the jungle stream Elerumaipallam is seasonal. The study area is located nearer to the reservoir (Bhavani Sagar) on the southern most side, flanked by Bhavani river from the western side and Elerumaipallam on the eastern side.

The type of soil in the area is predominantly red sandy soil with a pH range from near neutral to alkali. The nearest rain gauge station to the study area is Mettupalayam rain gauge station. The 70 year mean annual rainfall for the station is 925 mm. The season wise breakup is as follows:

Advancing monsoon (June to September) 170.70 mm

Retreading monsoon (October to December) 451.30 mm

Transitional period (January to May) 270.30 mm

SIV FARM - CHITTEPALAYAM

TOTAL AREA : 143.73 HA
LATTITUDE : 10° N
LONGITUDE : 77° E
ALTITUDE : 320 MSL
AREA : 143.73 HA

3.1.1. Geology

The study area is covered by crystalline rocks of Archean age. The rock type include complex genesic formation covered by top red sandy soil whose thickness ranges from 1.50 m to 3.00 m. The thickness of weathered zone extends up to 17 m. The depth of basement is expected at 20 m depth.

3.1.2. Important physico-chemical properties of soil in the study area (Table 3.1)

Physical properties

Bulk density (g. cm^{-3}) : 1.40 – 1.61

Particle density (g. cm^{-3}) : 2.39 – 2.48

Maximum water holding capacity (per cent) : 23.5 – 26.2

Volume expansion on wetting (per cent) : 2.64 – 2.97

Porespace (per cent) : 38.0 – 40.6

Particle size distribution (per cent)

Sand : 66.9

Silt : 22.4

Clay : 10.5

Soil type

Textural class : Sandy loam

USDA classification : *Typic Rhodustalf*

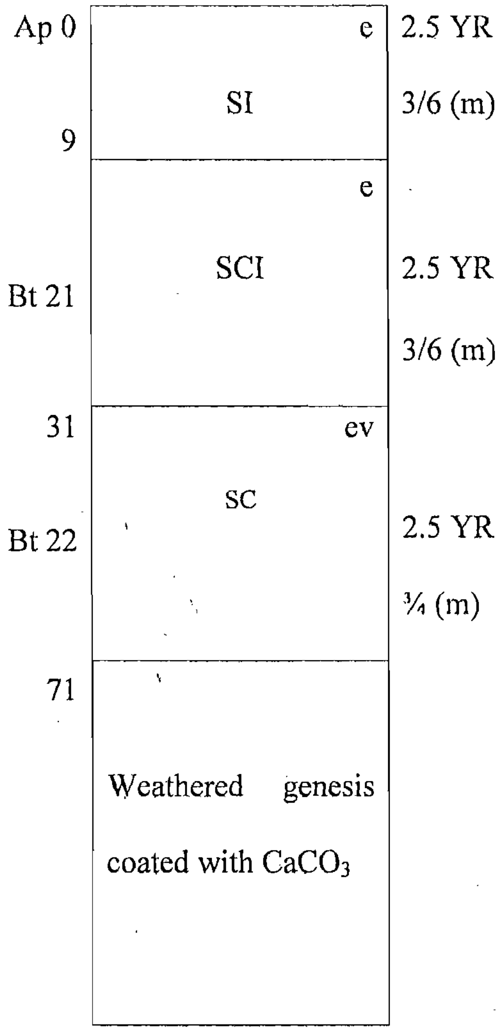
Chemical properties

pH : 6.44 – 7.8

EC (dSm^{-1}) : 0.01 – 0.7

Organic carbon (per cent) : 0.03 – 0.93

3.1.3. SIV – Field soil – Profile characteristics (Fig. 3.2)



Ap-0-9 cm	Dark red, sandy loam, moderate medium subangular blocky, slight effervescence, hard (dry) friable (moist), rapid permeability, medium pores, pH 7.9.
Bt 21-9-31 cm	Dark red, sandy clay loam, moderate medium subangular blocky, slight effervescence, hard (dry) friable (moist), slightly sticky (wet), thin clay films, moderate slow permeability, pH 7.9.

Bt 22-31-71 cm	Dark reddish brown, sandy clay with violent effervescence, strong, coarse subangular blocky, hard (dry), firm (moist), sticky and plastic (wet), thin clay films, moderate slow permeability, many kankar nodules 30-35 per cent, pH 8.5.
> 71 cm	Weathered genesis coated with $CaCO_3$.

3.2. Land application

Area : 355.00 acres

Quantity of effluent used for irrigation : 7500 – 9000 $m^3 . d^{-1}$

Frequency of irrigation / irrigation schedule : Once in five days

Cropping particulars : Block wise cropping particulars is given in the study area (SIV model farm) Map (Fig. 3.1)

3.3. Characterisation of soils of block A-E

Sampling was carried out in SIV model farm at five stages. The initial soil samples were collected (before effluent irrigation) from the blocks - A, B, C, D and E, covering all fields at 15 cm plough depth, using auger and stored in plastic bags. This was used for analyses for block wise comparison.

To investigate the impact of effluent irrigation on soil biological properties, the second, third, fourth and fifth set of samples were subsequently taken from the selected fields (B1-B15) of the blocks - A, B, C, D and E. Sampling was carried out on 30th, 60th, 90th and 120th day after effluent irrigation and compared with the initial samples taken before effluent irrigation.

3.3.1. Estimation of soil pH and electrical conductivity (EC)

The soils collected from each block (A, B, C, D and E) were mixed separately. From the mixture true representative samples were drawn. For the samples, pH and electrical conductivity were estimated according to the method prescribed by Jackson (1973), using pH meter (ELICO, India) and conductivity meter (ELICO, India) respectively.

3.3.2. Estimation of Bulk density (Bd)

Bulk density (Bd) of the soil samples for each block was determined by wax coating method (Piper, 1966) and expressed in g.cc^{-1}.

3.3.3. Estimation of soil organic carbon (per cent)

Organic carbon of the soil samples were estimated by chromic acid wet digestion method (Walkley and Black, 1934).

3.3.4. Enumeration of soil microorganisms

Total aerobic bacteria, fungi and actinomycetes were enumerated from the samples by serial dilution and pour plate method (Waksman and Fred, 1922). The methodology in detail has been described below.

3.3.4.1. Bacteria

Enumeration of soil bacteria was done in soil extract agar (Allen, 1953) by following serial dilution plate technique. The plates were incubated for 24 hrs at room temperature and the colonies were counted.

3.3.4.2. Fungi

Fungi in soil were enumerated in Martin's rose bengal agar (Martin, 1950). The samples were serially diluted. One ml of the 10^{-3} dilution was poured into the sterilized plates and covered with the medium. The plates were incubated at room temperature for 48 hrs and the colonies were counted.

3.3.4.3. Actinomycetes

Soil samples were serially diluted and 1 ml of 10^{-2} dilution was poured into sterilized plates and covered with Kenknight's medium (Allen, 1953). The plates were incubated for 5 days and the colonies were counted.

3.3.5. Assay of soil enzymes

Similar to the enumeration of microorganisms, assay of soil enzymes was also performed for the samples before and after irrigation on 30th, 60th, 90th and 120th day.

3.3.5.1. Dehydrogenase

Activity of the enzyme dehydrogenase was assayed according to the method prescribed by Casida *et al.* (1964). Five g of air-dried soil with 0.2 g of calcium carbonate was saturated with 1 ml of 3 per cent (W/V) solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC), 1 ml of 1 per cent sucrose, 2.5 Tris buffer (pH 7.0). After adding 0.25 ml of toluene to the above solution, the contents were incubated at room temperature. At the end of incubation period, hot methanol was added to the contents and filtered. The filtrate was made up to 100 ml with methanol and measured at 485 nm in a spectrophotometer (Electronics corporation of India Ltd, Hyderabad, India) using appropriate blank.

The amount of TPF (Triphenyl farmozan) formed was quantified from the known TTC. The dehydrogenase activity was expressed as µg of TPF released g^{-1} soil h^{-1}.

3.3.5.2. Phosphatase

Phosphatase activity in soil was estimated according to Halstead's procedure (Halstead, 1964). To 5 g air dried soil, 20 ml of 0.5 per cent disodium phenyl phosphate was added and incubated at 37°C for 1 h. Following the incubation, the samples were diluted with 100 ml of 0.3 per cent aluminium potassium sulphate, shaken and centrifuged (at 5000 rpm for 10 minutes). The amount of phenol released was determined in the supernatant and the activity of phosphatase was expressed as µg of phenol released g^{-1} soil h^{-1}.

3.3.5.3. Urease

Urease activity in soil was estimated according to the method prescribed by Tabatabai and Bremner (1972). To 5 gram of air-dried soil, 0.5 ml of toluene and 9 ml of 0.05 Molar Tris buffer were added and mixed well. One ml of 0.2 molar urea solution was added and incubated at 30°C for 2 h. After the incubation period, the volume was made up to 50 ml with potassium chloride-silver sulphate solution mixed well allowed to settle and then filtered through Whatman No. 42 filter paper. The ammonia liberated was estimated by Nessler's method (Jackson, 1973). To 1 ml of the filtrate, 2 ml each of 10 per cent potassium sodium tartrate and 10 per cent acidified sodium chloride solutions were added. To the contents, 1 ml of Nesslers reagent was added and the volume was made upto 25 ml with distilled water. The intensity of the yellow colour developed was measured at 595 nm in a spectrophotometer (Electronics corporation of India Ltd, Hyderabad, India). The amount of ammonia -N. liberated was calculated from known ammonium chloride standard and the enzyme activity was expressed as μg of ammonia-N released g^{-1} soil h^{-1}.

3.4. Characterization of pulp plant effluent

Viscose effluent was collected from effluent collection sump, which is located in the South Indian Viscose factory's model farm, in the vicinity of the factory. The collected sample was stored in plastic container (5 litre PVC can) at 4°C in cold room and the physical, chemical and microbial population were studied within 48 hours.

3.4.1. pH and EC

The pH and EC of the effluent was analysed at room temperature by using pH meter (ELICO, India) and conductivity meter (ELICO, India) respectively. The conductivity was expressed as dSm^{-1}.

3.4.2. Total Solids-Total Dissolved Solids (TDS) and Total Suspended Solids (TSS)

The total solids, total dissolved solids and total suspended solids in the effluent were calculated by the method prescribed by Patnaik (1997) and expressed in mg l^{-1} of the effluent.

3.4.3. Total phenols

One ml of the effluent was pipitted out into a clean dry test tube, with this 1 ml of Folin Ciocalteau reagent was added, followed by the 2.0 ml of 20 per cent sodium carbonate solution. The contents were boiled exactly for 1 minute, then cooled and the volume was made up to 25.0 ml with distilled water. The intensity of blue colour developed was measured at 650 nm using spectrophotometer (Electronics Corporation of

India Ltd, Hyderabad, India) (Bray and Thorpe, 1954). The amount of total phenols were expressed in µg of pyrogallol equivalents ml^{-1} of effluent.

3.4.4. Absorption maxima

The λ max (Absorption maxima) for the pulp plant effluent was detected by scanning the diluted effluent (1:3 ratio with distilled water) using microprocessor based UV-Visible spectrophotometer (Electronics Corporation of India Ltd, Hyderabad, India) at a wave length range 200-900 nm. Prior to scanning the effluent was centrifuged at 8000 rpm for 5 minutes (Kalaichelvan, 1997).

3.4.5. Enumeration of microbial population

Bacteria, Fungi and Actinomycetes were enumerated from the effluent (Chapter. 3.3.5.).

3.4.6. Identification of basic compounds in the pulp plant effluent

pH of the raw effluent (that was centrifuged at 8000 rpm for 5 minutes) was adjusted to less than 2 using orthophosphoric acid. The acidic effluent was extracted thrice with ethyl acetate @ 1: 3 ratio. The organic phase collected was evaporated at room temperature. The remaining residues were dissolved in 1:1 methanol water (HPLC grade) and used for compound identification (Kalaichelvan, 1997; Patnaik, 1997).

3.4.6.1. Compound Identification by HPLC

The sample prepared, by the manner above was analysed by high pressure liquid chromatography (Varian, 2000) using C-18 column. The predominant aromatic as detected by UV-detector at 254 nm wavelength. Methanol water (methanol, distilled water @ 1:1) was used as mobile phase (Boopathy, 1997). Standards were run at similar conditions. Based on the retention time of standard aromatic compounds (Table 3.2) and by area normalisation method, the components present in the samples were identified.

3.5. Influence of effluent irrigation on soil microbial population and enzyme activities

To investigate the impact of effluent irrigation on soil microbial population, the total aerobic microflora (bacteria, fungi, actinomycetes) were enumerated (Chapter 3.3.5.) from soil before effluent irrigation and 30th, 60th, 90th and 120th day after effluent irrigation.

The soil enzymes *viz.*, dehydrogenases, urease, phosphatase were assayed for the soil before effluent irrigation and 30th, 60th, 90th and 120th day after effluent irrigation (Chapter 3.3.6.).

3.6. Isolation, purification and screening of ligninolytic fungi for decolourisation of pulp plant effluent.

Soil samples were collected from an irrigation channel at SIV field trial and enrichment was carried out in conical flasks by keeping the collected soil and pulp plant effluent at 1:1 ratio. Before enrichment, the effluent was acidified to pH 6.0 using orthophosphoric acid. As a carbon source glucose was added @ 1 per cent. This was kept and maintained for three months and at the end of the third month, it was used for isolation of ligninolytic fungi. For isolation, 1 g enriched soil was taken and serially diluted (10^{-3}) and plated on Garren's (Garren, 1938) ligninolytic fungal media containing 0.2 per cent poly R-478 (Sigma, USA).

The plates were incubated at room temperature for 1 week. Colonies that produced straw yellow colour zones were selected and purified further by streak plate

Table 3.2. Retention time for standard aromatic compounds in HPLC

Standard		Retention time (mins.)
2, 4-dinitrophenol	-	3.638
4-nitrophenol	-	3.642
p-chlorophenoxy acetic acid	-	3.791
Benzoic acid	-	3.988
Ferulic aicd	-	3.358
Caffeeic acid	-	2.717
Syringic acid	-	3.162
Syringaldehyde	-	3.010
Syringaldazine	-	3.105
Picric acid	-	2.722
Pyrogallol	-	2.569
Pyrogallic acid	-	2.852
Pyrocatechol	-	2.786
Toluene	-	6.800
Benzene	-	6.275
Gentisic acid	-	3.122
Vanillic acid	-	3.238

method, and these were maintained on ligninolytic fungal media. Out of 12 types of cultures isolated, only four showed maximum decolourisation to poly R-478 treated medium and so were selected for further study.

The morphology of screened cultures were studied using slide culture technique (Kalaichelvan, 1987). The slides were observed under phase contrast microscope, the hyphal characters and spores were studied and photographed under appropriate magnification by Nikon microscope.

3.7. Decolourisation of viscose effluent using standard microbial cultures

In order to test the potentiality of various known microbial agents in decolourisation of South India viscose pulp-plant effluent, an experiment was conducted with mixed bacterial cultures (*Bacillus* sp. and *Pseudomonas* sp.), Fungal culture (*Fusarium* sp.) and Actizyme. The details of the experiment are given under.

3.7.1. Treatment particulars

T1 N1 - Control (100 ml effluent + carbon source)*

N2 - Control (100 ml effluent + carbon source+Nitrogen source)**

T2 N1 - 100 ml effluent + 0.5 ml of broth containing *Bacillus* sp.+ 0.5 ml of broth containing *Pseudomonas* sp.+ Carbon source

N2 - 100 ml effluent + 0.5 ml of broth containing *Bacillus* sp.+ 0.5 ml of broth containing *Pseudomonas* sp.+ Carbon source + Nitrogen source

T3 N1 - 100 ml effluent + 1.0 ml of broth containing *Fusarium* sp.+ carbon source

N2 - 100 ml effluent + 1.0 ml of broth containing *Fusarium* sp.+ carbon source + nitrogen source

T4 N1 - 100 ml effluent + Actizyme** + carbon source

N2 - 100 ml effluent + Actizyme + carbon source + Nitrogen source

* Glucose 0.1 gram 100 ml^{-1} effluent

** Diammonium sulphate 0.1 g 100 ml^{-1} effluent

*** Actizyme 0.5 gram 100 ml^{-1} effluent

3.7.1.1. Decolourization of Viscose effluent using composite culture of *Bacillus* sp. *Pseudomonas* sp.

The bacterial species (*Bacillus* sp. and *Pseudomonas* sp.) and the fungus (*Fusarium* sp.) used in this work were obtained from Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, India.

The bacterial cultures viz., *Bacillus* sp. and *Pseudomonas* sp. were maintained on Nutrient Agar slants and subsequently multiplied in 250 ml conical flask containing 100 ml of nutrient both supplied with beef extract 0.3 g; peptone 0.5 g; glucose 0.5 g; sodium chloride 0.5 g; pH 7.

On 3rd day after incubation 0.5 ml culture broth of each bacteria *viz.*, *Bacillus* sp. and *Pseudomonas* sp. was taken out from the culture and inoculated into 250 ml conical flask containing 100 ml effluent (pH 7) and the cultures were maintained under static condition.

3.7.1.2. Decolourisation of pulp plant effluent by *Fusarium* sp.

Czapek-Dox agar slants were used for the maintenance of the fungus *Fusarium* sp. Later it was allowed to grow as static culture in 250ml conical flask containing 100 ml of Czapek-Dox mineral solution (pH 6.5). The nutrient composition of the broth was sucrose 3.0 g; sodium nitrate 0.2 g; potassium hydrogen orthophosphate 0.1 g; magnesium sulphate 0.5 g; potassium chloride 0.05 g and Ferrous sulphate 0.001 g.

After 5 days, the mycelial pellets were washed with sterile phosphate buffer (pH 7.0) and then the mycelial mats were homogenized by grinding with acid washed sand. One ml of the fungal mycelial homogenate was added to each 250ml conical flask containing 100 ml (pH 6.5) of sterilized effluent. The cultures were maintained under static condition. Control without inoculation was run in parallel.

3.7.1.3. Decolourisation of pulp plant effluent using actizyme

Actizyme (pellets) @ 0.5 g were added to each conical flask containing 100 ml (pH 7) of sterilized pulp-plant effluent and incubated for colour removal. After two days of incubation period, the efficiency of colour removal was detected colorimetrically at 465 nm in Spectrophotometer (Electronics corporation of India Ltd, Hyderabad, India).

3.7.2. Decolourisation of pulp - plant effluent using screened ligninolytic fungal cultures

In order to decolourise the pulp-plant effluent effectively an efficient cultures were selected from the isolated fungal cultures that were isolated from the effluent enriched soil (C1 – C12). The selection was made based on the intensity of yellow colour zone that they were formed in poly R-478 added medium (3 per cent). The cultures C5, C8, C11 and C12 were selected and used for the decolourisation work.

3.7.2.1. Preparation of solid matrix for microbial immobilisation

Clay balls of 0.75mm size were made from the soil mixture, which was prepared by mixing red soil 60 per cent, clay 20 per cent and coir pith 20 per cent. After drying in the shade, all the balls were cooked in a kiln and used for fungal immobilisation.

3.7.2.2. Treatment details of batch study

Batch study was conducted in duplicates using efficient strains (C5, C8, C11 and C12) of ligninolytic fungus isolated from the effluent enriched soils. The cultures were inoculated in 100 ml conical flasks containing 25 ml of undiluted sterilized effluent, enriched with 0.3 (G1) and 0.6 (G2) per cent glucose, separately. Uniformly all the flasks were supplied with 10 numbers of clay balls, in order to act as supporting medium for mycelial attachment. The pH of the effluent was adjusted to 6.5 using orthophosphoric acid, then inoculated (1 ml of homogenised culture broth) and incubated (room temperature) at static condition.

Samples were drawn at 24 hours interval and the absorbance was measured at 465 nm, on 0th, 2nd, 4th, 6th, 8th, 10th and 12th day after incubation using spectrophotometer (Electronics Corporation of India Ltd.,). The details of the experiment are given under.

Experimental Details

S1 G1 - 25 ml effluent + clay ball 10 Nos. + 0.3 per cent glucose*
G2 - 25 ml effluent + clay ball 10 Nos. + 0.6 per cent glucose**
S2 G1 - 25 ml effluent + clay ball 10 Nos. + 0.3 per cent glucose + culture – C5
G2 - 25 ml effluent + clay ball 10 Nos. + 0.6 per cent glucose + culture – C5
S3 G1 - 25 ml effluent + clay ball 10 Nos. + 0.3 per cent glucose + culture – C8
G2 - 25 ml effluent + clay ball 10 Nos. + 0.6 per cent glucose + culture – C8
S4 G1 - 25 ml effluent + clay ball 10 Nos. + 0.3 per cent glucose + culture – C11
G2 - 25 ml effluent + clay ball 10 Nos. + 0.6 per cent glucose + culture – C11

S5 G1 - 25 ml effluent + clay ball 10 Nos. + 0.3 per cent glucose + culture – C12

G2 - 25 ml effluent + clay ball 10 Nos. + 0.6 per cent glucose + culture – C12

* G1 - 25 ml effluent with 0.3 per cent glucose

** G2 - 25 ml effluent with 0.6 per cent glucose

3.7.2.3. Decolourisation of pulp-plant effluent using ligninolytic fungal cultures - column study

Decolourisation study was conducted under laboratory condition using column packed with clay balls, red earth, charcoal and glass wool. The clay balls were immobilized with the ligninolytic fungal cultures.

The cultures (C5, C8, C11 and C12), used in the batch study were immobilised on clay balls and packed inside the column (Plate 2.7). Then the aerated effluent (pH 6.5) was passed through the column @ 4 ml min^{-1} (down flow) using peristaltic pump. The leachates (L1 - L5) collected at 0 (L1), 3 (L2), 6 (L3), 9 (L4) and 12 (L5) hours retention time, were analysed for colour at 465 nm. Similarly control was run without inoculation in parallel. In addition the change in pH of the treated effluent was also determined simultaneously. The details of the column (C1 – C5) and the treatment particulars are given under.

3.7.2.3.1. Particulars of column used in decolourisation study

Column type	-	Glass
Height (cm)	-	44
Diameter (cm)	-	6

Height up to which clay balls packed (cm)	-	34
Height up to which red earth packed (cm)	-	4
Height up to which charcoal packed (cm)	-	4
Height up to which glass wool packed (cm)	-	2
Void volume (cm^3)	-	3046.06

3.7.2.3.2. Treatment particulars of effluent decolourisation – Column study

C_1	-	Control (clay balls alone)
C_2	-	Clay balls immobilised with culture – C5
C_3	-	Clay balls immobilised with culture – C8
C_4	-	Clay balls immobilised with culture – C11
C_5	-	Clay balls immobilised with culture - C12

3.7.3. Characteristics of moringa (*Moringa oleifera*) seeds

Five grams of good quality shelled seeds were soaked in 100 ml of distilled water for 24 hours at room temperature in 250 ml glass beaker and stirred for 30 minutes, filtered through country filter paper, and the extract was determined for the following parameters.

3.7.3.1. pH and electrical conductivity

The pH and electrical conductivity (dSm^{-1}) of moringa seed extract were estimated, using pH meter (ELICO, India) and conductivity meter (ELICO, India) respectively. The amount of total dissolved solids (TDS) were measured directly by Cyber scan TDS meter and was expressed in mg l^{-1} of the extract.

3.7.3.2. Moisture content

Moisture content of the seed was calculated by drying a known weight of the seeds (5 grams) in an electric oven at 105°C for 15 minutes. Then the contents were cooled in a desiccator. The weight difference of the seed before and after drying was calculated and expressed in terms of percentage.

3.7.3.3. Total phenols

One ml of the moringa seed extract was pipetted out into a clean dry test tube. With this 1 ml of Folin Ciocalteau reagent was added, followed by the 2.0 ml of 20 per cent sodium carbonate solution. The contents were boiled exactly for 1 minute, then cooled and the volume was made up to 25.0 ml with distilled water. The intensity of blue colour developed was measured at 650 nm using spectrophotometer (Electronics Corporation of India Ltd, Hyderabad, India). The amount of total phenols were expressed in µg of pyrogallol equivalents ml^{-1} extract (Bray and Thorpe, 1954).

3.7.3.4. Protein

Protein content of the shelled moringa seeds (kernel) was estimated by Lowry's method (Lowry *et al.*, 1951) and was expressed in terms of mg g^{-1} kernel. Five hundred milligrams of the shelled moringa seed was extracted by grinding with phosphate buffer (pH 7).

Extraction of protein from the moringa seed was carried out with phosphate buffer (pH 7). The moringa seed (0.5 g) was ground by pestle and mortar with 10 ml of the buffer and the solution was centrifuged at 5000 rpm for 5 minutes. From the supernatant 0.1 ml and 0.2 ml were pipetted out into two clean test tubes. Volume was made up to 1 ml with buffer. To this five ml of reagent C (Alkaline copper solution) was added, mixed well and was allowed to stand for 10 minutes. Then 0.5 ml of reagent D (Folin Ciocalteau reagent) was added and incubated at room temperature in dark for 30 minutes. The intensity of blue colour was measured at 660 nm. A blank using phosphate buffer was run simultaneously along with the samples. Bovine Serum Albumin was used as standard. From the standard graph amount of protein was calculated and expressed in mg g^{-1} of kernel.

3.7.3.5. Separation, purification and quantification of proteins from moringa seed kernel

To explore the possibility of more efficient ways to use moringa seeds as a coagulant, coagulating agents were extracted using phosphate buffer (pH 7). The coagulating proteins were further purified by precipitation, dialysis as described by Ndabigengesere *et al.* (1995).

3.7.3.6. Identification of amino acids in moringa seeds by paper chromatography

To study the nature of amino acids present in the seed extract, Whatman No. 1 chromatography papers of 46 x 57 cm size were loaded with 100 µl of samples and ascending chromatography was performed (Plummer, 1990) with a solvent saturated atmosphere. The solvent system of n-butanol : acetic acid : water (4:1:5) was used. The chromatograms were developed by spraying 0.1 per cent ninhydrin reagent dissolved in acetone. Using known standards (Table 3.3.) the amino acid present in the extract were identified based on the Rf values.

3.7.4. Decolourisation of pulp-plant effluent using moringa seeds

An experiment was conducted using moringa seeds for decolourisation of the pulp plant effluent. The colour removal efficiency was tested by soaking the seeds at different

Table 3.3. Rf values for standard amino acid compounds in paper chromatography

Standard		Rf values
DL-α-alanine	-	0.906
DL-2-amino-n-butyric acid	-	0.952
L-arginine monohydrochloride	-	0.909
DL-aspartic acid	-	0.891
L-cysteine hydrohloride	-	0.903
L-cystine	-	0.883
DL-3, 4-dihydroxy phenyl alanine	-	0.933
Glycine	-	0.865
L-histidine monhydrochloride	-	0.886
L-hydroxy proline	-	0.921
L-leucine	-	0.947
DL-iso-leucine	-	0.948
L-lysine monohydrochloride	-	0.927
DL-methionine	-	0.918
DL-ß-phenyl alanine	-	0.912
L-proline	-	0.911
DL-serine	-	0.871
DL-threonine	-	0.903
DL-tryptophan	-	0.842
L-tyrosine	-	0.888

quantity at various soaking period. Colour reduction was colorimetrically (at 465 nm) estimated at 12, 24, 36 and 48 hours interval from the effluent in which different rates of the seeds were soaked. The details of the experiment is given below.

T_1 - 150 ml effluent (control)
T_2 - 150 ml effluent + 5 g seeds
T_3 - 150 ml effluent + 10 g seeds
T_4 - 150 ml effluent + 15 g seeds
T_5 - 150 ml effluent + 20 g seeds
T_6 - 150 ml effluent + 25 g seeds
T_7 - 150 ml effluent + 30 g seeds
T_8 - 150 ml effluent + 35 g seeds
T_9 - 150 ml effluent + 40 g seeds
T_{10} - 150 ml effluent + 45 g seeds
T_{11} - 150 ml effluent + 50 g seeds.

3.7.5. Decolourisation of pulp-plant effluent with soil packed columns 1 and 2

To detect the effluent carrying capacity of soils, red and sandy soils were filled separately in glass columns up to 30 cm height over the glass wool which was kept at the bottom of column up to 4 cm height.

Columns 1, 2 were saturated by passing the effluent continuously, until the leachate came out from the outlet. Effluent was passed @ 4 ml min^{-1} through the soil column beyond the saturation level, using peristalitc pumps. Each time the leachate collected (@ 200 ml) was analysed for pH, EC, TDS and colour. For the estimation of colour DR/2000 Hatch colorimeter was used and was measured at 455 nm.

In column 1 and 2, pH, EC (Jackson, 1973), organic carbon (Walkley and Black, 1934), enumeration of bacteria, fungi, actinomycetes (Waksman and Fred, 1922) and assay of soil enzymes *viz.,* urease (Tabatabai and Bremner, 1972) dehydrogenase (Casida *et al.*, 1964) and phosphatase (Halstead, 1964) were carried out for the soils (Red and Sandy soil) before and after treatment. For analysis, the soil samples were taken from the top (0 to 10 cm), middle (10 to 20 cm) and bottom (20 to 30 cm) regions of the columns (1 and 2).

Table 3.3. Experimental details of column study

Particulars	Column 1	Column 2
Soil type	Red	Sandy
Height (cm)	44	44
Diameter (cm)	6	6
Height upto which the soil filled (cm)	30	30
Weight of soil taken (g)	1000	1250
Effluent flow rate ml.min^{-1}	4	4
Volume of effluent Consumed by soil for saturation (ml)	420	140
Volume of the effluent treated (ml)	2600	2600
Void volume (cm^3)	3394.28	3394.28

3.8. Statistical Analysis

The data were statistically analysed by the method suggested by Panse and Sukhatme (1989). The critical differences were worked out at 5 per cent probability levels.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The impact of pulp plant effluent on soils chemical and biological properties and the decolourisation potentials of various colour removal methods was studied and the results are presented here.

4.1. Characteristics of soils of blocks A, B, C, D and E at South India Viscose plant site

4.1.1. Physico-chemical characteristics

The results of the physico-chemical properties viz., pH, EC, bulk density and organic carbon of the blocks studied (A-E) prior to effluent irrigation are presented in Table 4.1.

The pH of the blocks ranged between 6.81 and 6.97. Among the blocks, block 'D' had the highest pH, followed by block 'E'. The lowest pH was noticed in 'A' Block. Electrical conductivity of the blocks A-E ranged from 0.06 $dSm^{-1}$to 0.20 dSm^{-1}. Block E recorded the highest EC (0.20 dSm^{-1}) followed by D and A blocks, whereas, the lowest EC (0.06 dSm^{-1}) was observed in B block. Bulk density of soils in blocks A-E varied between 1.2 $g.cc^{-1}$ and 2.2 $g.cc^{-1}$. Block A registered the maximum bulk density (2.2 $g.cc^{-1}$) and the minimum was recorded in block B (1.2 $g.cc^{-1}$). The organic carbon content in the blocks studied was ranging from 0.35 per cent to 0.45 per cent and block C recorded the maximum (0.45 per cent).

4.1.2. Microbial population

The results of microbial population studied viz., bacteria, fungi and actinomycetes of the blocks studied (A-E) prior to effluent irrigation are given in Table 4.1.

The blocks A, B, C, D and E varied in bacterial population between 22 and 46 (x 10^5 g^{-1} soil). Block A had the maximum population (46 x 10^5 g^{-1} soil), followed by D block 38.5 (x 10^5 g^{-1} soil) and the minimum number of population was noticed in block B. Fungal population in the blocks A-E was ranging from 14 (CFU x $10^3.g^{-1}$ soil) to 23.5 (CFU x $10^3.g^{-1}$ soil). Among the blocks, block A had the highest population and the lowest population was observed in block B.

Actinomycetes population in blocks A, B, C, D, E was ranging from 8 (x $10^{2}.g^{-1}$ soil) to 14 (x $10^{2}.g^{-1}$ soil). Both A and C blocks registered the maximum number 14 (x $10^{2}.g^{-1}$ soil)) followed by D block, whereas, the least number of population was noticed in block 'B'.

4.1.3. Enzyme activity

The enzyme activities viz., dehydrogenase, phosphatase and urease in the blocks A-E, prior to effluent irrigation are given in table 4.1.

Activity of the enzyme dehydrogenase (µg of TPF released g^{-1} soil h^{-1}) in blocks A - E varied from 0.95 to 2.14. Block A recorded the maximum followed by block D and E. The activity was minimum in B block (0.95). Activity of the enzyme phosphatase (µg of inorganic phenol released g^{-1} soil h^{-1}) in all the blocks, ranged from 0.77 to 1.04.

The blocks A, B, C, D and E varied in urease activity (µg of ammonia –N released g^{-1} soil h^{-1}) from 4.90 to 6.30. The block A had the maximum activity (6.30) and the minimal activity was observed in D block (4.90).

Table 4. 1. Characteristics of soils of different blocks at South India Viscose site

Particulars	'A' block	'B' block	'C' block	'D' block	'E' block
pH	6.81 ± 0.04	6.92 ± 0.02	6.92 ± 0.02	6.97 ± 0.02	6.95
EC (dSm^{-1})	0.18	0.06	0.12 ± 0.01	0.18 ± 0.02	0.20
Organic carbon (per cent)	0.42 ± 0.04	0.38 ± 0.06	0.45 ± 0.02	0.35 ± 0.02	0.37 ± 0.06
Bulk density g.cc^{-1}	2.2 ± 0.14	1.2 ±0.14	1.7	1.5	1.7
Bacteria x 10^5 g^{-1} soil	46 ± 1.41	22 ± 1.41	36.5 ± 2.12	38.5 ± 0.71	27 ± 1.41
Fungi (CFU) x 10^3 g^{-1} soil	23.5 ± 0.71	14 ± 1.41	19	21 ± 1.41	16 ± 1.41
Actinomycetes x 10^2 g^{-1} soil	14 ± 1.41	8 ± 1.41	14 ± 1.41	13	11.5 ± 2.12
Dehydrogenase*	2.14 ± 0.73	0.95	1.39	1.68 ± 0.38	1.43 ± 0.38
Phosphatase**	1.04 ± 0.13	0.77 ± 0.08	0.77 ± 0.06	0.88 ± 0.042	0.80 ± 0.08
Urease***	6.30 ± 0.46	5.20 ± 0.18	6.25 ± 0.35	4.90 ± 0.35	5.55 ± 0.35

* - activity expressed in μg of TPF released g.$^{-1}$ soil h^{-1}
** - activity expressed in μg of inorganic phenol released g.$^{-1}$ soil h^{-1}
*** - activity expressed in μg of ammonia-N released g.$^{-1}$ soil h^{-1}

The pH and electrical conductivity of the sample were estimated after mixing soil and water suspension at 1:2.5 ratio (Jackson, 1973). Bulk density was estimated by wax coating method. The estimation of biological properties were performed immediately after sampling within 48 hours. All the values (mean of two replications with standard deviation) presented in the table show the properties of the soil before effluent irrigation and expressed on oven dry basis.

4.2. Characteristics of South India Viscose effluent

Physico-chemical properties and bacterial fungal and actinomycetes population were studied for the raw effluent and the results are presented in Table 4.2.

4.2.1. Physical characteristics

The brown coloured effluent had the sulfurous smell, which registered 5060 colour units (Pt. Co) at 455 nm. The absorption maxima (λ-max) of the raw effluent was 0.803 at the wave length of 248.5 nm (Fig. 4.1).

4.2.2. Chemical characteristics

The pH and EC (dSm^{-1}) of the raw effluent were 8.01 and 2.65 respectively. The amount of phenolics in the effluent (equivalents of μg of pyrogallol ml^{-1} effluent) was 2.55. The predominant aromatic compound present in the effluent was identified as caffeeic acid (2.717) picric acid (2.722) (Fig.4.2a and b)

4.2.3. Microbial population

The amount of total aerobic microflora, *viz.*, bacteria, fungi and actinomycetes in the raw effluent was 9.5 (x $10^5.g^{-1}$ soil), 3 (CFU x $10^3.g^{-1}$ soil) and 4 (x $10^2.g^{-1}$ soil) respectively.

4.3. Influence of effluent irrigation on soil microbial activities in blocks B1 – B15

The total number of bacteria, fungi and actinomycetes present in the soil (of blocks B1 – B15), before and after effluent irrigation (on 30th, 60th, 90th and 120th day) are presented in Fig. 4.2, 4.3 and 4.4 respectively.

Table 4. 2. Characteristics of South India pulp-plant effluent

Physical characteristics	
Colour (Pt. Co)	5060 (± 707.11)
Odour	Sulfurous smell
Absorption maxima / λ max (nm)	438
Chemical characteristics	
pH	8.01 ± 0.26
Electrical conductivity (dSm^{-1})	2.65 ± 0.78
Total solids ($mg.l^{-1}$)	2050 ± 117.0
Total suspended solids ($mg\ l^{-1}$)	125 ± 35.40
Total dissolved solids ($mg.\ l^{-1}$)	1925 ± 742.46
Total phenols (equivalents of µg of pyrogallol ml^{-1} sample)	2.55 ± 0.63
Predominant aromatic present in the effluent	Picric acid & Caffeeic acid
Microbial population	
Bacteria x 10^5 ml^{-1} effluent	9.5 ± 2.12
Fungi (CFU) x 10^3 ml^{-1} effluent	3 ± 1.41
Actinomycetes x 10^2 ml^{-1} effluent	4 ± 1.41

Effluent after sampling was stored at 4°C and the physical, chemical and microbial population were studied within 48 hours. The values given are mean of two replications with standard deviation.

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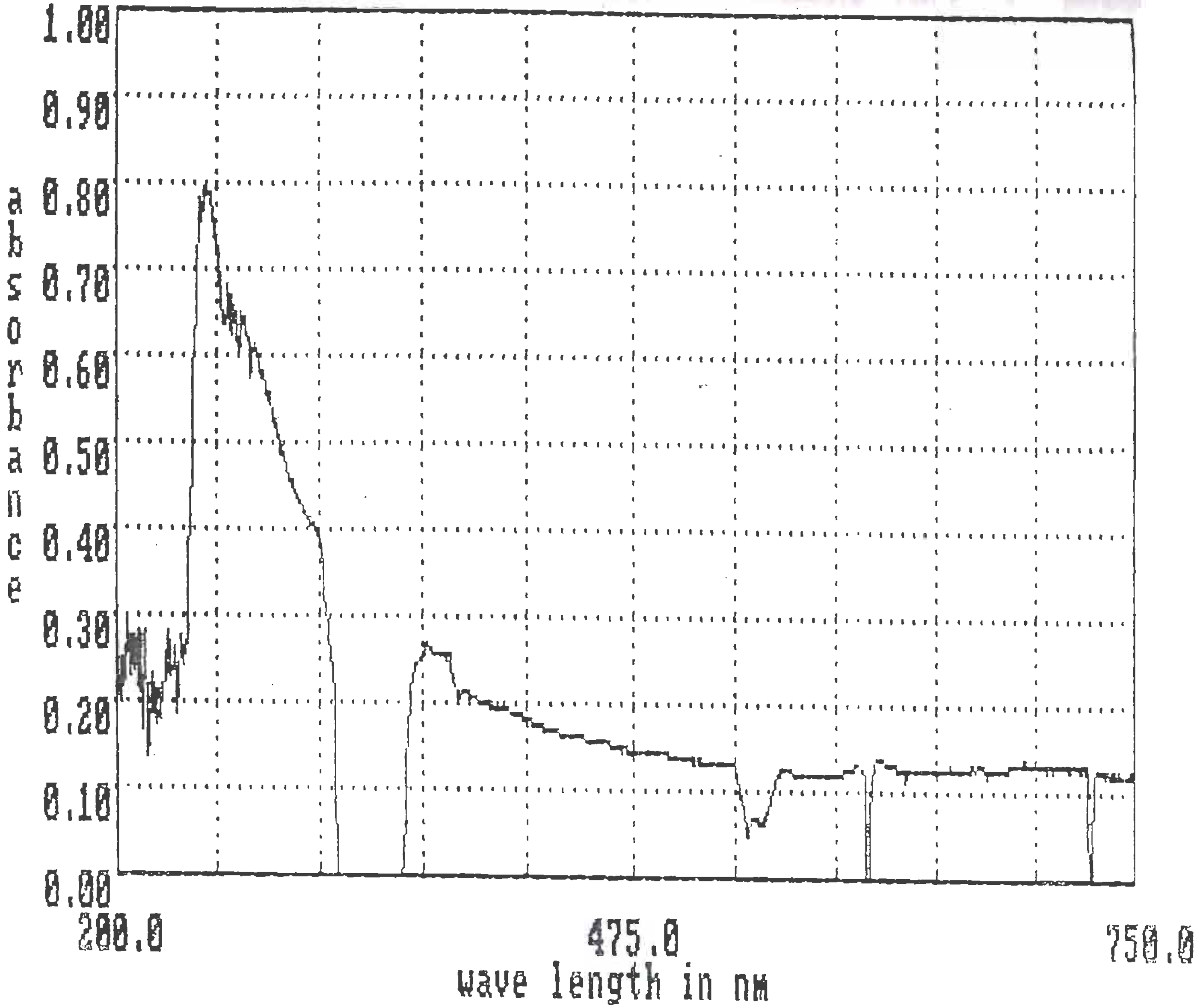
Fig.4.1. Absorption maxima (λ max) of pulp-plant effluent



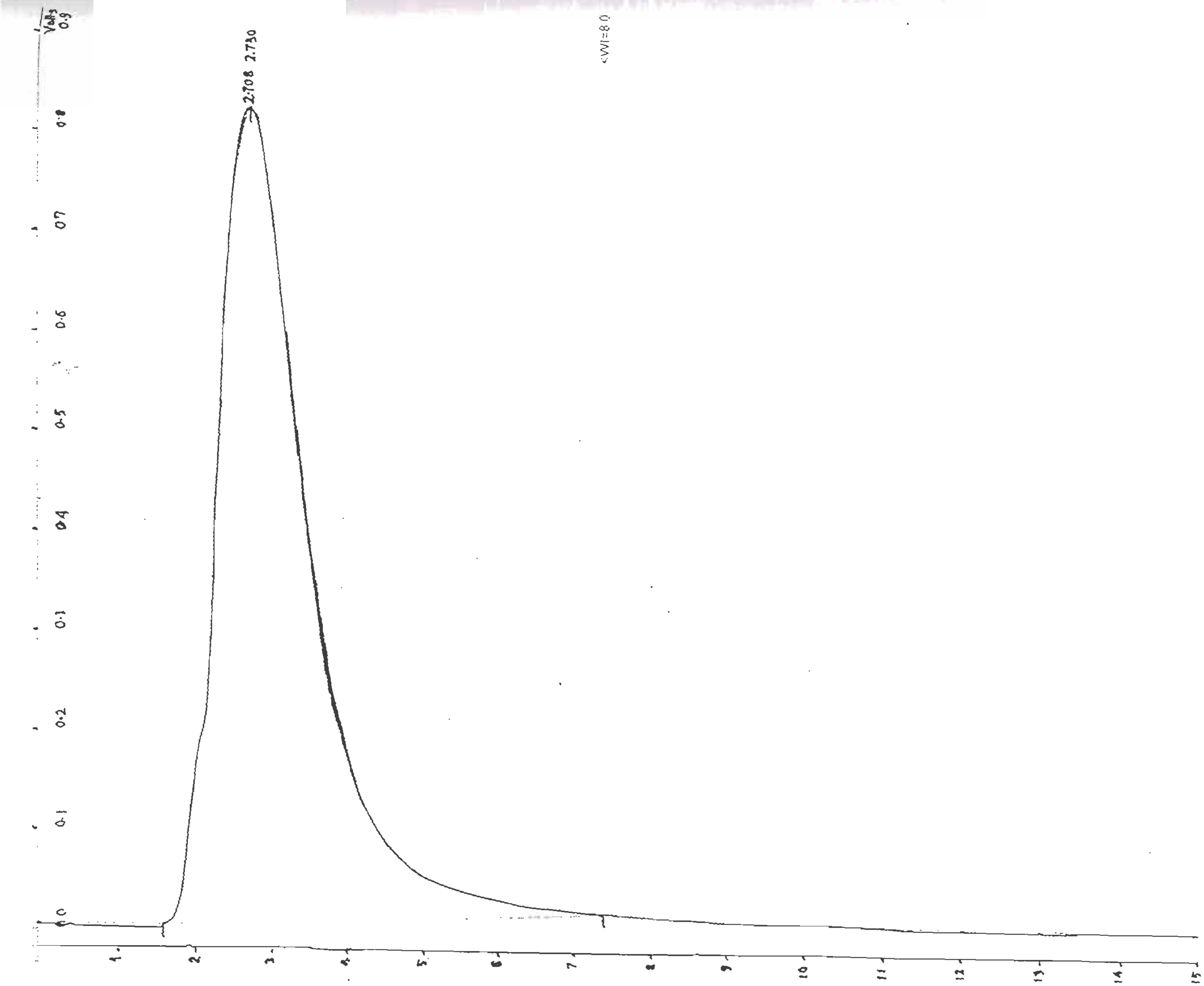
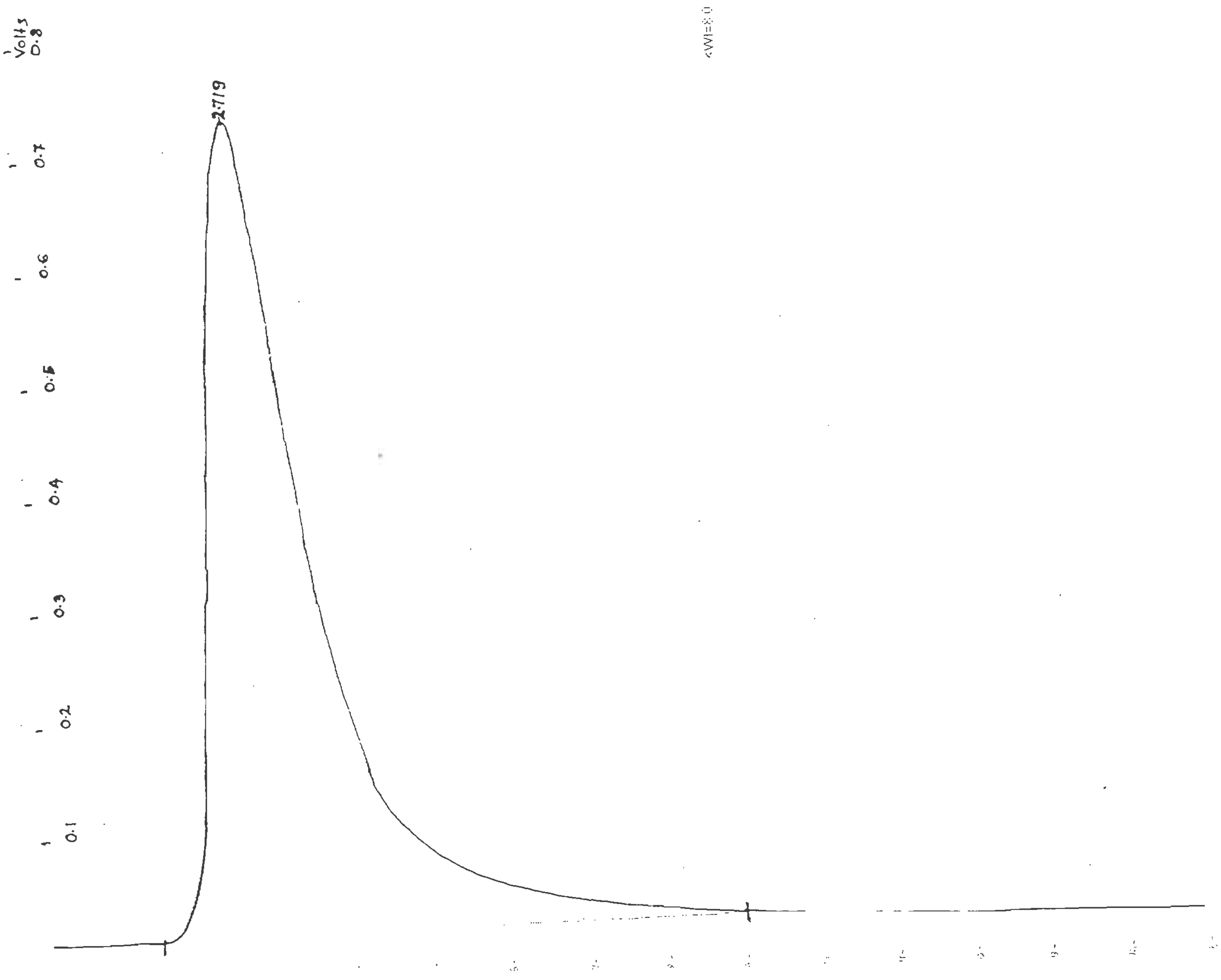


Fig. 4.28. Chromatogram of raw effluent (HPLC)

Fig. 4.2b. Chromatogram of extracted effluent (using ethyl acetate)



4.3.1. Bacterial population in effluent irrigated soils

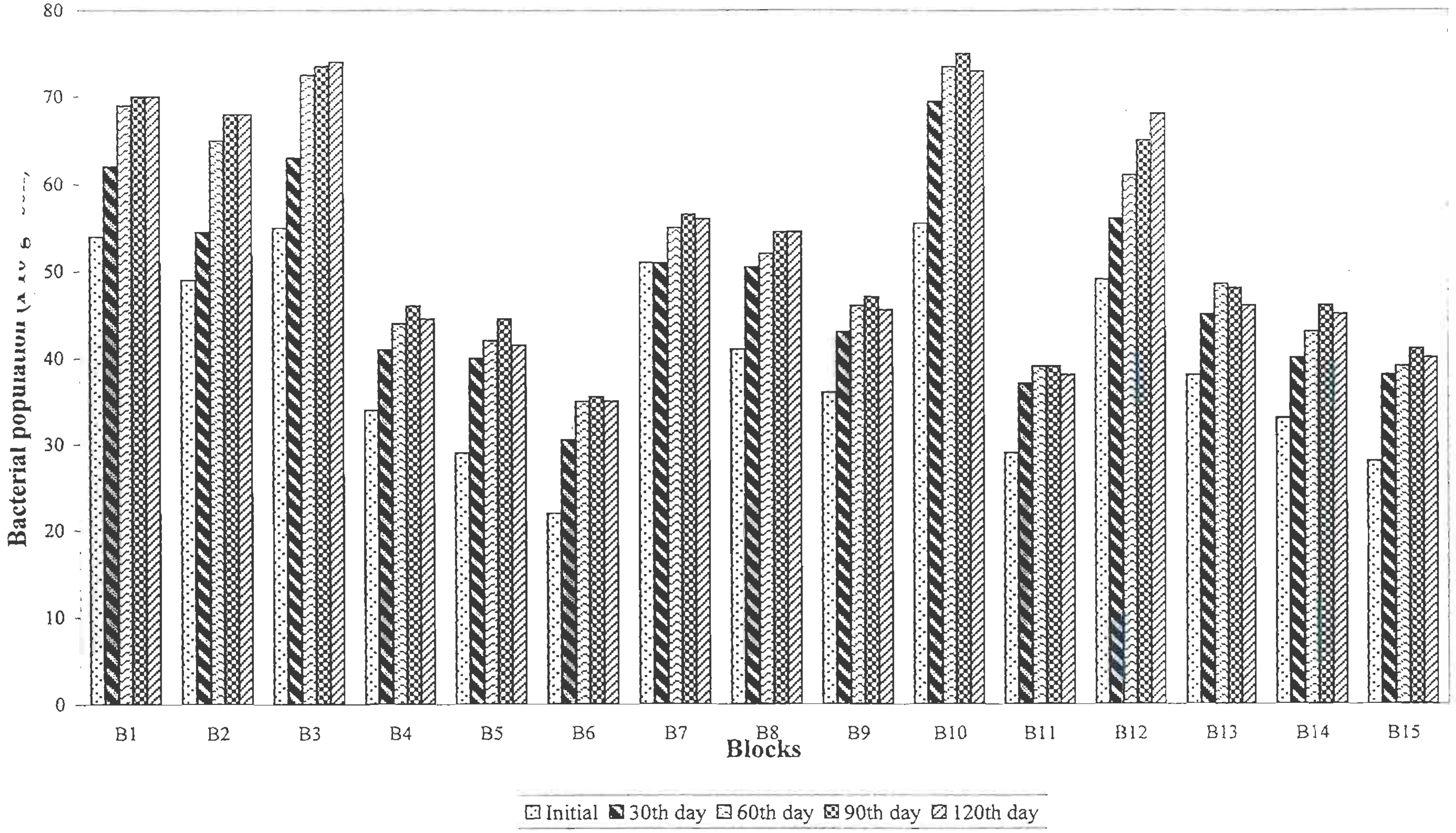
Population of bacteria in all the blocks, was ranging from 31.60 (x $10^5.g^{-1}$ soil) to 69.30 (x $10^5.g^{-1}$ soil). Among these, B10 had the highest population (69.30 x $10^5.g^{-1}$ soil) followed by B3 (67.60 x $10^5.g^{-1}$ soil) and B1 (65.00 x $10^5.g^{-1}$ soil). The bacterial population in all the blocks, during the study period (from initial to 120th day after effluent irrigation) ranged between 40.23 (x $10^5.g^{-1}$ soil) and 53.27 (x $10^5.g^{-1}$ soil). Initially (before effluent irrigation) the total number of bacteria in all the blocks was very less (40.23 x $10^5.g^{-1}$ soil) and increased after effluent irrigation till 90th day (53.97 x $10^5.g^{-1}$ soil) then decreased significantly on 120th day.

Regarding the effect of interaction between blocks and days on bacterial population. Except B7 all blocks showed significant increase in population on 30th day of effluent irrigation. In B7 no change was observed on 30th day. Among the blocks, population in B10 remained maximum till 90th day, later decreased significantly. At the end of experiment ie., on 120th day of effluent irrigation, B3 had the highest population (74.00 x $10^5.g^{-1}$ soil) followed by B10 (73.00 x $10^5.g^{-1}$ soil). The bacterial population in B6 was very minimum throughout the experiment. In B3, the population increased continuously till 120th day of effluent irrigation.

4.3.2. Fungal population in effluent irrigated soils

Fungal population in all the blocks varied between 19.60 (x $10^3.g^{-1}$ soil) and 37.30 (x $10^3.g^{-1}$ soil). Among the blocks, B10 had the maximum number (37.30 x $10^3.g^{-1}$ soil) followed by B3 (34.80 x $10^3.g^{-1}$ soil). The minimum was recorded in B6 (19.60 x $10^3.g^{-1}$ soil). The overall fungal population collectively in all the blocks both before and after

Fig. 4.2. Bacterial population in effluent irrigated soils



One gram soil sample was serially diluted and the bacterial population was enumerated by pour-plate method using soil extract agar medium. (Allen, 1953). Population was calculated on oven dry basis.

Fig. 4.2. Bacterial population in effluent irrigated soils

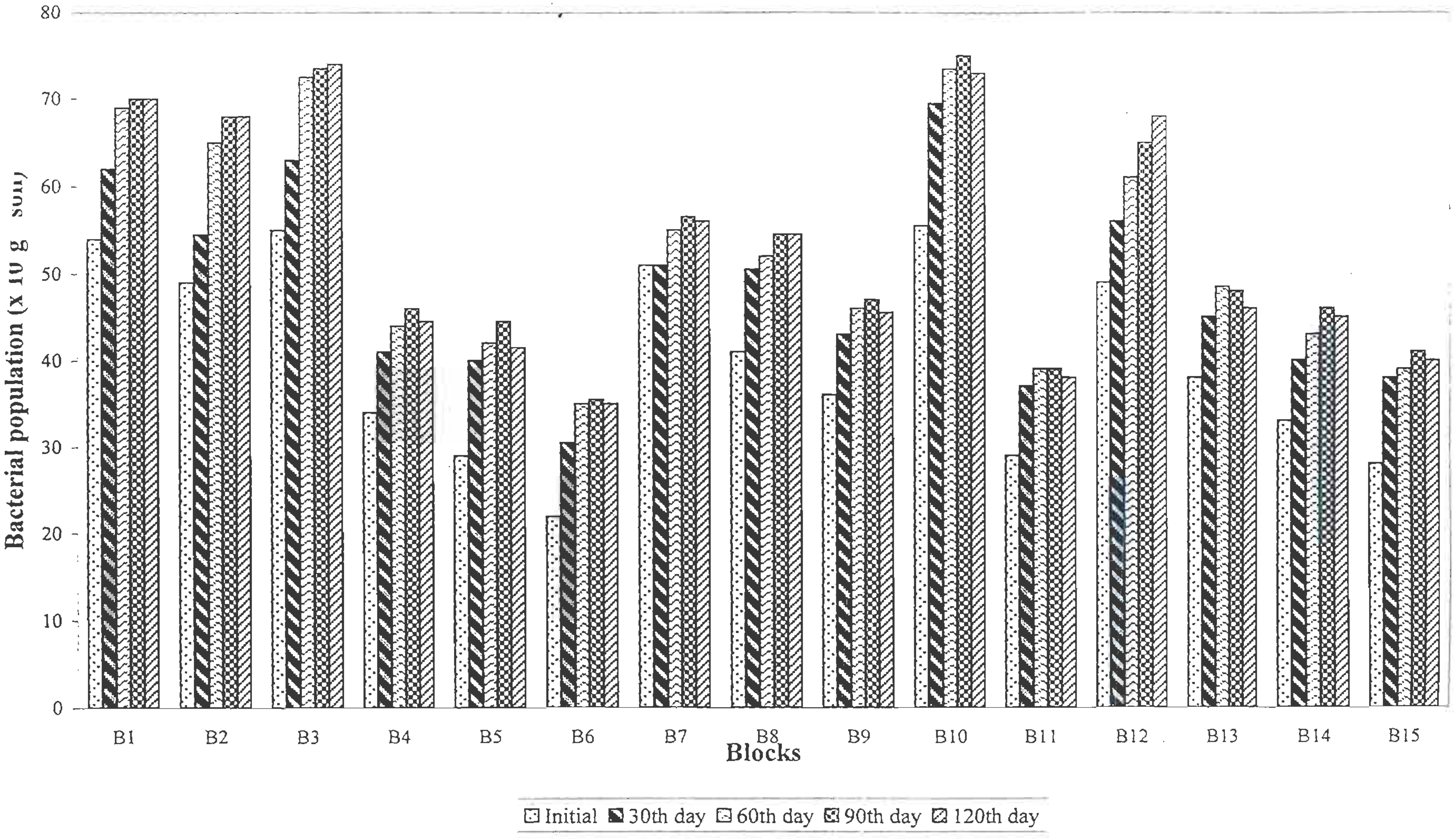


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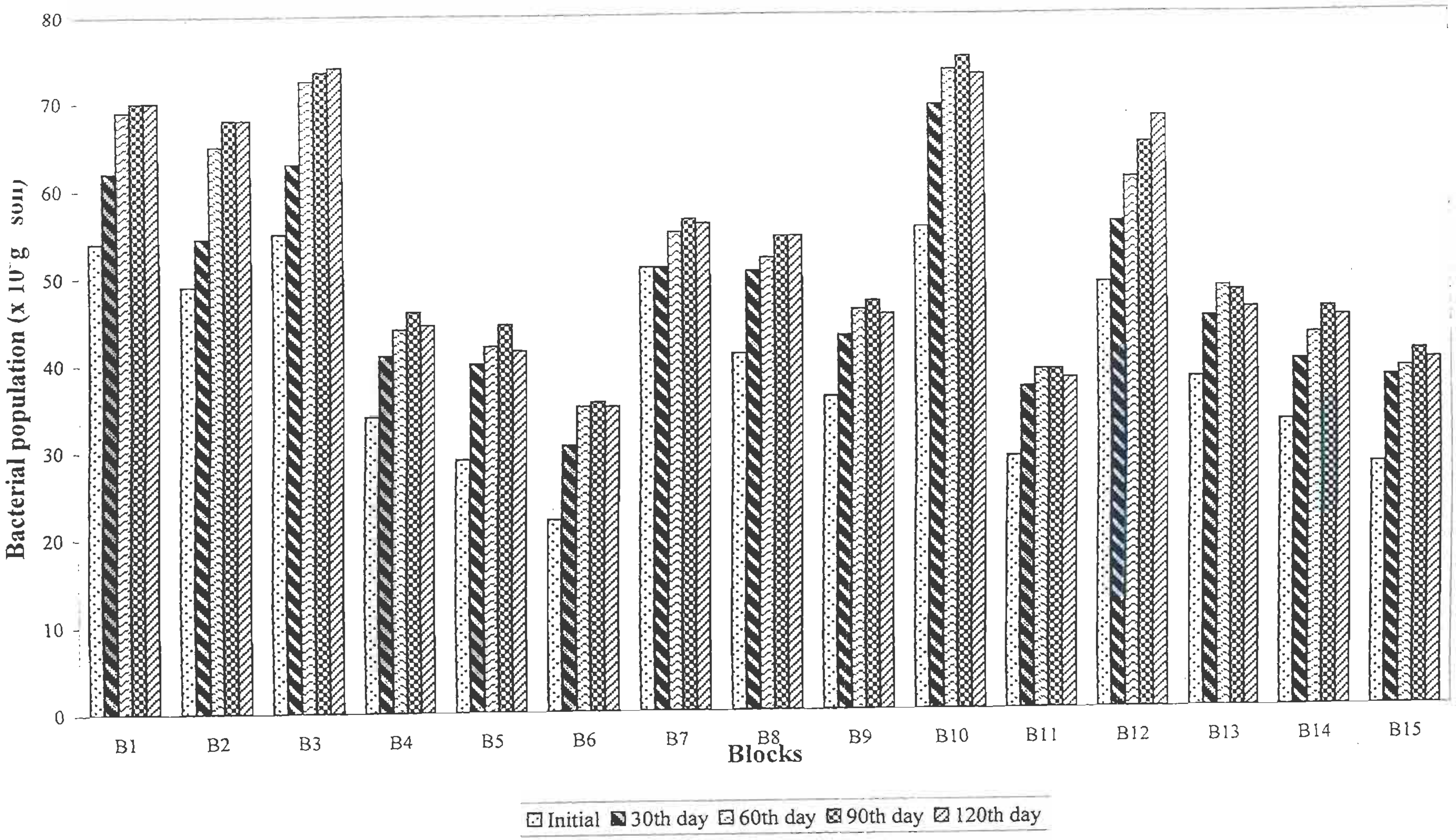
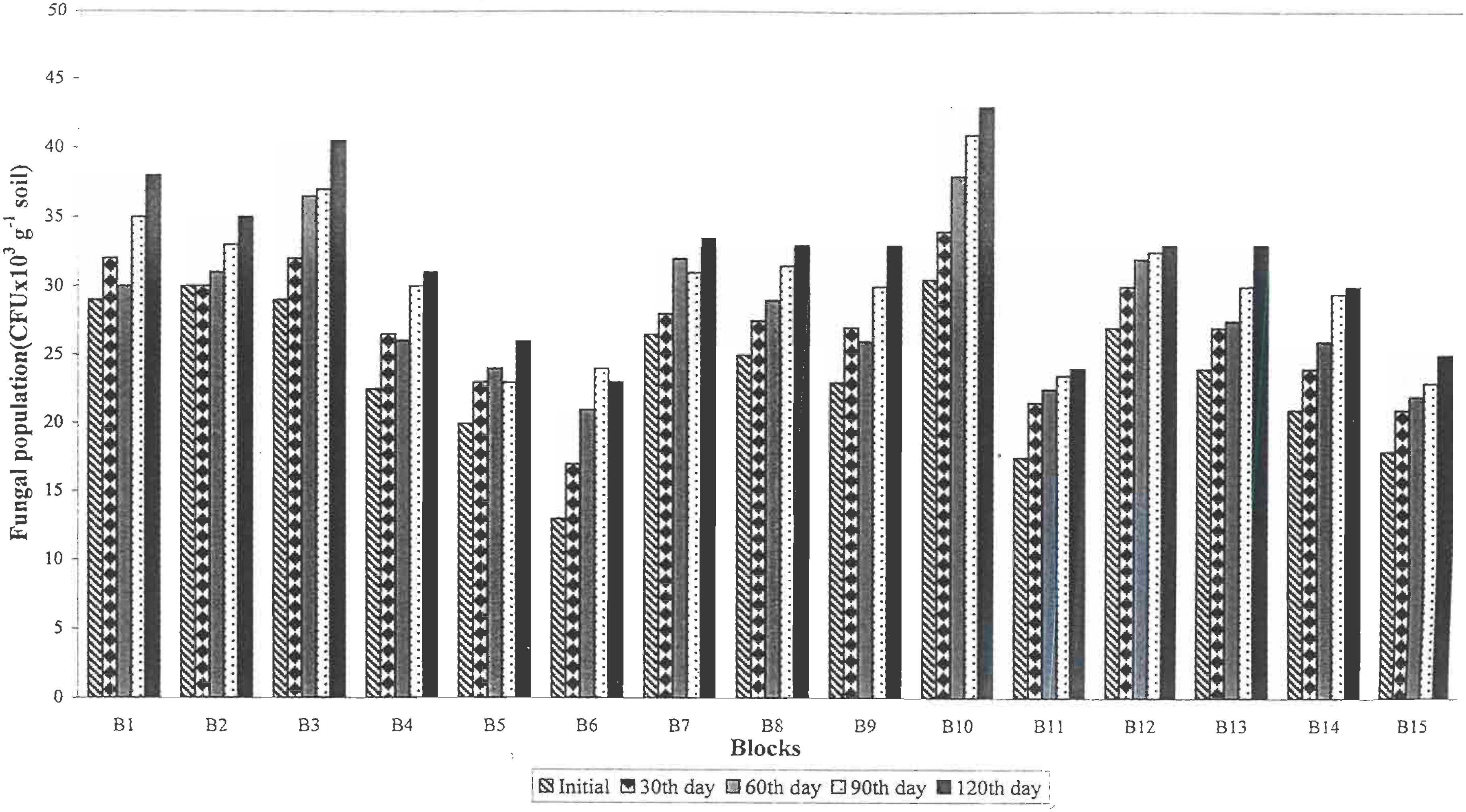
Fig. 4.2. Bacterial population in effluent irrigated soils



One gram soil sample was serially diluted and the bacterial population was enumerated by pour-plate method using soil extract agar medium. (Allen, 1953). Population was calculated on oven dry basis.

Fig. 4.3. Fungal population in effluent irrigated soils



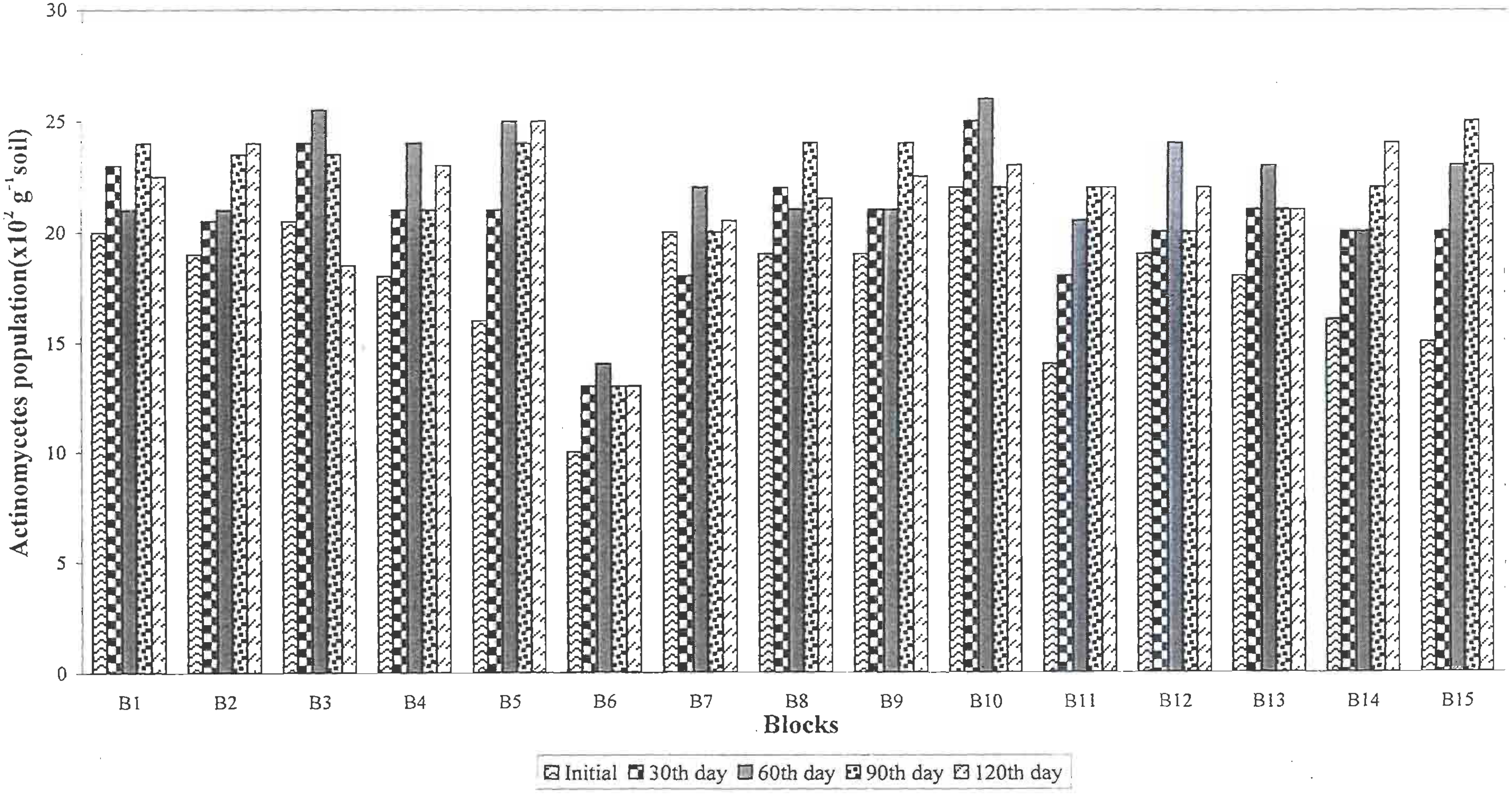
One gram soil sample was serially diluted and the fungal population was enumerated by pour-plate method using Martin's rose Bengal agar medium. (Martin, 1950). Population was calculated on oven dry basis effluent irrigation, was ranged from 23.66 (x $10^3.g^{-1}$ soil) to 32.06 (x $10^3.g^{-1}$ soil). The fungal population in the blocks (B1 – B15) before effluent irrigation was very less and increased significantly after effluent irrigation and reached the maximum on 120th day of effluent irrigation. Significant level of increase in population was observed throughout the study period. Regarding the interaction between blocks and days, highest population was observed in B10 (43.00 x $10^3.g^{-1}$ soil) and in B3 (40.5 x $10^3.g^{-1}$ soil) on 120th day of effluent irrigation.

The fungal population observed in the blocks B2, B3, B8 and B1- B15, before effluent irrigation showed significant increase on 30th, 60th, 90th and 120th day of effluent irrigation. Whereas in blocks B1, B4 - B6, B7 and B9, it did not increase steadily instead, a fluctuation was observed.

4.3.3. Actinomycetes population in effluent irrigated soils

The blocks B1 - B15 varied in actinomycetes population ranged between 12.60 (x $10^2.g^{-1}$ soil) and 23.60. Among the blocks, B10 recorded the maximum (23.60 x $10^2.g^{-1}$ soil), followed by B3, B1 and B5. Parity was observed among B1, B3 and B5. The population of all the blocks both before and after effluent irrigation (on 30th, 60th, 90th and 120th day) was ranging from 17.70 (x $10^2.g^{-1}$ soil) to 22.06 (x $10^2.g^{-1}$ soil). In which, the highest population was noted on 60th day (22.06 x $10^2.g^{-1}$ soil), followed by 90th day (21.93 x $10^2.g^{-1}$ soil). Significant difference in population was observed between initial and 30th day of effluent irrigation. Whereas, parity was noted among 60th, 90th and 120th day.

Fig. 4.4. Actinomycetes population in effluent irrigated soils



One gram soil sample was serially diluted and the actinomycetes population was enumerated by pour-plate method using Kenknight's agar medium. (Allen, 1953).
Population was calculated on oven dry basis

4.4. Influence of effluent irrigation on soil enzyme activities in blocks B1 – B15

The activity of soil enzymes *viz.,* dehydrogenase, phosphatase and urease in the blocks B1 – B15 before (initial) and after effluent irrigation (on 30th, 60th, 90th and 120th day) are given in table 4.3, 4.4 and 4.5 respectively.

4.4.1. Dehydrogenase activity in effluent irrigated soils

The dehydrogenase activity (μg of TPF released g^{-1} soil h^{-1}) in blocks B1-B15 was ranging between 0.71 and 2.83. Among the blocks, B3 recorded the maximum activity (2.83) followed by B10 and B1.

The dehydrogenase activity collectively in all the blocks during the study period showed significant difference and varied from 1.55 to 2.02. The enzyme activity was minimum in all the blocks prior to effluent irrigation and increased after effluent irrigation and attained maximum on 90th day of effluent irrigation. Among the blocks, B3 recorded maximum activity (3.20) on 60th day of effluent irrigation. A very low activity was noted in B6 throughout the study period.

4.4.2. Phosphatase activity in effluent irrigated soils

Phosphatase activity (μg of inorganic phosphate released g^{-1} soil h^{-1}) in blocks B1-B15 ranged between 0.88 and 2.75. Among the blocks, the enzyme activity was the highest in B3 (2.75) followed by B13 and B12. The lowest activity was noted in B10 (0.88) which was on par with B14 (0.92).

The enzyme activity collectively in all the blocks both before and after effluent irrigation ranged from 0.91 to 1.73. The activity of the enzymes after effluent irrigation

Table 4.3. Dehydrogenase activity in effluent irrigated soils

Blocks	Dehydrogenase activity (µg of TPF released g^{-1} h^{-1} soil)					Mean
	Initial	30th day	60th day	90th day	120th day	
B1	ij 2.22	fg 2.62	efg 2.68	de 2.75	def 2.71	b 2.59
B2	opqr 1.92	ijk 2.20	i 2.25	h 2.35	h 2.36	c 2.21
B3	i 2.23	c 2.92	b 3.07	a 3.20	de 2.74	a 2.83
B4	~♣♦ 1.32	opqr 1.92	nopq 1.95	mno 2.02	tuvw 1.73	j 1.79
B5	♣♦♥, 1.27	z{|| 1.56	vwxy 1.68	vwxy 1.67	♦♥; 1.23	j 1.48
B6	’’ 0.54	f 0.74	f 0.76	f 0.79	f 0.80	$ 0.73
B7	vwxy 1.68	k$m 2.10	jk$ 2.12	ijk 2.16	stu 1.79	e 1.98
B8	|} 1.49	nopq 1.95	mnop 2.00	$mn 2.04	stuv 1.77	f 1.85
B9	~♣ 1.34	rst 1.83	opqr 1.93	nop 1.99	wxyz{ 1.64	h 1.75
B10	i 2.24	efg 2.66	def 2.71	d 2.79	g 2.59	b 2.60
B11	’ 1.17	wxyz{ 1.63	tuvw 1.73	uvwx 1.71	de 2.74	g 1.79
B12	pqr 1.90	ijk 2.16	ijk 2.18	hi 2.26	pqr 1.90	d 2.08
B13	{~ 1.41	stuv 1.77	qrs 1.85	nopq 1.94	xyz{ 1.60	h 1.71
B14	’ 1.19	{| 1.55	yz{| 1.59	xyz{ 1.61	♦, 1.21	k 1.43
B15	♣♦♥ 1.31	vwxyz 1.66	stuv 1.77	stu 1.79	yz{ 1.59	i 1.63
Mean	e 1.55	c 1.95	b 2.02	a 2.07	d 1.89	1.90

	SEd	CD (0.05)
D	0.29	0.57
B	0.50	1.00
D x B	1.12	2.24

Any two means having a common letter are not significantly different at 5 per cent level of significance.

Assay of dehydrogenase activity was carried out for soil samples at pH 7 (Casida *et al.*, 1964). TTC at 3 per cent level was used as substrate. The amount of TPF (Triphenyl Farmozan) released was assayed after incubation period of 24 hours. The results were expressed on oven dry basis. The values given are mean of two replications.

Table 4.4. Phosphatase activity in effluent irrigated soils

Blocks	Phosphatase activity (µg of inorganic phenol released g^{-1} soil h^{-1})					Mean
	Initial	30th day	60th day	90th day	120th day	
B1	xyz{| 0.94	cdef 1.89	bcde 1.96	bcd 2.01	bc 2.03	c 1.77
B2	~♣ 0.68	nopqrst 1.44	opqrst 1.43	nopqrs 1.46	mnopqr 1.47	g 1.29
B3	efghij 1.78	a 2.93	a 3.02	a 3.02	a 3.00	a 2.75
B4	z{|} 0.89	ijk$mno 1.62	ghijk$ 1.68	fghijk 1.71	fghijk 1.71	e 1.52
B5	{|}~ 0.80	defgh 1.82	bcde 1.91	bcde 1.93	cdefg 1.87	d 1.67
B6	{~♣ 0.71	$mnopqr 1.48	k$mnop 1.55	k$mnop 1.58	jk$mno 1.60	f 1.38
B7	{~♣ 0.71	nopqrs 1.46	k$mnopq 1.54	k$mnop 1.57	ijk$mno 1.60	f 1.37
B8	vwxy 1.11	bcde 1.92	bcde 1.97	bc 2.02	bcd 2.00	c 1.80
B9	|}~ 0.76	jk$mnop 1.59	hijk$m 1.64	hijk$m 1.66	fghijk 1.71	e 1.47
B10	♦ 0.47	{|}~ 0.80	{|}~ 0.86	yz{| 0.92	qrstu 1.35	j 0.88
B11	♣♥ 0.52	uvw 1.22	rstuv 1.29	rstu 1.32	stuv 1.27	i 1.12
B12	k$mnop 1.56	bcde 1.96	bc 2.02	bc 2.07	defghi 1.81	b 1.88
B13	k$mnop 1.56	bcde 1.98	bc 2.05	b 2.11	bc 2.06	b 1.95
B14	♣♦ 0.52	yz}| 0.92	wxyz 1.06	vwx 1.12	xyz{ 0.99	j 0.92
B15	~♣ 0.67	rstuv 1.30	nopqrs 1.45	pqrstu 1.39	tuv 1.25	h 1.21
Mean	c 0.91	b 1.62	a 1.69	a 1.73	a 1.71	1.53

	SEd	CD (0.05)
D	0.02	0.04
B	0.03	0.07
D x B	0.08	0.16

Any two means having a common letter are not significantly different at 5 per cent level of significance.

To assay phosphatase activity, 20 ml of 0.5 per cent di-sodium phenyl phosphate was used as substrate. Amount of phenol released was estimated after an hour of incubation at 37°C (Halstead, 1964). Results were expressed on oven dry basis. The values represent the mean of two replications.

increased up to 90th day, later on it decreased. With reference to the days and blocks interaction, maximum activity was observed in B3 on 60th and 90th day of effluent irrigation (3.02).

4.4.3. Urease activity in effluent irrigated soils

The blocks B1 - B15 varied in urease activity (µg of ammonia – N released g^{-1} soil h^{-1}) and ranged from 5.73 to 8.83. Among the blocks B5 had the highest activity (8.83) followed by B1 and B4.

The urease activity collectively in all the blocks observed during the study period, was ranging from 5.61 to 8.22. Initially (prior to effluent irrigation) all the blocks showed only minimum activity (5.61), after effluent irrigation, the activity increased and reached maximum on 60th day followed by 90th day of effluent irrigation. Among the blocks, urease activity was maximum in B3 (9.52) which was observed on 90th day of effluent irrigation. Where as it was very less in B2 before effluent irrigation.

4.5. Morphological features of ligninolytic fungi isolated from soil enriched with pulp plant effluent

All the morphological key features of the screened ligninolytic fungi C1 – C12 are given in the table 4.6. Among the 12 strains (Plates 4.3 (a-l) and 4.4 (a-k)) C5, C8, C11 and C12 showed the maximum poly-R dye degradation.

4.6. Treatments of pulp plant effluent using various colour removing agents

Using bacteria, fungi, actizyme, natural coagulants etc. the colour of the pulp-plant effluent was removed under laboratory condition. And the results of the each experiment are discussed in detail.

Table 4.5. Urease activity in effluent irrigated soils

Blocks	Urease activity (μg of ammonia – N released g^{-1} soil h^{-1})					Mean
	Initial	30th day	60th day	90th day	120th day	
B1	v 6.78	gh 8.66	e 8.97	f 8.88	gh 8.70	b 8.40
B2	# 3.94	f 5.47	♣♦ 6.14	vw 6.73	{| 6.38	m 5.73
B3	… 4.51	f 8.86	c 9.27	a 9.52	ef 8.93	c 8.22
B4	u 6.90	wx 6.66	c 9.26	v 9.43	f 8.86	c 8.22
B5	u 6.90	d 9.08	b 9.40	a 9.52	c 9.27	a 8.83
B6	{|} 6.34	j 8.38	f 8.86	gh 8.71	i 8.47	d 8.15
B7	♦ 6.10	k$ 8.09	g 8.75	gh 8.66	q 7.38	e 7.80
B8	~♣ 6.19	k 8.12	i 8.48	j 8.37	r 7.29	f 7.69
B9	’’ 5.00	vw 6.72	t 7.03	uv 6.81	z{ 6.43	k 6.40
B10	… 4.50	|} 6.32	xy 6.59	v 6.79	{| 6.41	$ 6.12
B11	♦ 5.78	o 7.72	k$ 8.10	$ 8.01	t 7.11	h 7.34
B12	t 4.05	}~ 6.27	h 8.64	k$ 8.09	rs 7.23	j 6.86
B13	’ 5.64	p 7.53	mn 7.84	u 6.90	x 6.61	i 6.90
B14	♦ 6.05	m 7.90	k 8.15	k$ 8.10	s 7.20	g 7.48
B15	f 5.55	pq 7.45	no 7.80	u 6.89	yz 6.51	j 6.84
Mean	e 5.61	c 7.55	a 8.22	b 8.09	d 7.52	7.40

	SEd	CD (0.05)
D	0.01	0.02
B	0.01	0.03
D x B	0.04	0.08

Any two means having a common letter are not significantly different at 5 per cent level of significance.

Assay of urease activity was carried out for soil samples at pH 7 (Tabatabai and Bremner (1972)). One ml of 0.2 M urea solution was used as substrate, incubated at 30°C for 2 h. The ammonia liberated was estimated by Nessler's by method (Jackson, 1973). The enzyme activity was expressed as μg of ammonia-N released g^{-1} soil h^{-1}. The values given are mean of two replications.

Plates 4.3 (a-f). Ligninolytic fungal strains isolated from pulp-plant effluent enriched soil

C1

(a)

C2

(b)

C3

(c)

C4

(d)

C5

(e)

C6

(f)

Plates 4.3 (g-1). Ligninolytic fungal strains isolated from pulp-plant effluent enriched soil

C7

(g)

C8

(h)

C9

(i)

C10

(j)

C11

(k)

C12

(l)

Plate 4.4 (a-d). Morphological features of isolated ligninoltytic fungal strains

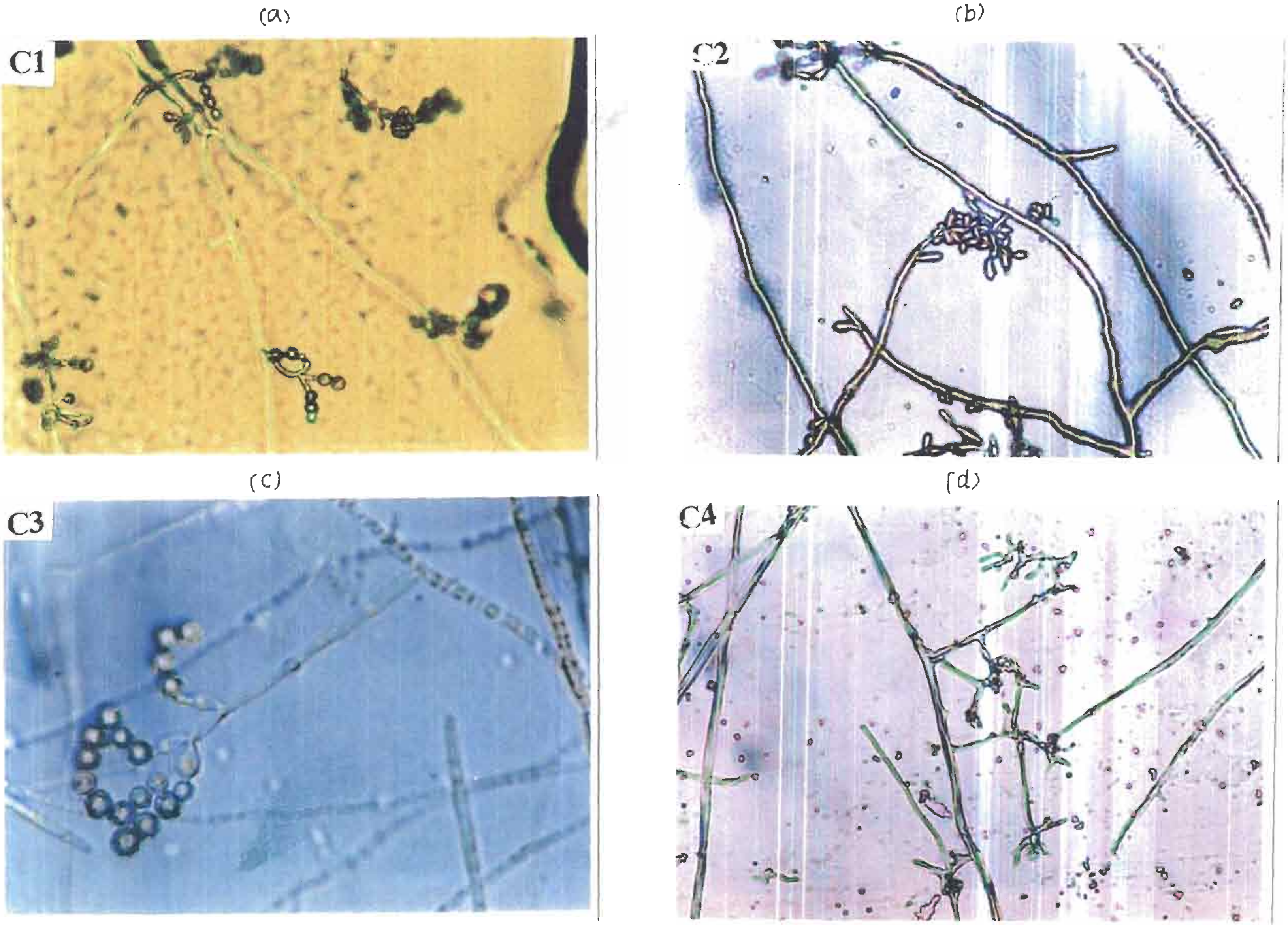


Plate 4.4 (e-h). . Morphological features of isolated ligninoltytic fungal strains

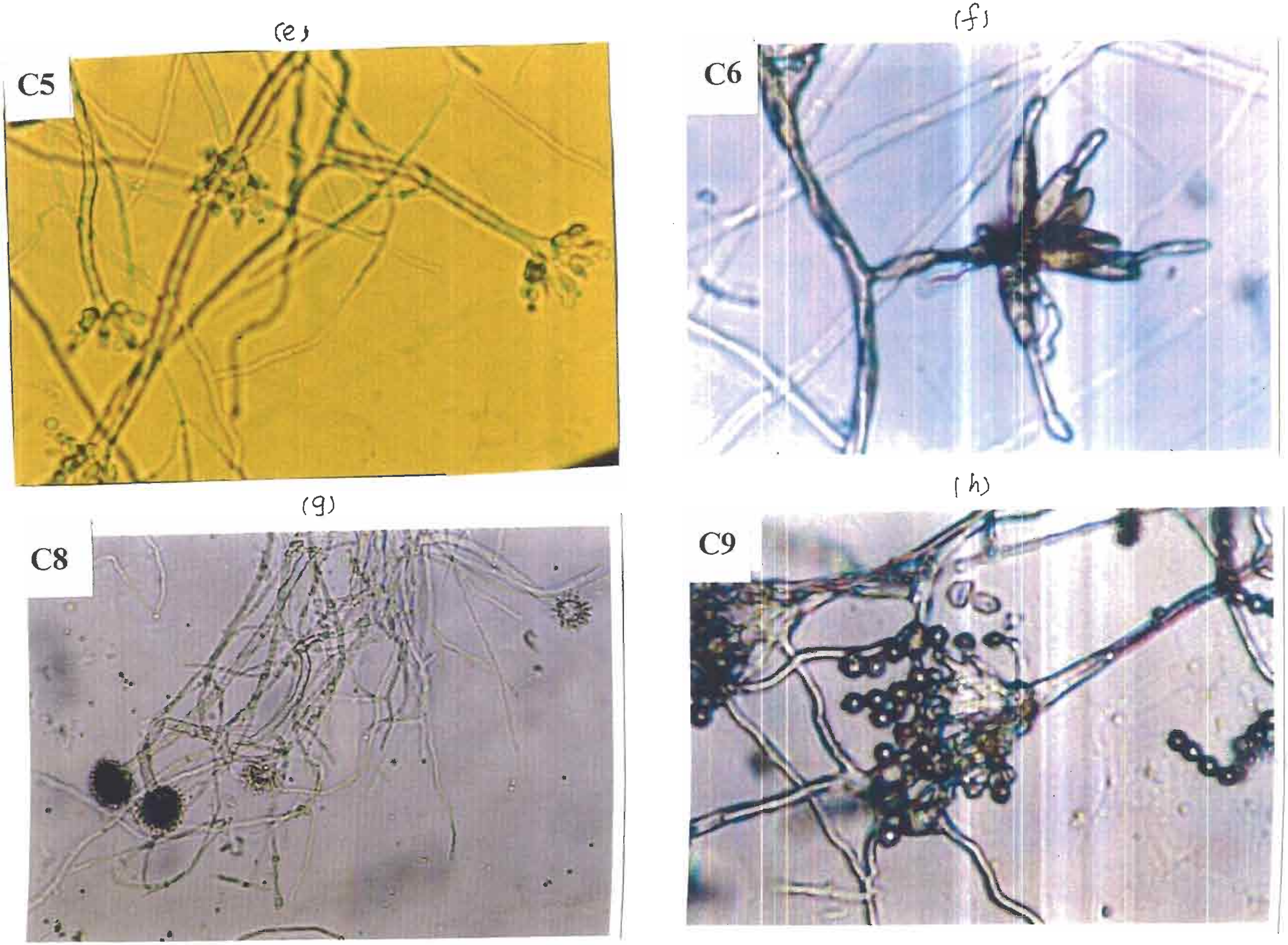
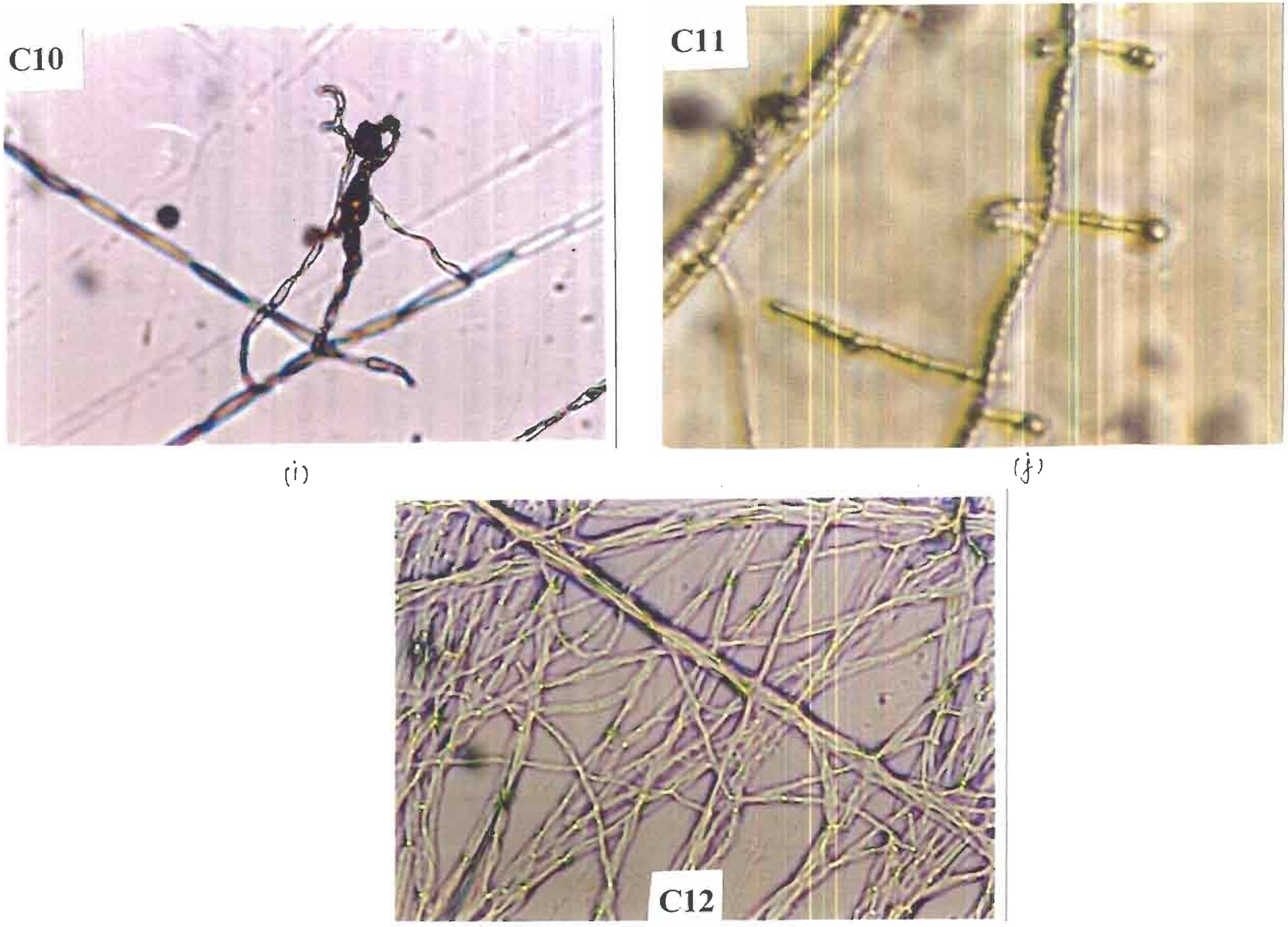


Plate 4.4 (i-k). Morphological features of isolated ligninoltytic fungal strains



(i) (j) (k)

Table 4.6. Morphological features of ligninolytic fungi isolated from effluent enriched soils

Culture number	Morphology
C1	Colonies on Czapeck's agar were floccose and spreading. Surface, initially white became off-white later. Trichotomously branched conidiophores raised from aerial mycelium. Branches were short which bore round and smooth conidia.
C2	Colonies on Czapeck's agar which were floccose and having initially blue-green surface, became dark brown after 5 days. Reverse became white to yellowish. Conidiophores, raised in branches from mycelium on one side, bore ovate conidia.
C3	Colonies on Czapeck's agar, which were white, became almost black in seven days. Conidiophores, raised from mycelium at right angles, bore four to nine round shaped conidia.
C4	Colonies on Czapeck's agar were pure white and spreading. Conodiophores were erect, delicate, septate and with branchletes in an alternate or opposite manner. Conidia, formed at the tip of the branchlets were tiny and globose.
C5	Colonies on Czapeck's agar, which were white, became gray with loose floccose and septate mycelium. Phialides were short, elongated and bulged towards apex. One or two conidia in chains arranged at the tip of the phialides.
C6	Colonies on Czapeck's agar were dull white and powdery. From the septate mycelia erect conidiophores were formed. Spindle-shaped conidia were formed at the tip of the secondary conidiophores.
C7	Fungus on Czapeck's agar produced only restricted growth. Only few transparent hyphae formed over the medium and fruiting bodies were could not be able to visualize even under 100x magnification.
C8	Colonies on Czapeck's agar which were white, slowly became yellowish-green with white margin and became black after ten days. Conidiophores raised from mycelia were enlarged at the apex and bore round conidia in chains
C9	Colonies on Czapeck's agar were gray-green to dark green and densely matted. Their surface was hard, folded and wrinkled. After seven days the colour turned to dark brownish green. Reverse and agar became more or less brownish. Conidiophores were enlarged at the apex and bore round conidia in chains
C10	Colonies on Czapeck's agar, which were pure white, became smokish black on aging. On growing, septate mycelium became bulged and got detached.
C11	Colonies on Czapeck's agar were initially pure white and brownish-black discolouration, from margin towards centre, was observed in old cultures. From mycelium conidiophores were raised at right angles bore single and round conidia.
C12	Colonies on Czapeck's agar were pure white. After ten days margins became wine-brown colour. Mycelia were densely woven. Fruiting bodies were could not be able to visualize even under 100 x magnification.

All cultures from C1 to C12 were maintained in agar slants under room temperature. For identification, they were cultured by "slide culture technique". Photographs were taken with appropriate magnification using camera attached microscope.

4.6.1. Effect of various cultures and nitrogen addition on absorbance of pulp plant effluent – Batch study (Plate 4.5)

Colour of the pulp-plant effluent was removed using bacteria, fungi and actizyme. T1 was maintained as control. T2 was inoculated with mixed cultures of *Bacillus* sp. and *Pseudomonas* sp. In T3 the fungi *Fusarium* sp. was used. In T4 actizyme was used. The efficiency of decolourisation of the treatments (T1 – T4) was tested without nitrogen addition (N1) and with nitrogen addition (N2). The absorbance of the effluent at two days interval was measured upto 16th day of incubation period and the results are given in Fig. 4.5.

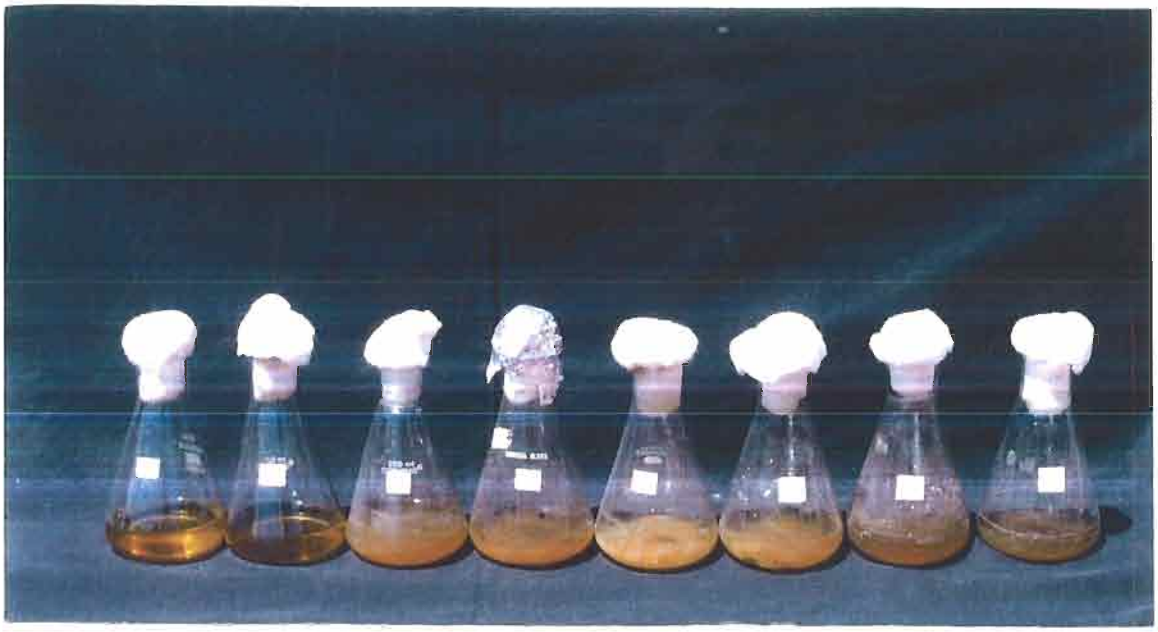
All the treatments irrespective of nitrogen addition showed significant change in the absorbance of the effluent. The absorbance of the effluent in T1 – T4 at various incubation period (D0, D2, D4 … D14 and D16) was ranging from 0.252 - 0.506 nm. The absorbance of the effluent decreased with increase of incubation period. Among the treatments (T1 – T4) T3 (*Fusarium* sp.) recorded maximum reduction in absorbance (0.235 nm) which was observed on 16th day of incubation period (D16).

4.6.2. Effect of various ligninolytic fungal strains and glucose levels on the absorbance of pulp-plant effluent – Batch study (Plates 4.6 a-c)

The results of the decolourisation experiment of pulp-plant effluent with various fungal culture isolated from effluent enriched soil are presented in Fig. 4.6. Treatment S1 was control without microbial inoculam, whereas the treatments S2, S3, S4 and S5 were inoculated with fungal culture – C5, C8, C11 and C12 respectively.

The absorbance of the effluent in treatments S1 – S5 irrespective of glucose levels (G1 – 0.3%, G2 – 0.6%) ranged between 0.349 – 0.503 nm. Among the treatments, S3

Plate 4.5. Decolourisation of pulp plant effluent with standard microbial cultures



T1 N1 - Control (100 ml effluent + carbon source)*

N2 - Control (100 ml effluent + carbon source+Nitrogen source)**

T2 N1 - 100 ml effluent + 0.5 ml of broth containing *Bacillus* sp.+ 0.5 ml of broth containing *Pseudomonas* sp.+ Carbon source

N2 - 100 ml effluent + 0.5 ml of broth containing *Bacillus* sp.+ 0.5 ml of broth containing *Pseudomonas* sp.+ Carbon source + Nitrogen source

T3 N1 - 100 ml effluent + 1.0 ml of broth containing *Fusarium* sp.+ carbon source

N2 - 100 ml effluent + 1.0 ml of broth containing *Fusarium* sp.+ carbon source + nitrogen source

T4 N1 - 100 ml effluent + Actizyme** + carbon source

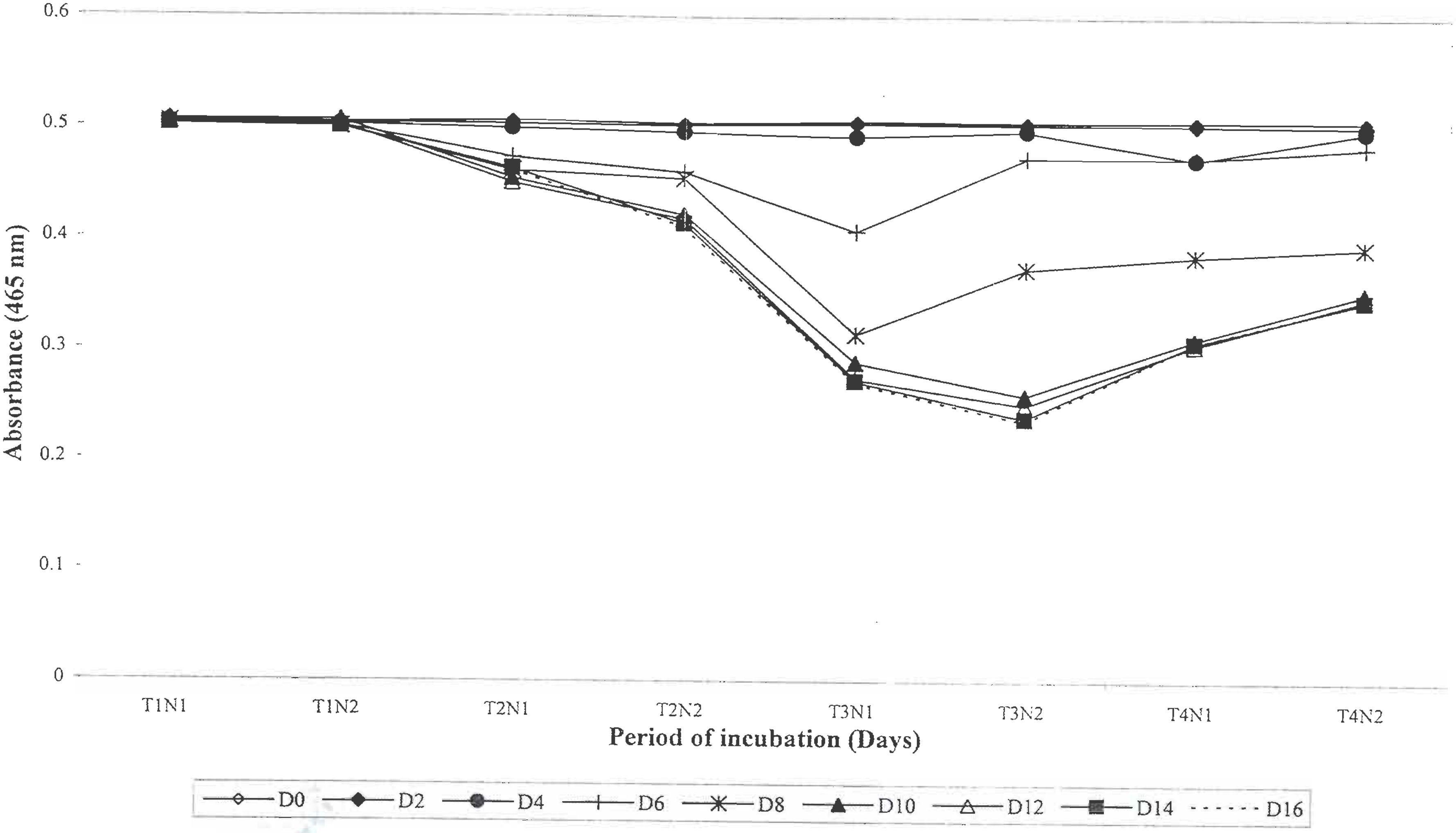
N2 - 100 ml effluent + Actizyme + carbon source + Nitrogen source

* Glucose 0.1 gram 100 ml^{-1} effluent

** Diammonium sulphate 0.1 g 100 ml^{-1} effluent

*** Actizyme 0.5 gram 100 ml^{-1} effluent

Fig. 4.5. Effect of various cultures and nitrogen addition on the absorbance of pulp-plant effluent



The decolourising ability of the bacteria, fungi and actizyme were investigated with and without nitrogen (N1 and N2). The colour reduction was analysed by measuring the absorbance at 465nm. All the values given are mean of two replications (culture - 8) recorded maximum reduction in absorbance followed by S5 (culture - 12). Very poor reduction was noticed in S1 – control (0.503 nm).

Among the two levels of glucose addition (G1 0.3%; G2 0.6%) G1 registered maximum reduction in colour of the effluent.

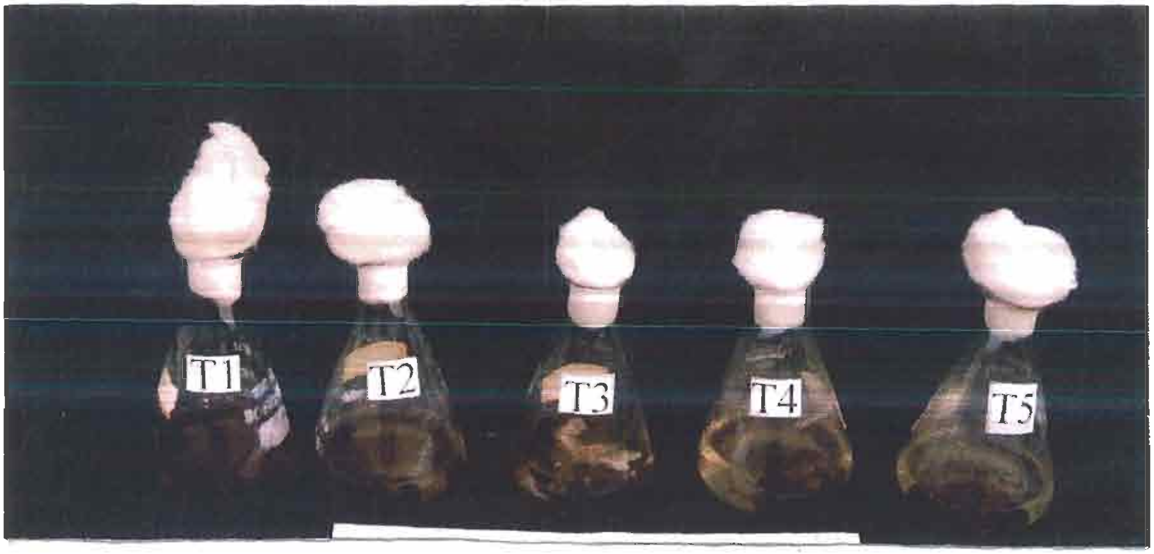
Among the incubation periods (D0, D2, D4 ..... D10 and D12) the absorbance of the effluent was very much reduced on 12th day (D12). The effect of addition of glucose @ 0.3% (G1) and 0.6% (G2) on the absorbance of effluent in treatments S1 – S5 ranged between 0.335 nm (S3G1) and 0.504 nm (S1G1).

The change in absorbance of the effluent treatments S1, S2, S3, S4 and S5 at incubation periods D0 – D12 ranged between 0.230 nm - 0.507 nm. Maximum reduction was observed in S3 (culture - 8) and S5 (culture - 12) on 12th day of incubation (D12). The effect of glucose levels on absorbance of effluent at different incubation periods (D0 – D12) showed the maximum reduction in G1 (0.3% glucose) on 12th day of incubation period - D12 (0.295 nm).

The changes observed in absorbance of the effluent influenced by the interaction of all the three factors *viz.*, treatments (S1 – S5), glucose levels (G1 and G2) and incubation period (D0 – D12) showed significant reduction. Among the treatments, the maximum reduction in absorbance was noticed in S3 (culture - 8) under 0.3% glucose level (G1) on 12th day (D12) and the absorbance of effluent was 0.203 nm.

Plates 4.6 (a and b). Decolourisation of pulp plant effluent with ligninolytic fungal cultures isolated from effluent enriched soil – Batch study

(a)



(b)

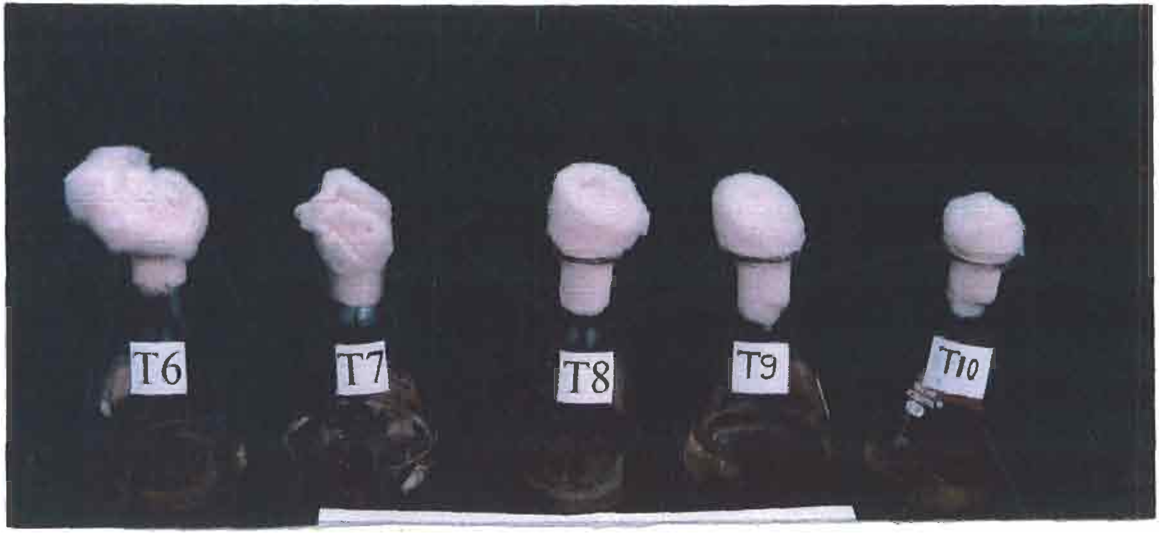
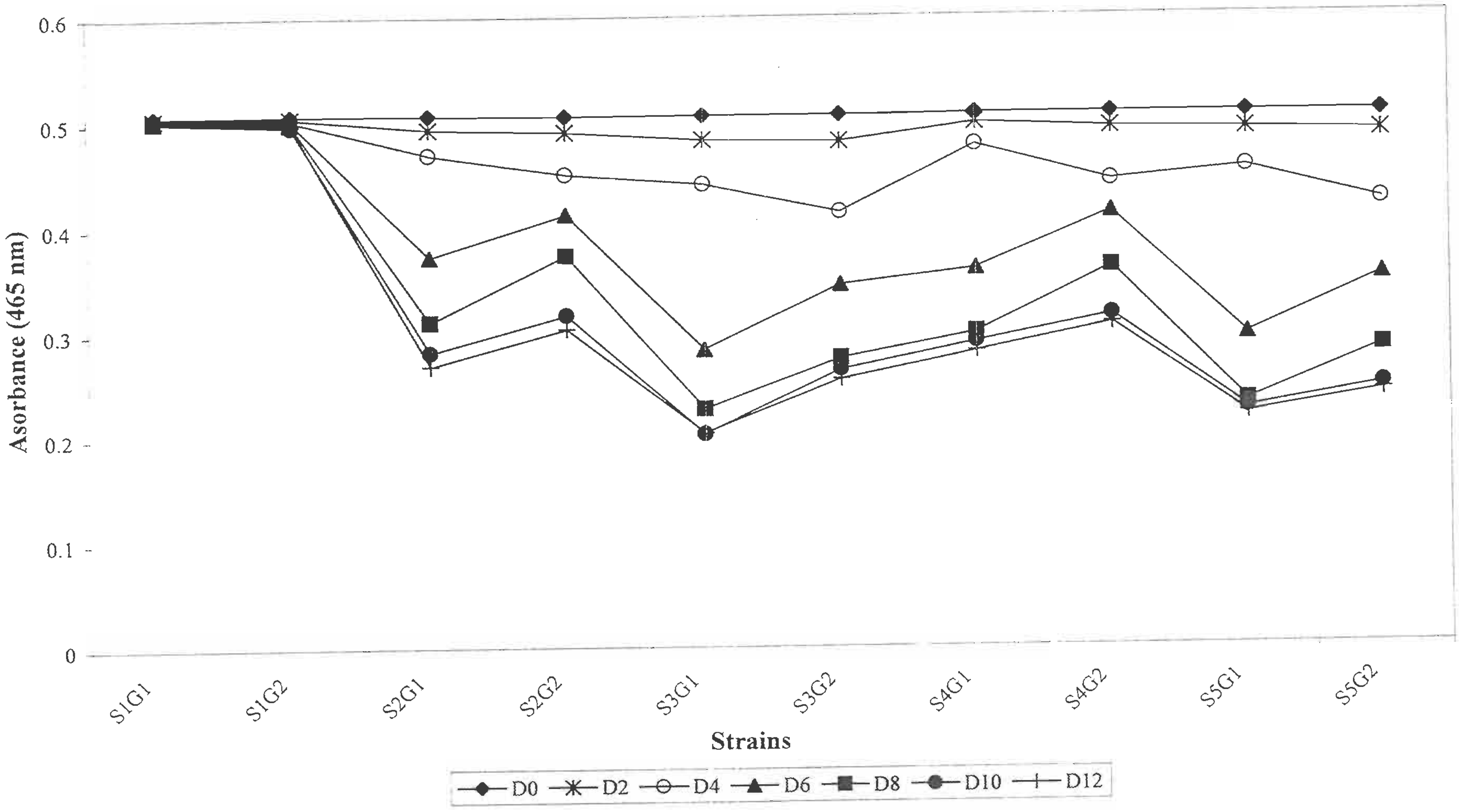


Plate 4.6 (c). Close up view of solid matrix adhered by mycelial biomass

Fig. 4.6. Effect of various ligninolytic fungal strains and glucose levels on the absorbance of pulp plant effluent - Batch experiment



The efficient strains (C5, C8, C11 and C12) were immobilised on solid matrix and cultured in flasks containing sterilised, untreated effluent supplied with glucose at two levels (0.3 and 0.6 per cent). The rate of colour reduction was measured at 465 nm in alternate days upto 12 days incubation period.

4.6.3. Effect of ligninolytic cultures on absorbance of the pulp-plant effluent – Column study (Plates 4.7 a&b)

The absorbance of the pulp-plant effluent of treatments C1, C2, C3, C4 and C5 was observed at 3 hours interval (H0, H3, H6, H9 and H12). Treatment C1 was maintained as control without inoculum. Whereas C2, C3, C4 and C5 were inoculated with ligninolytic fungal cultures – C5, C8, C11 and C12 respectively. The results are presented in Table 4.7.

The absorbance (nm) of the effluent in treatments C1, C2, C3, C4, and C5 ranged from 0.232 to 0.371 nm. The maximum reduction in absorbance of effluent was noticed in C2 (culture-5), C4 (culture-11) and C3 (culture-8) and the absorbance were 0.232 nm, 0.239 nm and 0.239 nm respectively.

The absorbance of the effluent at different retention times (H0, H3, H6, H9 and H12) irrespective of treatments (C1 – C5) showed significant reduction. Initially (H0) the absorbance was very high (0.505 nm) and later showed decreasing trend with retention time and at 12th hour (H12) it was very much reduced (0.112 nm).

The absorbance of effluent recorded in treatments C1 – C5 of different retention time showed significant reduction in colour. Initially (H0) the absorbance of the effluent was very high in all the treatments later it was very much reduced and the maximum reduction was noted in treatments C5 (culture-12), C4 (culture-11) and C3 (culture-8) at 12th hour (H12) and the absorbance was 0.062 nm, 0.064 nm and 0.067 nm respectively.

Plate 4.7 (a). Decolourisation of pulp plant effluent with ligninolytic fungal culture isolated from effluent enriched soil – Column study

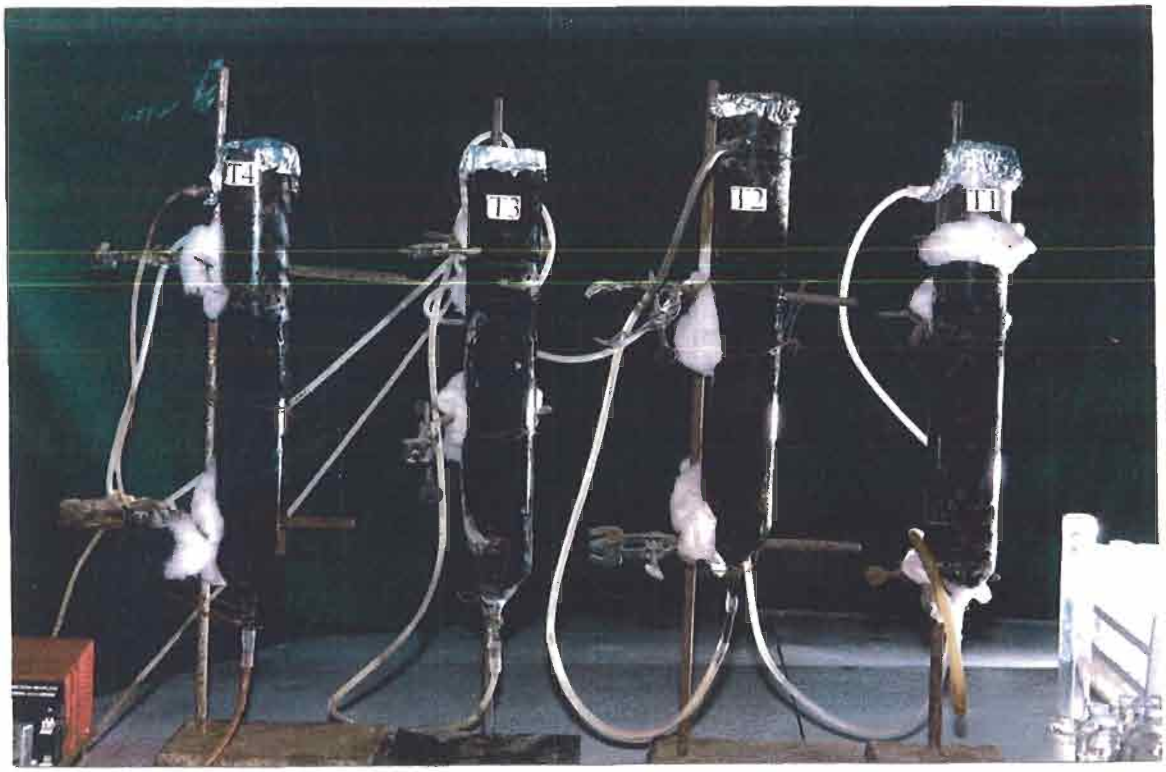


Plate 4.7 (b). . Decolourisation of pulp plant effluent with ligninolytic fungal culture isolated from effluent enriched soil – Column study

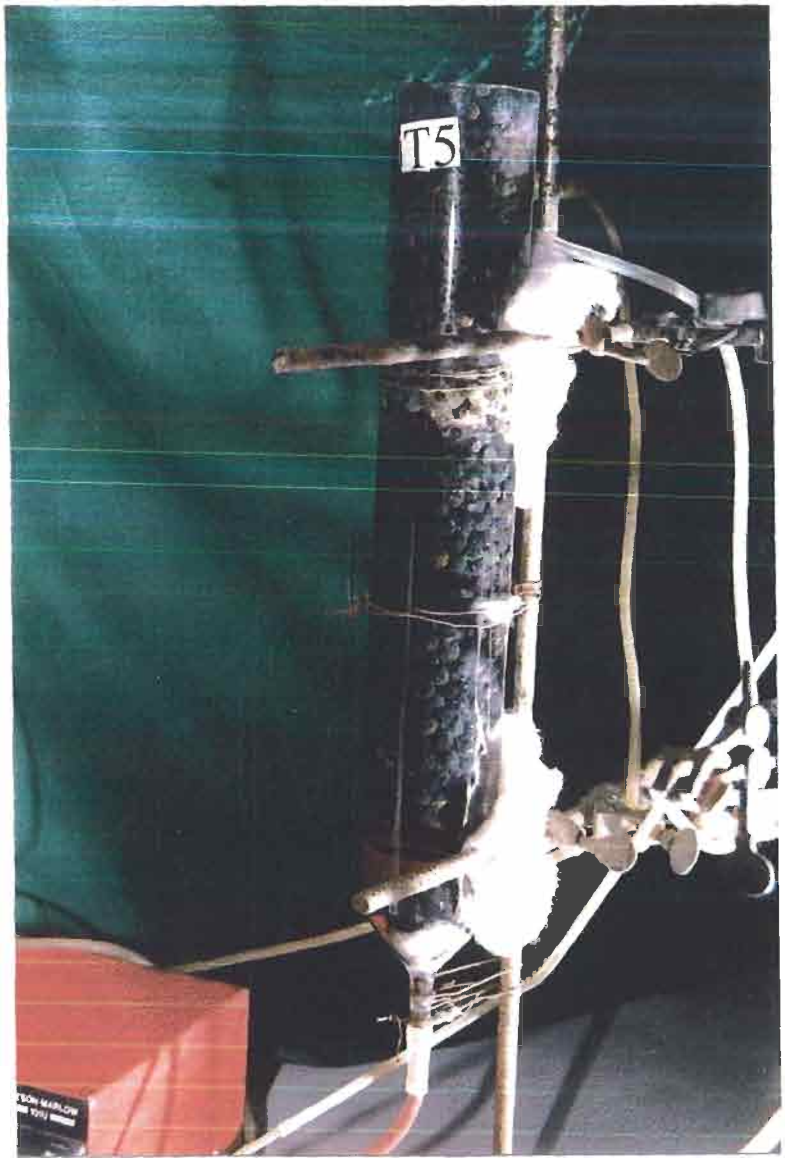


Table 4.7. Effect of various ligninolytic fungal strains on the absorbance of pulp plant effluent -Column study

Treatment	Absorbance (at 465 nm) at different retention times (hours)					
	H0 (Initial)	H3	H6	H9	H12	Mean
C1 (control)	0.509^{a}	0.456^{b}	0.325^{c}	0.294^{d}	0.272^{e}	0.371^{a}
C2 (culture 5)	0.505^{a}	0.276^{de}	0.145^{i}	0.139^{i}	0.095^{j}	0.232^{c}
C3 (culture 8)	0.504^{a}	0.292^{d}	0.169^{gh}	0.164^{h}	0.067^{k}	0.239^{c}
C4 (culture 11)	0.503^{a}	0.284^{de}	0.173^{gh}	0.171^{gh}	0.064^{l}	0.239^{c}
C5 (culture 12)	0.505^{a}	0.275^{de}	0.234^{f}	0.184^{g}	0.062^{l}	0.252^{b}
Mean	0.505^{a}	0.317^{b}	0.209^{c}	0.190^{d}	0.112^{e}	0.267

	SEd	CD (0.05)
C	0.003	0.007
H	0.003	0.007
C x H	0.008	0.017

Any two means having a common letter are not significantly different at 5 per cent level of significance

The raw effluent (pH adjusted to 6.5 with orthophosphoric acid) was passed through the column @ 4ml min^{-1} using peristaltic pumps. The change in colour was estimated at different retention times

4.6.3.1. Effect of ligninolytic cultures on pH of the pulp-plant effluent – Column study

The pH of the effluents after treating with ligninolytic fungal cultures in various retention times are presented in table 4.8.

The change in pH of the effluent observed in all the treatments (C1 – C5) varied from 6.41 to 7.55. The pH observed in treatments C3 (culture-8), C4 (culture-11) and in C5 (culture-12) was 6.44, 6.41 and 6.42 respectively and they differed significantly from one another.

The pH of the treatments recorded under different retention times (H0, H3, H6, H9 and H12) showed significant difference. The maximum pH was recorded in control (C1) and showed increasing trend with retention time till 6th hour (H6). The maximum pH (7.73) was recorded in control (C1) at 6th hour (H6), whereas it was very low (6.33) in treatment C4 (culture-11) at 12th hour (H12).

4.6.4. Characteristics of moringa seed (extract)

The nature of moringa seed extract in detail is presented in table 4.9. Moisture content (per cent) of the moringa seed was 3.13 (per cent). The amount of protein and total phenols found in the seed extract were 144 ($mg.g^{-1}$ kernel) and 1.2 (equivalents of μg of pyrogallol ml^{-1} extract) respectively.

The pH and TDS of the extract were found to be 6.04 and 1290 ($mg\ l^{-1}$ extract) respectively. The Rf values of the protein extract of the seeds were (i) 0.948 (ii) 0.927 and (iii) 0.883 (cm). The amino acids identified based on the Rf value were isoleucine, lysine monohydrochloride and L. cystine (Plates 4.9 a-f).

Table 4.8. Effect of various ligninolytic cultures on pH of the pulp plant pulp plant effluent at different retention time - column study

Treatment	pH at different time intervals (hours)					
	H0 (Initial)	H3	H6	H9	H12	Mean
C1 (culture 1)	7.05^{e}	7.55^{d}	7.73^{a}	7.70^{c}	7.71^{b}	7.55^{a}
C2 (culture 5)	6.47^{g}	6.45^{j}	6.41^{q}	6.40^{s}	6.36^{x}	6.42^{d}
C3 (culture 8)	6.50^{f}	6.47^{h}	6.44^{l}	6.40^{t}	6.40^{v}	6.44^{b}
C4 (culture 11)	6.45^{i}	6.45^{k}	6.40^{r}	6.40^{u}	6.33^{y}	6.41^{e}
C5 (culture 12)	6.42^{p}	6.44^{m}	6.43^{n}	6.43^{o}	6.40^{w}	6.42^{c}
Mean	6.58^{e}	6.67^{b}	6.68^{a}	6.67^{c}	6.64^{d}	6.65

	SEd	CD (0.05)
C	0.01	0.02
H	0.01	0.02
C x H	0.02	0.05

Any two means having a common letter are not significantly different at 5 per cent level of significance

The raw effluent (pH adjusted to 6.5 with orthophosphoric acid) was passed through the column @ 4ml min^{-1} using peristaltic pumps. The change in pH was estimated at different retention times

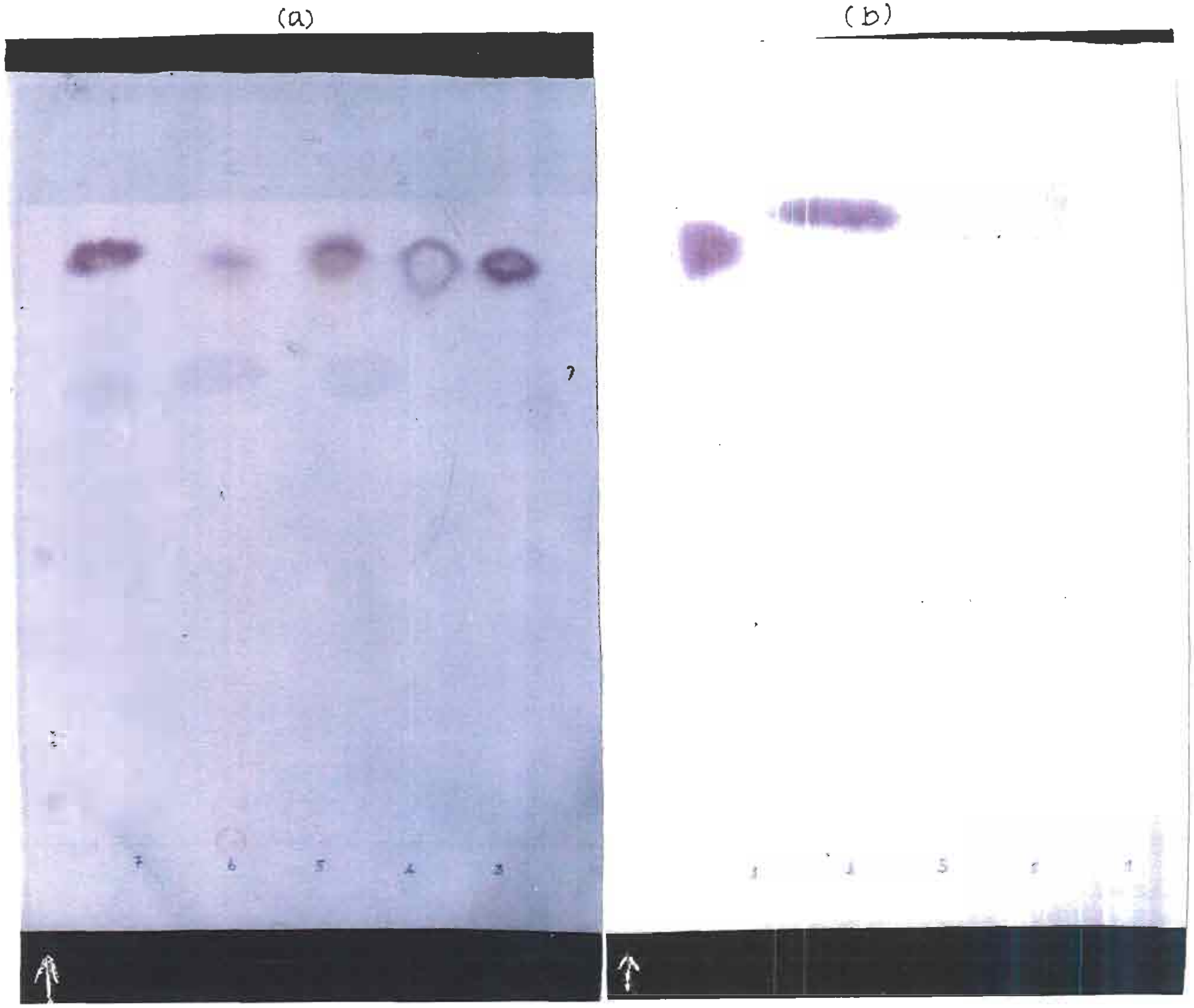
Table 4. 9. Characteristics of moringa seed (extract)

pH	6.04 ± 0.01	
EC (dSm^{-1})	2.1 ± 0.14	
TDS (mg l^{-1} extract)	1290 ± 14.14	
Moisture content (per cent)	3.13 ± 0.02	
Total phenols (equivalents of µg of pyrogallol ml^{-1} extract)	1.2 ± 0.14	
Protein content (mg g^{-1} kernel)	144 ± 6.36	
Amount of protein purified from moringa seed (mg g^{-1} kernel)	129 ± 3.53	
*Amino acids (Rf. values in cm)		
Isoleucine	i.	0.948
Lysine monohydrochloride	ii.	0.927
L-cystine	iii.	0.883

* Chromatograms were given in plates (4.9 a – f)

Aqueous extract of moringa seeds was prepared by soaking the seeds in distilled water for 24 hours. for the extraction of proteins, phosphate buffer (pH 7) was used and the coagulating proteins were precipitated with the solution of 100 per cent saturated ammonium sulphate and purified further by dialysis. The amino acids in seed proteins were identified by chromatographic methods. Values given in table are mean of two replications with standard deviation.

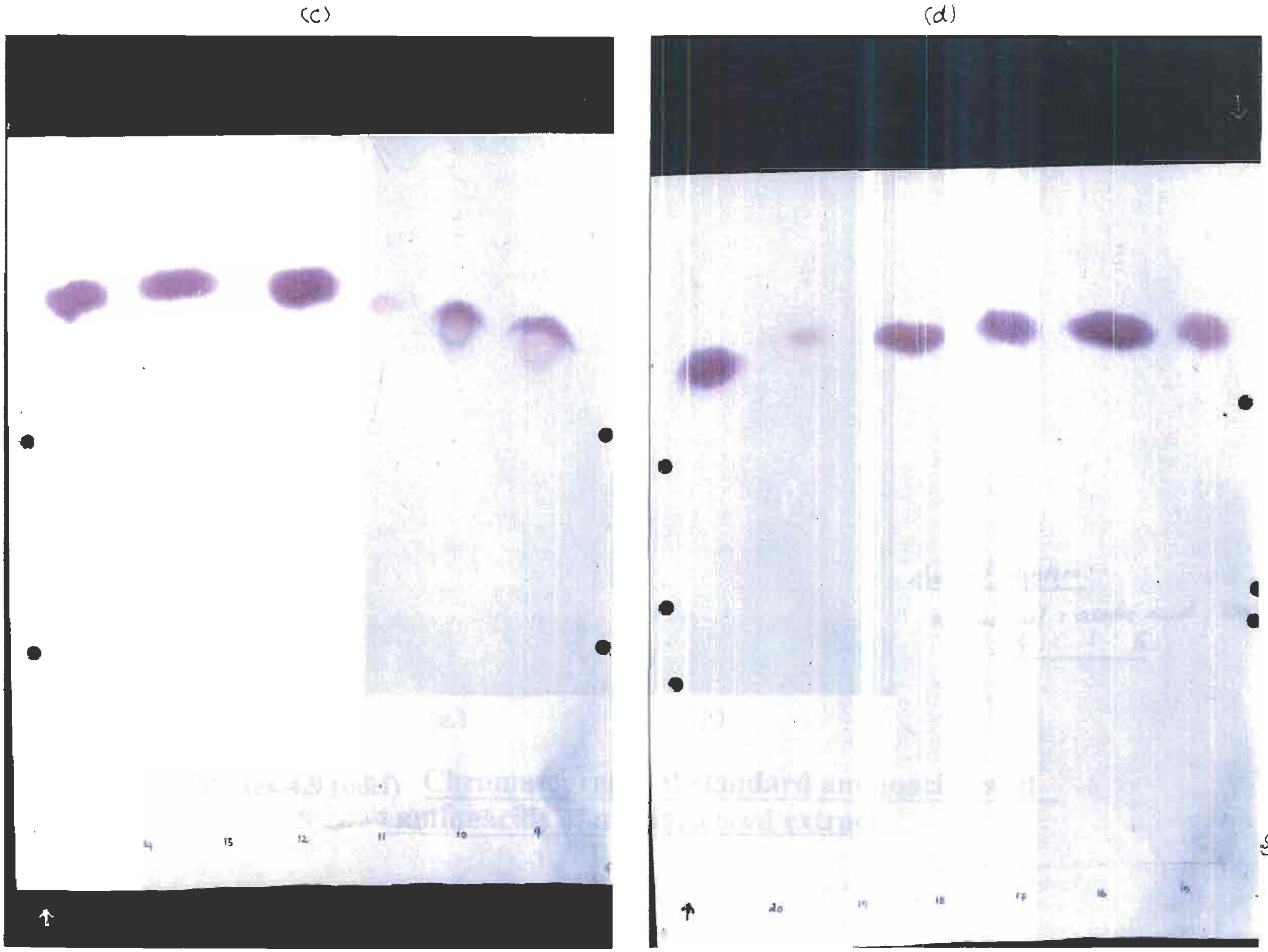
Plates 4.9 (a&b). . Chromatograms of standard aminoacids and aminoacids of moringa seed extract



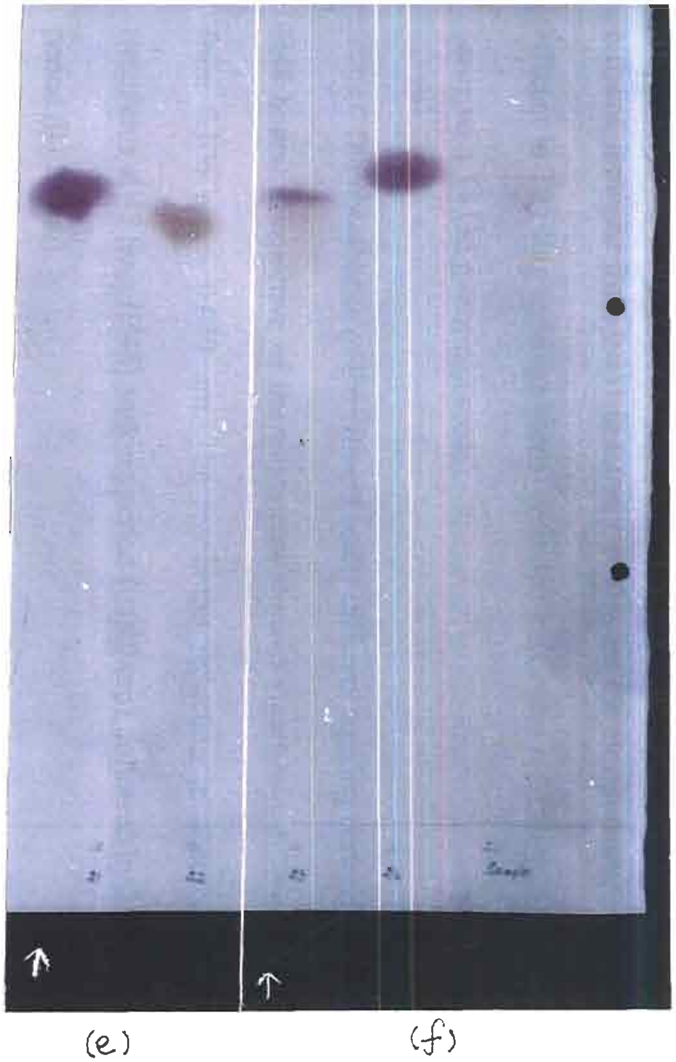
S = moringa seed extract

Solvent system : n-butanol : acetic acid : water : 4 : 1 : 5

Plates 4.9 (c&d). **. Chromatograms of standard aminoacids and aminoacids of moringa seed extract**


(c)
(d)

Solvent system:
n butanol: acetic aci
4:1:5



Solvent system:
n-butanol : acetic acid : water
4 : 1 : 5.

Plates 4.9 (e&f). Chromatograms of standard aminoacids and aminoacids of moringa seed extract

4.6.5. Decolourisation of pulp-plant effluent using moringa seeds (Plates 4.8 a-c)

Decolourisation study was carried out by soaking moringa seeds in different quantity in pulp-plant effluent. The change in absorbance, pH and EC of the effluent at various soaking periods (H12, H24, H36 and H48) were recorded and are presented in Fig. 4.7, Table 4.10 and fig.4.8 respectively.

4.6.5.1. Effect of moringa seeds on absorbance of the effluent

Significant difference was observed between the absorbance of treated and untreated effluents. The absorbance of the untreated effluent was 0.504 nm. Whereas in treated effluent, it ranged from 0.031 to 0.096 nm. Among the eleven treatments the absorbance of effluent in T9 (40 g moringa seeds) recorded maximum reduction (0.031 nm) and showed parity with T8 (35 g moringa seeds).

The absorbance of the effluent under different soaking periods (H12, H24, H36 and H48 hours) irrespective of the treatments showed significant difference and was ranging from 0.106 nm – 0.080 nm. The maximum reduction in absorbance was noticed in treatments at 48 hour (H48) soaking period (0.080 nm), followed by 36 hour (H36) soaking period (0.084 nm).

Regarding the interaction effect between treatments and soaking periods, (T x H), the maximum reduction in absorbance was recorded in T9 (40 g moringa seeds) under 48 hours soaking period which was 0.027 nm.

Plates 4.8 (a-c). Decolourisation of pulp plant effluent using moringa seeds

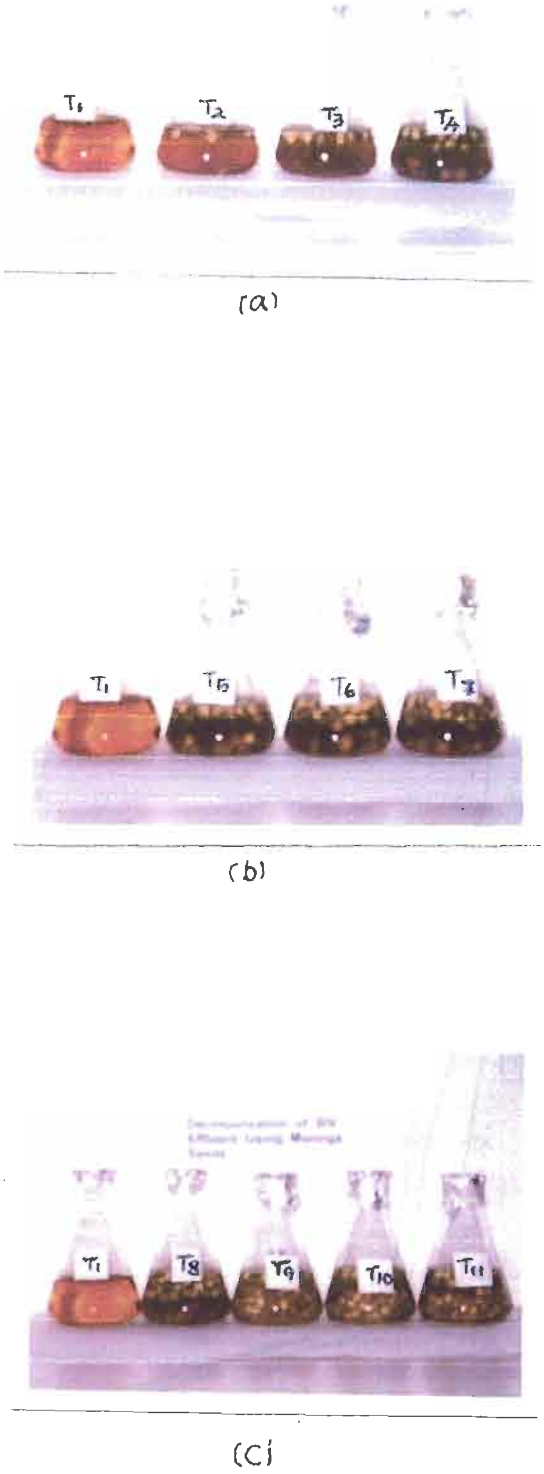
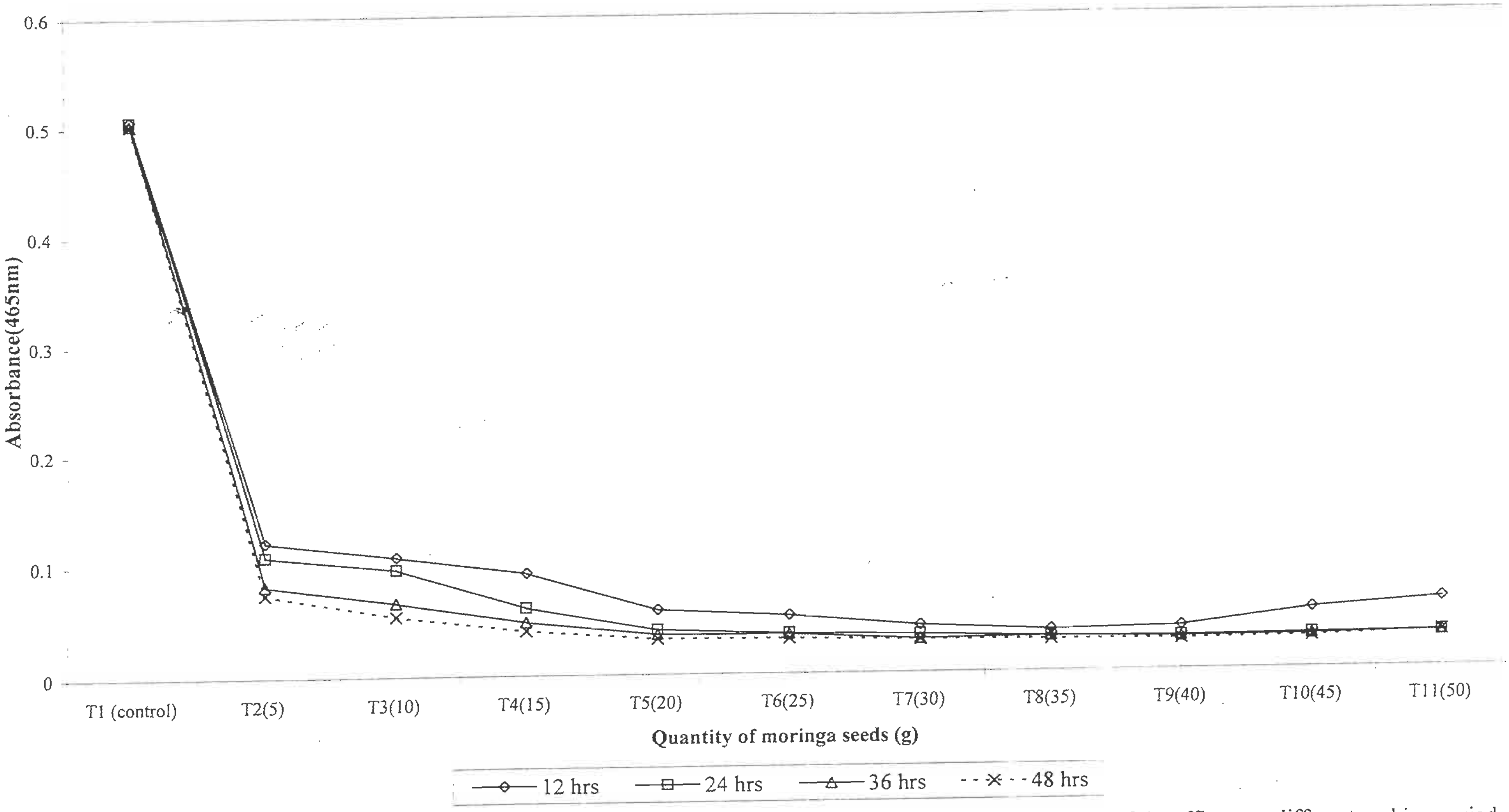


Fig. 4.7. Effect of moringa seeds on the absorbance of the effluent



romophoric substances from effluent were removed by soaking the seeds with effluent .The changes in the colour of the effluent at different soaking period were analysed by measuring the absorbance at 465nm.

4.6.5.2. Effect of moringa seeds on pH of the effluent

The pH of the pulp-plant effluent of treatments T1 – T11 was measured in 12, 24, 36 and 48 hour soaking intervals and the results are given in table 4.10.

Significant difference was observed between the pH of treated and untreated effluent. The change in pH was highly significant in treated effluents (T2 - T11) and ranged between 6.27 and 7.96. In control (T1) pH was 8.04. Slightly acidic pH (6.27) was recorded in T11 (50 g moringa seed), followed by T9 (40 g moringa seeds) and T10 (45 g moringa seeds). Whereas in T2 (5 g moringa seed) it was slightly in alkaline range (7.96) and neutral pH (7.00) was observed in T7 (30 g moringa seeds).

The pH of effluent observed under different soaking periods (H12, H24, H36 and H48) showed significant difference and varied between 6.84 and 7.47. The change observed in pH of the effluent was maximum at 48th hour (H 48).

Regarding the interaction effect between treatments and soaking periods (T x H) on pH of the effluent, T11 (50 g moringa seed) recorded very low pH (5.85) in 48 hour soaking period (H48).

4.6.5.3. Effect of moringa seeds on the electrical conductivity (EC) of the effluent

The EC of the pulp-plant effluent of treatments T1 – T11 was measured in 12, 24, 36 and 48 hour soaking intervals and the results are given in fig.4.8.

Electrical conductivity of the effluent irrespective of soaking periods, ranged from 2.16 dSm^{-1} to 4.40 dSm^{-1}. It was maximum (4.4 dSm^{-1}) in T11 (50 g moringa seeds) followed by T10 (45 g moringa seeds) and T9 (40 g moringa seeds). Whereas the EC was

Table 4. 10. Effect of moringa seeds on the pH of the pulp plant effluent

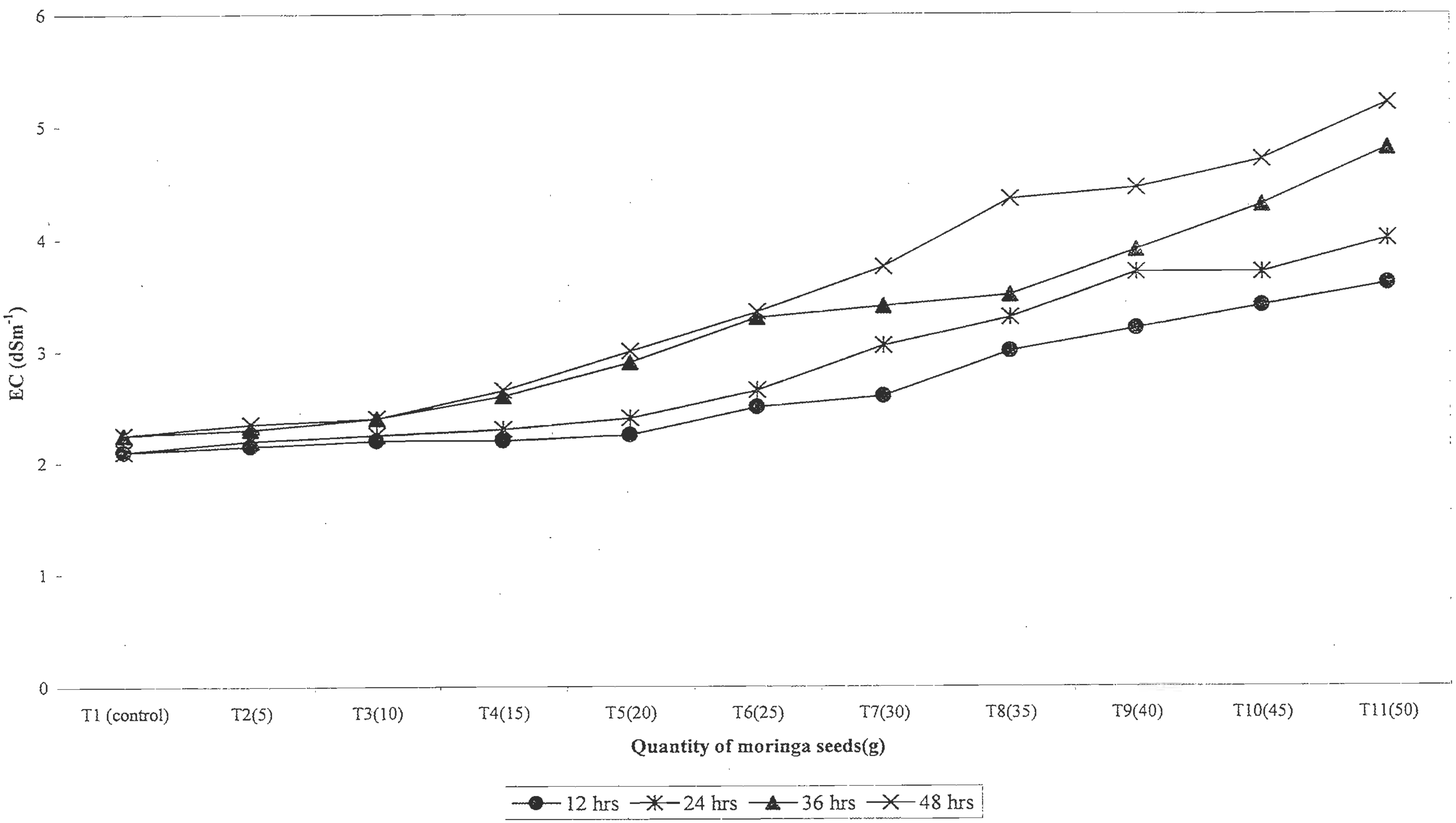
Qty. of moringa seeds (g)	pH at different time intervals (hours)				Mean
	H_1 (12)	H_2 (24)	H_3 (36)	H_4 (48)	
T1 (control)	8.10^{a}	8.10^{a}	8.00^{b}	7.95^{c}	8.04^{a}
T2(5)	7.97^{bc}	7.97^{bc}	7.96^{bc}	7.96^{c}	7.96^{b}
T3(10)	7.90^{d}	7.87^{d}	7.66^{f}	7.42^{j}	7.71^{c}
T4(15)	7.71^{e}	7.69^{ef}	7.41^{jk}	7.20^{n}	7.50^{d}
T5(20)	7.61^{g}	7.60^{gh}	7.34$^{\$}$	7.14^{o}	7.42^{e}
T6(25)	7.56^{h}	7.38^{k}	7.13^{o}	6.93^{q}	7.25^{f}
T7(30)	7.47^{i}	7.25^{m}	6.83^{r}	6.46^{u}	7.00^{g}
T8(35)	7.09^{p}	7.20^{n}	6.60^{t}	6.29^{v}	7.80^{h}
T9(40)	7.08^{p}	6.90^{q}	6.04^{y}	5.92^{z}	6.48^{j}
T10(45)	6.92^{q}	6.80^{r}	6.25^{w}	6.12^{x}	6.52^{i}
T11(50)	6.71^{s}	6.59^{t}	5.92^{z}	5.85$^{\{}$	6.27^{k}
Mean	7.47^{a}	7.39^{b}	7.01^{c}	6.84^{d}	7.18

	SEd	CD (0.05)
H	0.005	0.011
T	0.009	0.018
H x T	0.018	0.037

Any two means having a common letter are not significantly different at 5 per cent level of significance.

Raw effluent was treated by soaking the moringa seeds in different rates at 12,24 ,36 and 48 hours. The change in pH of the effluent after treatments was studied using pH meter. The values given in table are mean of two replications.

Fig. 4. 8. Effect of moringa seeds on the EC of the effluent



Raw effluent was treated by soaking the seeds in effluent for 12,24,36 and 48 hours. The change in electrical conductivity (at various soaking period) for the treated effluent was analysed by conductivity meter.

very less in T1 (control) followed by T2 (5 g moringa seeds). Electrical conductivity of the effluent collectively in all the treatments observed in different time intervals, showed significant change. The change was observed to be maximum in 48 hours soaking period (H48) and minimum in 12 hours soaking period (H12). EC of the effluent of treatments T1 - T11 in 12, 24, 36 and 48 hour soaking periods varied between 2.10 dSm^{-1} and 5.20 dSm^{-1}. It was maximum (5.20 dSm^{-1}) in T11 (50 g moringa seeds) at 48th hour soaking periods (H48) and was very less in control (T1) of all soaking periods studied. In addition, the EC observed in control did not differ significantly with EC of the effluent observed at 12 hour soaking period in T2 (5 g moringa seeds), T3 (10 g moringa seeds), T4 (15 g moringa seeds) and T5 (20 g moringa seeds).

4.6.6. Treatment of pulp plant effluent using soil columns (Plate 4.10)

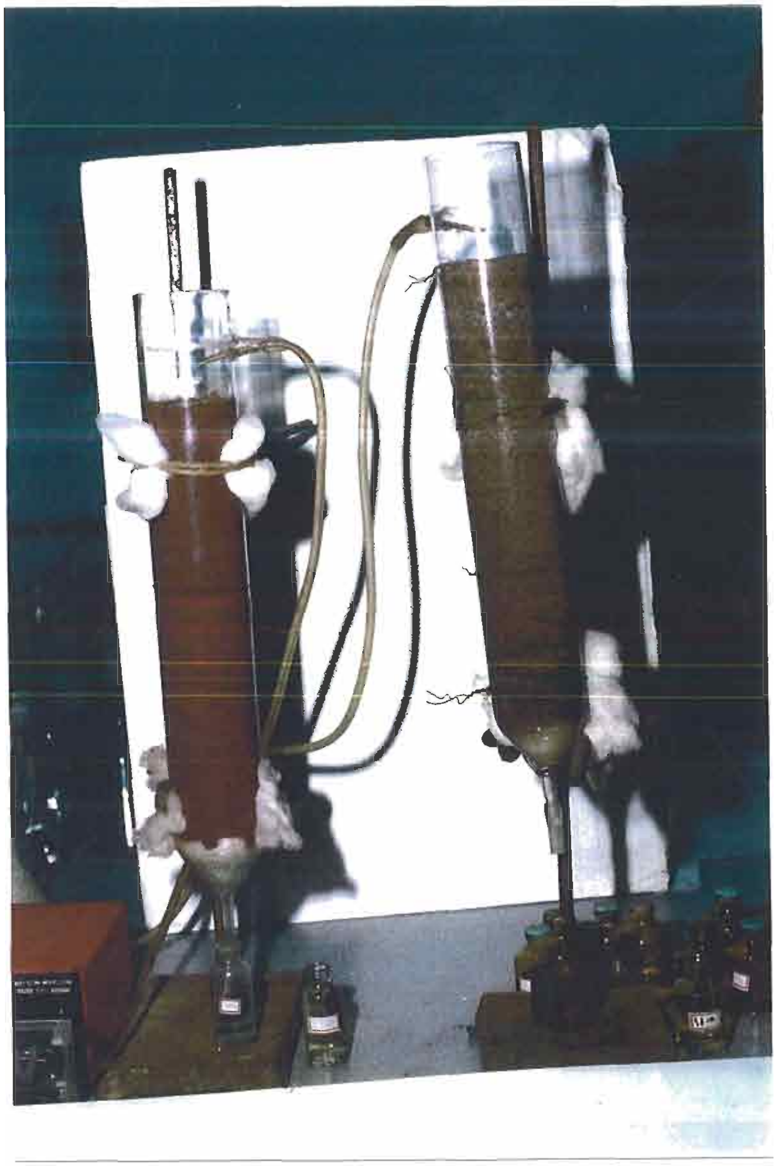
Colour of the pulp-plant effluent was removed by passing the raw effluent through columns packed with red soil (column 1) and sandy soil (column 2). Each time the leachate collected (L1 – L13) @ 200 ml was analysed for pH, EC, colour and TDS and the results are presented in Fig. 4.9, Fig.4.10, Fig. 4.11 and Fig.4.12 respectively.

4.6.6.1. Characteristics of effluent treated using column 1

pH of the raw effluent was 8.25. After passing through the column 1 (red soil) reduction in pH was noted in the leachates (effluent collected from outlet after passing through the column) collected upto 600th ml (L3). After that, a fluctuation was observed in the pH of leachates upto 1400th ml (L7) of the effluent. Whereas from 1800th ml (L9) onwards pH showed increasing trend and exceeded the initial value of 8.25. At the end of the treatment (L13) the pH of the treated effluent (2600th ml) was 10.2.

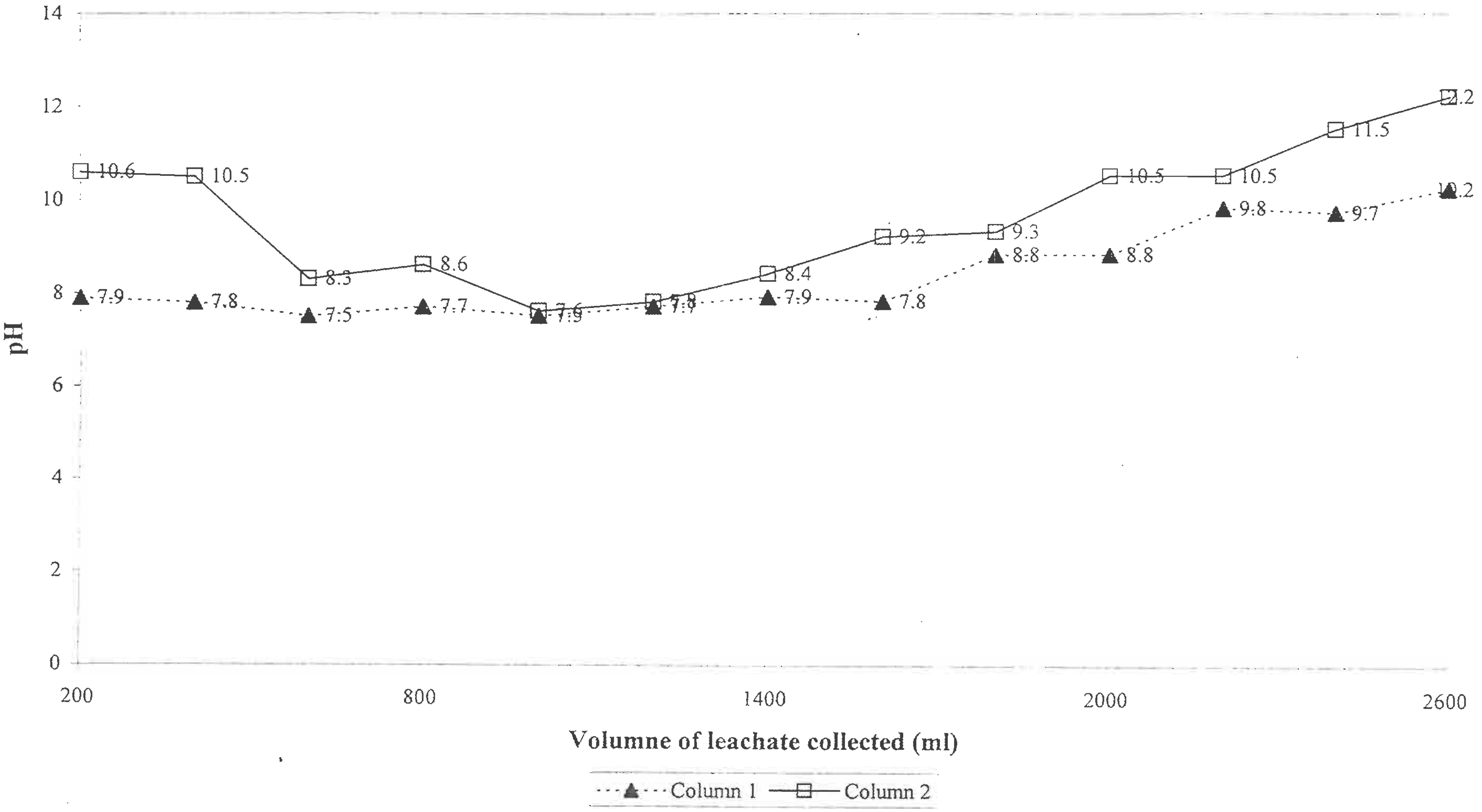
Plate 4.10. Decolourisation of pulp plant effluent using the columns packed with red and sandy soils

Column 1 & Column 2



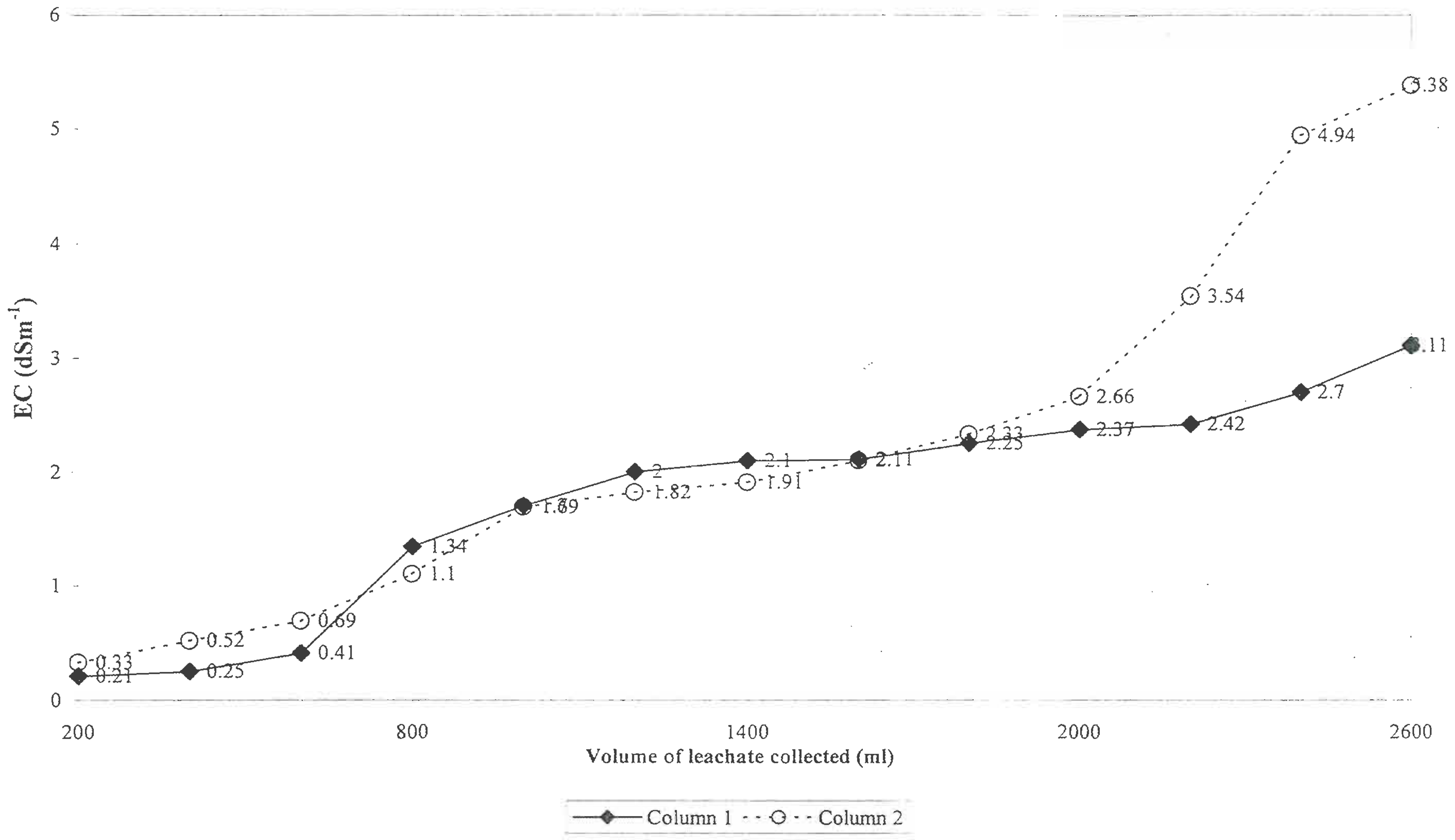
Foot note : Column 1 and 2 were filled separately with red and sandy soils upto 30 cm height over the glass wool which was kept at the bottom of column upto 4 cm height. Effluent was continuously passed through the column @ 4 ml. min^{-1}. Each time the leachate collected @ 200 ml was analysed for colour, pH, EC and TDS.

Fig. 4.9. pH of the effluent, treated through columns 1 and 2



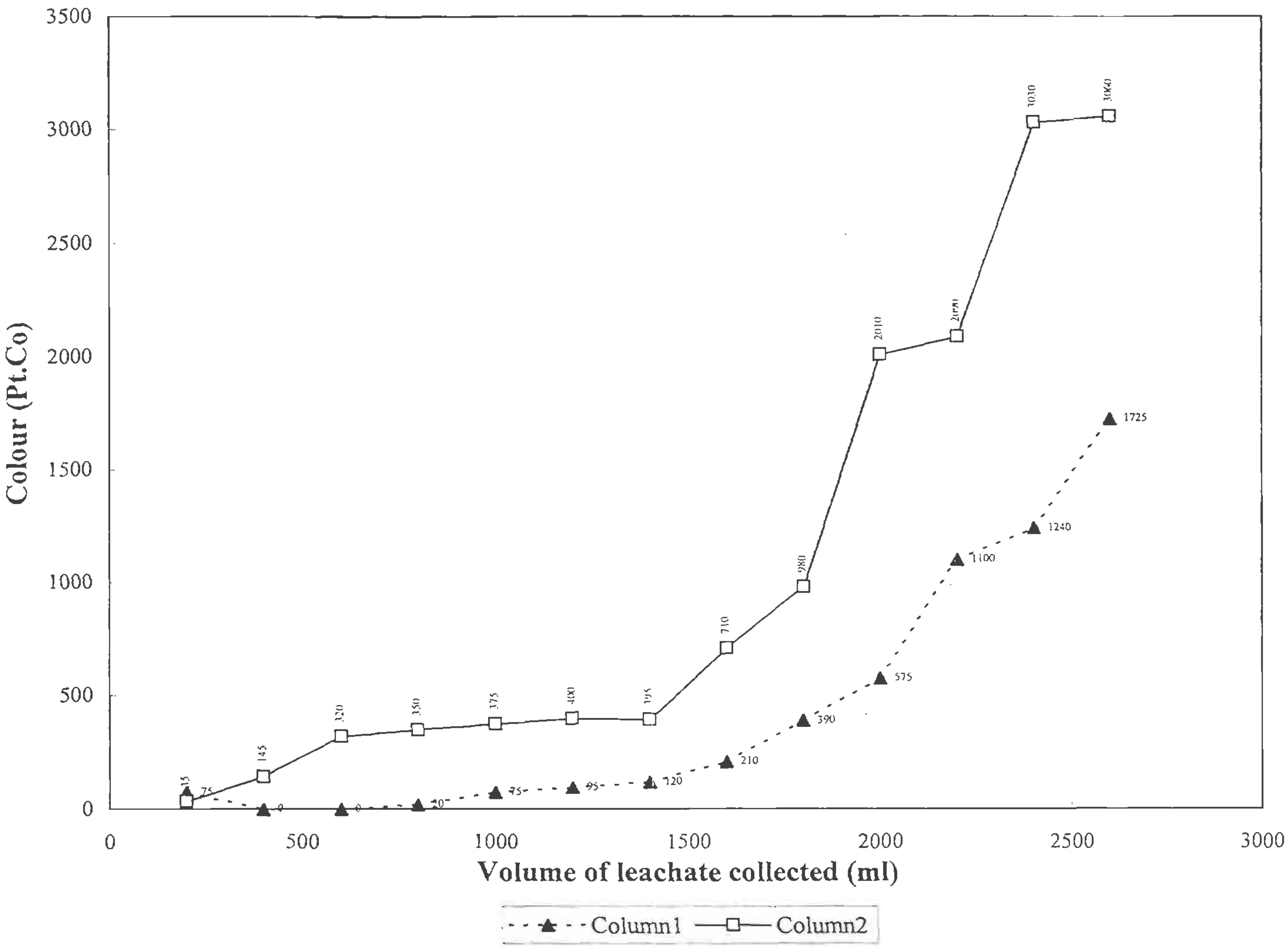
The raw effluent was treated by passing through columns 1 and 2, which were packed with red and sandy soils respectively. Each time the leachate collected at the rate of 200 ml was analysed for pH

Fig. 4.10. EC of the effluent, treated through columns 1 and 2



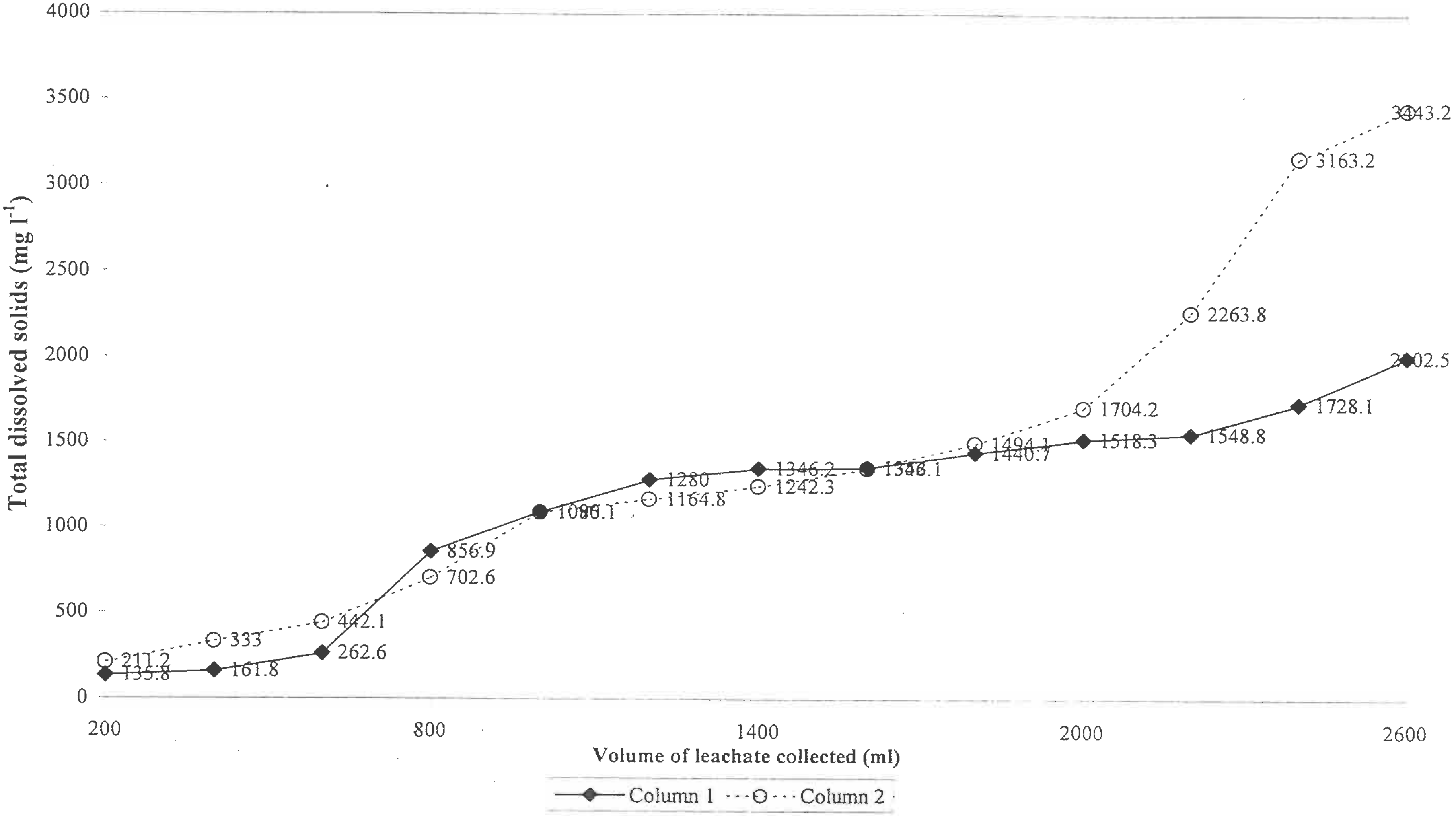
The raw effluent was treated by passing through columns 1 and 2, which were packed with red and sandy soils respectively. Each time the leachate collected at the rate of 200 ml was analysed for EC

Fig. 4. 11. Colour of the effluent, treated through columns 1and 2



The raw effluent was treated by passing through columns 1 and 2, which were packed with red and sandy soils respectively. Each time the leachate collected at the rate of 200 ml was analysed for Colour (with help of DR/2000 Hatch colorimeter at 455 nm)

Fig. 4.12. Total dissolved solids of the effluent, treated through columns 1 and 2



The raw effluent was treated by passing through columns 1 and 2, which were packed with red and sandy soils respectively. Each time the leachate collected at the rate of 200 ml was analysed for Total dissolved solids

The raw effluent initially had an electrical conductivity of 2.65 dSm^{-1}. After passing through the column 1 (red soil) it was decreased to 0.21 dSm^{-1} in the leachate collected upto 200 ml collected (L1) and then increased continuously till the end of the treatment (2600th ml – L13). EC of the leachate L12 (2400th ml) and L13 (2600th ml) was found to be higher than the initial value of the raw effluent before treatment. Among the leachates collected, the amount of EC was maximum (3.11 dSm^{-1}) in L13 (2600th ml). Total dissolved solids of the untreated raw effluent was 1925 (mg l^{-1} effluent). After treatment it was ranging from 135.8 – 2002.5 (mg l^{-1} effluent).

In general, the amount of TDS was increased in all the leachates. The amount of TDS in 200th ml (L1) was 135.8 (mg l^{-1} effluent), whereas at the end of the treatment, (L13- 2600th ml), it became 2002.5 (mg l^{-1} effluent). TDS level was maximum in 2400th ml (L13) which was higher than the initial value (1925), of untreated effluent. The raw, untreated effluent initially had 5,500 units of colour (Pt. Co. units). The colour of the leachates collected (effluent) after passing through the column fluctuated between 75 - 1,725 Pt. Co. units. Drastic colour reduction was observed in all the leachates from 200th ml – 2600th ml (L1 - L13). Whereas, it was totally absent in 400th ml (L2) and 600th ml (L3). Even though the leachates from 800th ml – 2600th ml (L4 - L13) showed increase in colour, no one exceeded the initial colour (5060 Pt. Co. units) of untreated effluent.

4.6.6.2. Characteristics of soil (red soil) packed in column 1

The pH, EC, organic carbon, microbial population and enzyme activities of soil packed in column 1 (red soil) before and after treatment with pulp-plant effluent was studied and the results are presented in Table 4.11.

Table 4. 11. Characteristics of soil (red soil) packed in column I before and after treatment with pulp plant effluent

Particulars	Top		Middle		Bottom	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Chemical properties						
pH	8.37 ± 0.14	10.8 ± 0.14	8.37 ± 0.14	11.5	8.37 ± 0.14	9.75 ± 0.212
EC (dSm^{-1})	0.15 ± 0.014	3.5	0.15 ± 0.014	3.7 ± 0.14	0.15 ± 0.014	2.85 ± 0.212
Organic carbon (per cent)	0.46 ± 0.02	0.61 ± 0.06	0.46 ± 0.02	0.50 ± 0.02	0.46 ± 0.02	0.52 ± 0.02
Microbial population						
Bacteria x 10^5 g^{-1} soil	13 ± 1.41	22 ± 1.41	13 ± 1.41	25 ± 1.41	13 ± 1.41	18.5 ± 2.12
Fungi (CFU) x 10^3 g^{-1} soil	8	10.5 ± 2.12	8	10 ± 1.41	8	9
Actinomycetes x 10^2 g^{-1} soil	5 ± 1.41	8 ± 1.41	5 ± 1.41	7	5 ± 1.41	5.5 ± 2.12
Enzymes activity						
Dehydrogenase*	1.55 ± 0.06	1.89 ± 0.08	1.55 ± 0.06	1.81 ± 0.020	1.55 ± 0.06	1.67 ± 0.127
Phosphatase**	0.94 ± 0.021	1.44 ± 0.042	0.94 ± 0.021	1.74 ± 0.02	0.94 ± 0.021	1.54 ± 0.020
Urease***	5.55 ± 0.091	7.57 ± 0.004	5.55 ± 0.091	7.64 ± 0.020	5.55 ± 0.091	7.89 ± 0.020

* - activity expressed in µg of TPF released $g.^{-1}$ soil h^{-1}
** - activity expressed in µg of inorganic phenol released $g.^{-1}$ soil h^{-1}
*** - activity expressed in µg of ammonia-N released $g.^{-1}$ soil h^{-1}

Chemical and biological properties were studied for the soil packed in column 1 both before and after the effluent treatment. Soil samples were collected from top, middle and bottom regions of the soil column. The microbial population and enzyme activities were carried out 24 h after experiment. Values represent mean of two replications and expressed on oven dry basis with standard deviation

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In column 1, prior to effluent treatment pH, EC and organic carbon were 8.37, 0.15 (dSm^{-1}) and 0.46 (per cent) respectively. After treating the effluent change was observed in all the three parameters studied. Regarding pH and EC middle region recorded maximum value than the top and bottom region of the soil column. Whereas organic carbon was the maximum in upper part of the soil column and was 0.61 (per cent).

With respect to microbial population studied, the number of bacteria in top, middle and bottom region of the column, prior to effluent treatment was 13 ($x\ 10^5\ g^{-1}$ soil). Increase in number after the effluent treatment was noticed and was 22 ($x\ 10^5\ g^{-1}$ soil), 25 ($x\ 10^5\ g^{-1}$ soil) and 18.5 ($x\ 10^5\ g^{-1}$ soil) in top, middle and bottom portion of the soil column respectively. Whereas the population of fungi and actinomycetes did not increase very much. Regarding soil enzyme activities, the phosphatase and urease showed maximum change than the enzyme dehydrogenase. The activity of phosphatase observed in observed in top, middle and bottom region of the column were 1.44 (μg of inorganic phenol released g-1 soil h^{-1}), 1.74 (μg of inorganic phenol released g-1 soil h^{-1}) and 1.54 (μg of inorganic phenol released g-1 soil h^{-1}) respectively. With respect to urease the maximum activity was registered in bottom region of the column 7.89 (μg of ammonia-N released g^{-1} soil h^{-1}).

4.6.6.3. Characteristics of effluent treated, using soil (sand) column 2

The pH of the raw effluent was 8.25. After passing through the soil column 2 (sand) fluctuation in pH was observed in the leachates (treated effluent) from 600th ml – 1200th ml of the leachates (L3 – L6). Whereas from 1400th ml onwards pH was increased steadily and reached maximum (12.2) at the end of the treatment.

The electrical conductivity of the effluent was continuously increased after passing through the soil column 2 (sand) and was ranging from 0.33 to 5.38 (dSm^{-1}). EC of the leachates exceeded 2.65 dSm^{-1} from 2200th ml (L11) onwards.

Total dissolved solids (TDS) of the effluent after passing through the column 2 was ranging from 211.2 – 3443 ($mg\ l^{-1}$ effluent). The TDS was increased from 2200th ml of the leachate onwards (L11) and became maximum at the end of the treatment (2600th ml – L13).

Colour of the untreated pulp-plant effluent was 5060 (Pt.Co units). Effluent after passing through the column 2, colour reduction was observed and it was ranging from 35 - 3060 (Pt. Co units).

4.6.6.4. Chemical and biological properties of soil (sandy soil) packed in column 2 (Table 4.12)

pH of the soil initially in all the three regions (top, middle and bottom) was 10.8. At the end of the experiment, pH was increased in all the three regions and was maximum in middle region of the column (12.7).

The EC of the soil initially in all the three regions was 0.17 (dSm^{-1}). After treatment it was increased in all the three regions and the maximum was observed in middle region of the column. Regarding organic carbon in top, middle and bottom, the maximum amount was observed in top (0.37 per cent).

The total number of aerobic bacteria, fungi and actinomycetes in top, middle and bottom regions of the column (before the treatment) was 6 ($\times\ 10^5\ g^{-1}$ soil), 3 (CFU $\times\ 10^3\ g^{-1}$ soil) and 2 ($\times\ 10^2\ g^{-1}$ soil) respectively. After treatment, the fungal and actinomycetes population was maximum in the middle part of the column and was 7

Table 4.12. Characteristics of soil (sand) packed in column II before and after treatment with pulp plant effluent

Particulars	Top		Middle		Bottom	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Chemical properties						
pH	10.8 ± 0.21	12.5 ± 0.14	10.8 ± 0.21	12.7	10.8 ± 0.21	12.5 ± 0.14
EC (dSm^{-1})	0.17 ± 0.02	5.65 ± 0.61	0.17 ± 0.61	5.67 ± 0.02	0.17 ± 0.02	5.54 ± 0.02
Organic carbon (per cent)	0.25 ± 0.04	0.37 ± 0.02	0.25 ± 0.04	0.35 ± 0.02	0.25 ± 0.06	0.35 ± 0.06
Microbial population						
Bacteria x 10^5 g^{-1} soil	6	10.5 ± 2.12	6	12 ± 1.41	6	12.5 ± 0.71
Fungi (CFU) x 10^3 g^{-1} soil	3	5 ± 1.41	3	7	3	5.5 ± 0.71
Actinomycetes x 10^2 g^{-1} soil	2	2.5 ± 0.71	2	4 ± 1.41	2	3.5 ± 0.71
Enzymes activity						
Dehydrogenase*	0.78 ± 1.4	0.83 ± 0.06	0.78 ± 1.4	0.91 ± 0.72	0.78 ± 1.4	0.98 ± 0.04
Phosphatase**	0.21 ± 0.10	0.39 ± 0.02	0.21 ± 0.10	0.39 ± 0.10	0.21 ± 0.10	0.37 ± 0.02
Urease***	2.72 ± 0.02	2.73 ± 0.01	2.72 ± 0.02	2.75 ± 0.02	2.72 ± 0.02	2.76 ± 0.02

*	-	activity expressed in µg of TPF released g.$^{-1}$ soil h^{-1}
**	-	activity expressed in µg of inorganic phenol released g.$^{-1}$ soil h^{-1}
***	-	activity expressed in µg of ammonia-N released g.$^{-1}$ soil h^{-1}

Chemical and biological properties were studied for the soil packed in column I both before and after the effluent treatment. Soil samples were collected from top, middle and bottom regions of the soil column. All the parameters except pH, EC and organic carbon were studied 24 h after the treatment. Values represent mean of two replications and expressed on oven dry basis with standard deviation.

(CFU x 10^3 g^{-1} soil) and 4 (x 10^2 g^{-1} soil) respectively. Whereas, the bacterial population was found to be maximum in the lower part of the column (12.5 x 10^5 g^{-1} soil) only.

The activity of the enzymes, *viz.*, dehydrogenase phosphatase and urease in the soil (collected from top, middle and bottom regions of the column 2) before treatment was 0.78 (μg of TPF released g^{-1} soil h^{-1}), 0.21 (μg of inorganic phenol released g^{-1} soil h^{-1}) and 2.72 (ammonia -N released g^{-1} soil h^{-1}) respectively. After treatment, the enzymes studied in all the three regions showed increased activity. The enzyme urease was slightly increased in soil after treatment and the activity was in top 2.73 (ammonia -N released g^{-1} soil h^{-1}), middle 2.75 (ammonia -N released g^{-1} soil h^{-1}) and in bottom 2.76 (ammonia -N released g^{-1} soil h^{-1}). The activities of dehydrogenase and phosphatase were maximum in middle region and was 0.98 (μg of TPF released g^{-1} soil h^{-1}) and 0.39 (μg of inorganic phenol released g^{-1} soil h^{-1}) respectively.

DISCUSSION

V. DISCUSSION

Recently, reuse of partially treated pulp plant effluents in agricultural land is considered as common practice. Adverse effects of this practice on soil biota remains unknown. There are several studies dealing almost exclusively with crop productivity aspects. However, the impact of the effluent disposal on soil biota remains unknown. To quantify the risks associated with land application of effluent a thorough understanding of the physicochemical and biological behaviour of effluent components in soil system is necessary. Since the colour of the pulp plant effluent mainly arises from toxic chlorolignin compounds, the complete removal prior to land application is necessary. Though several colour removal technologies are available, considering the retention time and cost, they are beyond the reach of industries. This initiated the development of the present investigation with an objective of study of effluent disposal *in situ* and its consequence on soil biological properties. Over and above the study involving removal of chromophoric substances was given major consideration. Analysis of the SIV study area showed that all the blocks had, the acidic pH, with very low level of organic carbon (less than 5 per cent) content. The soil microbial properties, *viz.*, the population of bacteria, fungi, actinomycetes and activity of enzymes such as dehydrogenase, phosphatase and urease were found to be high in A block. The SIV model farm in which the impact study of effluent irrigation was carried out was bought from the farmers. So block to block variation observed in the preliminary analysis might be due to the varied agricultural practices practiced by the farmers in the field earlier.

The pH of the effluent was alkaline in nature and the EC was 2.65 (dSm^{-1}) and it did not exceed the critical limit (critical limit fixed for irrigation is 3 dSm^{-1}). The amount of total dissolved solids were within the permissible limit for land disposal as prescribed by ISI (1981). The absorption maxima of the raw effluent confirmed the presence of lignin derived components in the effluent, because, it had the absorption maxima (λ max) nearer to the UV range (248.5 λ) (Kalaichelvan, 1997). The chromatograms of raw and extracted effluents revealed the presence of picric acid and caffeeic acid in the effluent. The effluent used for irrigating the crops in model farm was treated already in the pulp-plant by conventional methods. So the presence of aerobic microflora in the effluent might be due to the less BOD and COD of the effluent (Kannan *et al.*,1990).

The impact study of effluent irrigation on soil microbial population revealed that the number of bacteria, fungi and actinomycetes were maximum in block -10. Compared to initial (population prior to effluent irrigation) all the blocks showed increased population, immediately after effluent irrigation (on 30th day). This might be due to the creation of favourable environment by agricultural activities. The activities such as levelling, ploughing and wetting creates favourable environment, which help the organisms to flourish well was reported by Johnson and Ryder (1988). Sankaran and Subbiah (1991) have reported that aerobic organisms thrive well when the soil is adequately aerated by cultivation and become inactive when the aeration of the soil gets reduced. Eventhough all the blocks were irrigated with same effluent, the microbial population did not increase uniformly in all the blocks throughout the study period. With respect to bacterial population, reduction in number was noticed in blocks – B4, B5, B6, B7, B9, B10, B11, B13, B14 and B15 on 120th day of effluent irrigation. Regarding actinomycetes, decreased activity was observed on 90th day of effluent irrigation in blocks B3, B4, B5, B7, B10, B12. In Blocks B3, B8, B9 and B15 the reduction was noticed on 120th day of effluent irrigation whereas, progressive increase in number was observed in fungal population in most of the blocks. The reason for the population reduction observed in the aforesaid blocks is not clearly known. Brezny *et al.* (1993) reported 40 per cent decline in gram positive bacteria in the chlorolignin compounds treated soil on 30th day of incubation period. Since the lignin content was not quantified in the effluent irrigated soil, the reason for the decreased actinomycetes and bacterial population is not known and require further investigation.

The activity of enzyme dehydrogenase both before and after effluent irrigation showed that among the 15 blocks B3 was superior than rest and reached the maximum on 90th day of effluent irrigation. Whereas, B6 was very poor in dehydrogenase activity throughout the experimental period. Ross (1971) stated that oxygen concentration, temperature and pH of the soil played a major role in deciding the activity of the enzymes. In addition, he reported that high soil pH coupled with water logged condition improved the dehydrogenase activity in soil. .

The urease activity in the blocks showed maximum activity up to 60th day of effluent irrigation. Afterwards it declined till the end of the study period. The reduction in urease activity observed after 60th day of effluent irrigation may be due to the application of nitrogenous fertilizers in the soil during the period of cultivation as reported by Fang *et al.* (1988) and Abdel-Rahman *et al.* (1992). They have reported that application of nitrogenous fertilziers suppressed the urease activity in the soil.

Laidler and Hoare (1949) reported that the urease activity increased linearly with nitrogen concentration, reached the maximum and then subsequently declined when nitrogen concentration increased at very high level.

The impact study of effluent irrigation on soil phosphatase activity showed that except blocks B5, B11, B12, B13, B14 and B15 all the blocks exhibited increased activity after effluent irrigation. Maximum activity was observed on 60th,90th and 120th day of effluent irrigation. The phosphatase activity in general in all the blocks showed considerable improvement after the blocks were irrigated with effluent. Gasser (1964) and Hutchinson and Viets (1969) stated that release of ammonia in excess by soil urease, caused rise in pH, and triggered phosphatase activity. It can be concluded that in the short run, the reuse of treated effluent on soil enzyme system did not show any toxic effect.

The effect of addition of bacteria (T2-composite culture of *Bacillus* sp. and *Pseudomonas* sp.), fungi (T3-*Fusarium* sp.) and Actizyme (T4) on decolourisation of pulp plant effluent was studied in the presence of carbon source viz., glucose. The colour removal efficiency of all the three treatments (T2, T3 and T4) was tested in the presence and absence of nitrogen source. The results revealed that among all the treatments, T3 (*Fusarium* sp.) was superior over the rest. It removed 53.65 per cent of the colour from the effluent in the presence of nitrogen source. The colour reduction was maximum on 16th day of incubation period. Next to control, T2 (*Bacillus* sp. and *Pseudomonas* sp.) recorded minimum colour reduction. It was reported that the glucose addition @ 1 per cent (w/v) triggered the decolourisation potential of *Phanerocheate chrysosporium* (Satwinder *et al.*, 1998). Kannan (1990), Prasad and Joyce (1987) observed an enhanced rate of decolourisation in the presence of carbon and nitrogen supplements. The efficiency of colour removal by *Schizophyllum commune* (Belsare and Prasad, 1988) and *Trametes versicolor* (Modi *et al.*, 1998) in glucose supplemented effluent was reported to be further improved by addition of nitrogen in the form of ammonium chloride and ammonium nitrate respectively.

Regarding decolourisation study with isolated ligninolytic fungal cultures, the maximum colour reduction was in S3 (culture-8), which removed 59.96 per cent of colour from the effluent followed by S5 (culture-12). The efficiency of colour removal was maximum in 0.3 per cent glucose level. Reduction of colour of the effluent was observed in all the treatments on day 4 (D4) and the maximum colour reduction was observed on 10th (D10) and 12th (D12) day of incubation period.

Kirk *et al.* (1978) have reported that a medium containing an unlimited level of glucose and a limiting dose of nitrogen stopped primary growth and stimulated on set of ligninolytic activity during late stationary phase. Keyser *et al.* (1978) have reported that the process of lignin degradation is completed in two phases. In the initial primary phase the ligninolytic enzyme system is synthesized while during the secondary phase lignin is degraded.

Changes in colour of effluent observed in the column study revealed that all the treatments effected considerable reduction in colour. The per cent colour reduction observed in the control was 46.56. It might be due to the adsorption of chromophoric substances by the surface of solid matrix, charcoal and red earth. Among the treatments, the maximum colour reduction was noticed in C5 (culture-12), C4 (culture-11) and C3 (culture-8) and the per cent colour reduction noticed was 87.82, 87.42 and 86.83 respectively. Maximum colour reduction was observed during 12 hour retention time.

Kirk*et al.* (1986) have reported that the process of lignin degradation is strictly an oxidative process and increasing the oxygen level in culture has a strong activating effect on the rate of lignin degradation. In submerged cultures of *Phanerochate chrysosporium* grown under nitrogen – limiting conditions, the primary growth slowed down between days 2 and 3 due to nitrogen depletion has been reported by Livernoche *et al.* (1983)

The peak lipase (LiP) activity on day 9 rapidly declined, followed by no detectable activity by the twelfth day has been reported by Marchand (1978).

The pH of the effluent observed in column under different retention time (H0 – H12), ranged from 6.33 to 7.73. In control column, pH of the effluent was near neutral to slightly alkaline throughout the experiment (H0 – H12). Whereas, treatments C2 (culture-5), C3 (culture-8), C4 (culture-11) and C5 (culture-12) reduced the pH of the effluent significantly. In addition it was also observed that the degree of acidity increased with increase in retention time. Among all the treatments, the maximum acidity was recorded in C4 (culture 11) under 12 hour retention time (H12).

The release of organic acids by the fungus during biodegradation could be the reason for the acidity of the effluent. During biodegradation, CO_2 is released, which may combine with water and may get converted to carbonic acid, which may be responsible for the acidity. Since the control column was free from mycelial mat, exchangeable cations like Na^{2+}, Ca^{2+} etc. present in the solid matrix may undergo hydrolysis with OH ions.

Since the control column was uninoculated with ligninolytic fungal culture, there is no acid production to counter the effect of OH ions. This could be the reason for alkaline pH of control column.

The results of the decolourisation study involving moringa seed as the natural coagulant revealed that addition of moringa seeds in pulp plant effluent removed significant amount of colour. The per cent colour reduction was found to be increased with increase in amount of seed added and with duration of soaking periods. Among all the treatments, the maximum colour reduction was in T9 (40 g moringa seed) at 48 hours (H48) of soaking period and the per cent colour reduction achieved was 94.70. Control and T2 (5 g moringa seed) showed minimum colour reduction especially at 12 hours (H12) soaking period. Numerous laboratory studies have so far shown that *Moringa oleifera* seeds possess effective coagulation properties (Muyibi and Evison, 1995) and that they are not toxic to humans nor animals (Berger *et al.*, 1984). The dry seeds of *Moringa oleifera* is one of the natural coagulants and dimeric cationic proteins are act as a active agents of coagulation which has been reported by Ndabigengesere *et al.* (1995).

In addition to colour, pH and EC of the effluent were also changed during the treatment. The pH of the effluent decreased with increasing soaking hours. The quality of the seeds soaked and the soaking duration also favoured the acidification of the effluent. Among all the treatments, the maximum degree of acidification was recorded in T11 (50 g moringa seed) at 48 hours soaking period. The acid pH of the effluent after treatment might be due to the release of H^+ ions from the seed kernel during soaking. Because, the aquous seed extract of the moringa seed was also acidic (pH 6.04) after soaking in water for 24 hours. The acidity might have also been developed, due to the microbial degradation of the seed during soaking. This was observed in treatments where, the seeds were soaked more than 36 hours (H36) in the effluent. However, Ndabigengesere and Subbanarasiah (1998) have reported that the use of moringa seed in drinking water treatment did not affect the pH of the water significantly. Compared to drinking water, effluent might have contained more amount of microorganisms. Which might have caused degradation of seeds during soaking period. However it requires further investigation.

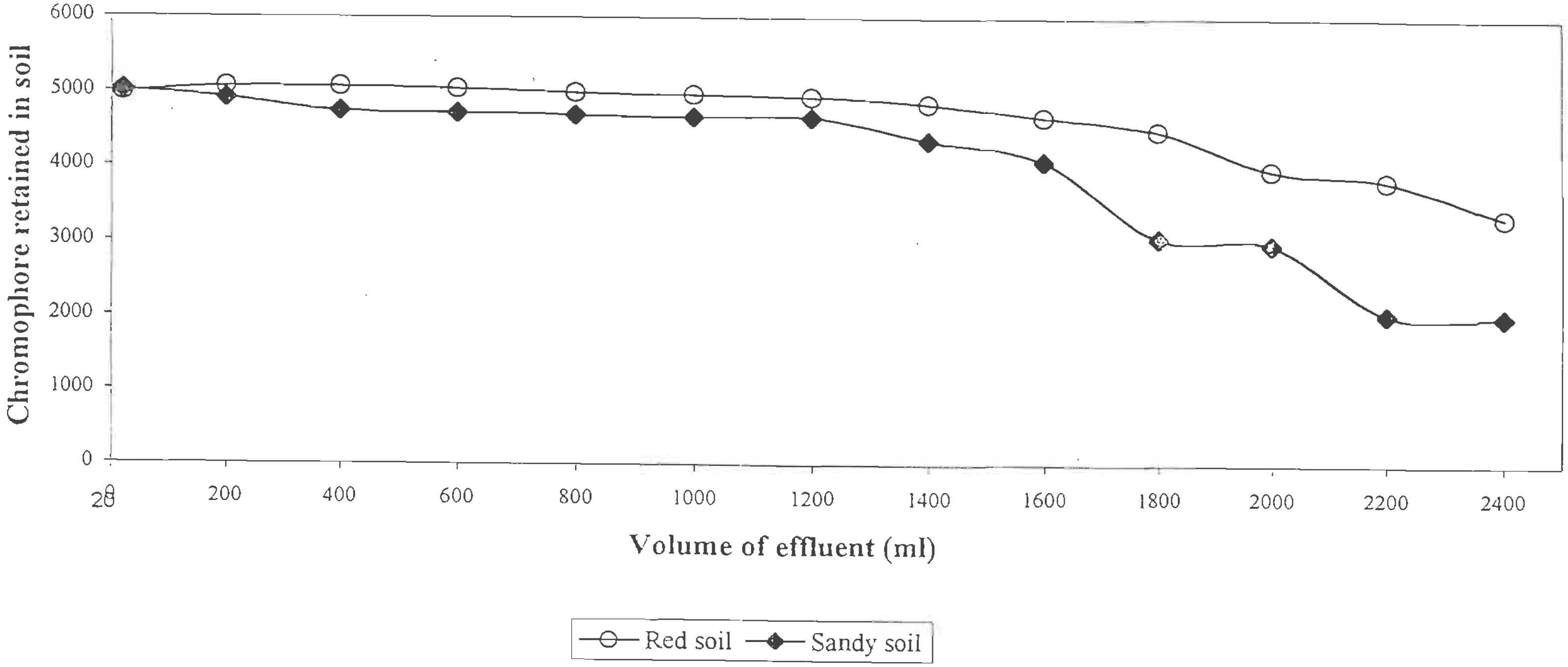
The degree of increase in EC was found to be directly proportional to the amount of seed used and also the soaking duration. Next to control, T2 (5 g moringa seed) and T3 (10 g moringa seed) showed very negligible increase in EC. Whereas it did not exceed 3 (dSm^{-1}) after 12 hours of soaking period in treatments T1 (control) – T8 (35 g moringa seed). However, it was maximum in T11 (50 g moringa seed) after 48 hours of soaking period (H48).

Though the aim of the soil column study was colour removal, consideration was paid to chemical properties like pH, EC and TDS of the effluent. The results of column study revealed that significant colour reduction was achieved in both the columns. But, column 1 (red soil) was found to be superior over column 2 (sandy soil), because the former achieved maximum colour reduction (65.9–100 per cent) than the latter. The reason behind the lower reduction in column 2 may be the low organic carbon (0.25 per cent only) of the sorbent, *viz.*, sandy soil. Soil organic matter has often been shown to have a dominant effect on the sorption of non-ionic, hydrophobic organic compounds (Rao *et al.*, 1990). Clay content in soils provides equally important sorption surface for toxic organic compounds (TOC_s). The extent and reversibility of sorption processes and subsequent mobility of TOC_s in soils can be greatly influenced by other factors, such as pH, solute concentration, and the presence of organic contaminants and competitive ions (Green and Karif kholf, 1990; Koskinsen and Harper, 1990).

As stated above, the colour of the treated effluent (leachates L1-L13) found in both the columns seemed to increase with increase in pH. Though colour reduction was observed in highly alkaline pH (> 10.5), the per cent removal was less. It has been reported that the colour of the effluent is pH dependent, and it increased with increase in pH (Vandenbusch, 1992). In addition, he has also reported that, as the pH approaches 10.5, a precipitate begins forming in the effluent which, when removed, results in some degree of colour removal. This was supported by the pH data of leachates from L5 (1000 ml) to L13 (2600 ml) in both the columns.

The sorption of chromophore by red soil was declined gradually after addition of effluent from 600th – 1800th ml (L3) to (L9), later it dropped suddently in 2000th ml (L11). Slight stability was observed in 2200th ml (L12) and again reduced further. When it was correlated with pH of the leachates of 2000th ml (L10) and 2200th ml (L11), a sudden increase in pH of one unit was noticed. This may be one of the reasons for the fluctuation observed in sorption curve (Fig. 5.1). Even the same trend was also observed in 1600th ml (L8) and in 1800th ml (L9), it did not influence the sorption behaviour of soil. This may be due to the lesser degree of alkaline pH compared to L10 (2000th ml) and L11 (2200th ml). In addition, the saturation of clay surface with sorbate molecules should also be taken into consideration. According to Langmuir equation adsorption of

Fig.5.1 . Effect of continuous flow of effluent on sorption behaviour of soil columns



The raw effluent was treated by passing through columns 1 and 2, which were packed with red and sandy soils respectively.
The amount of chromophore retained by the soils was calculated by deducting the colour of the leachates from the initial colour of the effluent and expressed in Platinum - cobalt units (Pt. Co)

organic matter on clay surface tends to reach the maximum limit (Biswas and Mukherjee, 1997). It is compatible with the fact that soil and clay don't have an infinite capacity to adsorb but will sooner or later be saturated.

Adsorption of compounds, such as humic acid and the like, following the longmuir type of equation has also been reported by Inoue and Wada (1973) and Tan *et al.* (1975). With respect to sandy soil, though the colour eventually increased, it did not exceed the colour of the untreated effluent. In addition, even though the infiltration rate of sandy soils are high, due to the presence of organic carbon (0.25 per cent), considerable decrease in colour was achieved and the per cent colour reduction ranged between 39.53 and 99.31.

In column 1 (red soil), when the per cent colour reduction observed in treated effluent was compared with the pH and EC of the treated effluent, upto 1400 ml, the effluent can be treated safely. Beyond this level, the pH and EC of the treated effluent exceeded the limit prescribed by ISI, 1981. In addition it also (addition of more than 1600 ml) affect the soil's effluent carrying capacity.

The results of decolourisation study using soil column 1 and 2 showed that the passage of effluent changed the pH, EC and microbial activities of soils of both column 1 and 2. Among the regions *viz.*, top (0-10 cm), middle (10-20 cm) and bottom (20-30 cm), pH and EC of the middle zone exhibited maximum change but the magnitude of increase was found to be very high in column 2. Compared to column 1, the varied buffering capacity of the red and sandy soils might have caused such variation between them. Biswas and Mukherjee (1997) reported that the soil organic matter improves the buffering capacity of soil, making it less amenable to pH changes by acids and bases, mainly because of preferential adsorption of H+ ions. In addition they have also reported that the concept of buffering capacity of soil is not only limited to the reaction of the soil but also makes the soil to act as a filter for dissolved and colloidal contaminants.

The distribution and adsorption of dissolved solids varies from soil to soil depending upon their hydraulic conduction and retention time (Nvozamsky *et al.* 1976). As the effluent was passed from top towards bottom the upper layer retained more organic carbon followed by middle and lower portion of the column.

Regarding microbial population, both the columns exhibited considerable increase in number after the effluent was passed through it. Though they differed significantly with each other, only very negligible amount of difference was observed between each regions. In addition, the changes of microbial population in various zones were not clearly understood. In column 1 the dehydrogenase and phosphatase activity was found to be high in middle region, where the pH and electrical conductivity were found to be high. Whereas, urease activity was recorded maximum in the bottom portion of the column only. In addition, it is likely to decrease with decrease in depth of the column and no correlation was observed between the total microbial load and enzyme activity investigated.

Regarding column 2 (sand column), urease and dehydrogenase activities were observed to be maximum only in the lower portion of the column, in which the availability of oxygen is found to be less. The activity of enzyme phosphatase was maximum in both upper and middle part of the column, where, pH and EC were maximum. When compared to column 1 (red soil) the column 2 showed some variation in chemical and biological properties. Because, in column 1, red soil was used as adsorbent, in which the organic carbon was 0.46 (per cent), whereas the column 2 was packed with sandy soil low in organic carbon (0.25 per cent). Compared to column 2, the per cent clay content was more (>20 per cent) in column 1 (red soil). In addition, the hydraulic conductivity is more in sandy soils, whereas in the red soil hydraulic retention time is high (Newson, 1985). All these factors might have caused more than 95 per cent of colour reduction in column 1 (red soil). Biswas and Mukherjee (1977) have reported that water retention is always higher for the soil with high organic matter as compared to one with low organic matter.

Considering the cost, applicability and time requirement, decolourisation using ligninolytic fungal isolates was found to be the best followed by land treatment. Though moringa seeds removed the colour of the effluent, significantly, it's dose, soaking period, persistence of odour in the treated water and disposal of used seeds limits their usage under large scale (Muyibi and Evison, 1995).

SUMMARY

VI. SUMMARY

To meet the objectives, the changes in the soil biological properties *viz.*, total aerobic microflora (bacteria, fungi and actinomycetes) and the enzyme activities (dehydrogenase, phosphatase and urease) were studied under effluent irrigated condition in SIV model farm,. In addition various colour removal methods were also tested for their efficiency of colour reduction and the results are summarised.

- Use of partially treated effluent for irrigation did not seem to affect the population of bacteria, fungi and actinomycetes. Though the population found to be reduced on 120th day of effluent irrigation, it was not significant compared to the initial population level.

- The enzyme activity in the soil both before and after effluent irrigation revealed that all the three enzymes assayed *viz.*, dehydrogenase, phosphatase and urease did not get affected by the effluent disposal. In addition, all the blocks showed considerable increase in the enzyme activities, after effluent irrigation.

- Among the 12 isolated ligninolytic fungal cultures, the C5, C8, C11 and C12 were found to have considerable amount of ligninolytic activity than the others.

- With respect to decolourisation with known bacterial, fungal cultures and the enzyme viz., Actizyme, the fungus *Fusarium* sp. removed maximum amount of colour (53.65 per cent) from the effluent during 16 days of incubation period. This was achieved only in the presence of 0.1 per cent diammonium sulphate.

- Among the isolated ligninolytic fungal strains from the effluent enriched soil, C8 showed the maximum colour reduction (59.96 per cent) in the presence of 0.3 per cent glucose. In addition, the efficiency of colour removal was found to be reduced in the presence of excess glucose (0.6 per cent).

- The same ligninolytic fungal strains showed improved performance when the decolourisation study was carried out in columns packed with immobilised strains. All the strains except C5, showed equality in the per cent colour reduction, when the aerated effluent with 0.3 per cent glucose was used. About 86.83 to 87.82 per cent of the colour was removed from the effluent during 12 hour retention time.

- The results of the effluent decolourisation using moringa seeds (at different doses) showed significant colour reduction (more than 75 per cent) in all the treatments. The maximum colour reduction (94.70 per cent) was achieved with 40 g. seed (for 150 ml of effluent). The pH and EC of the effluent in all the treatments found to be increased with seed rate and soaking periods. Considering the seed cost, soaking period, changes in pH and EC of the treated effluent, 5 g. seed was found to be best (for treating 150 ml effluent).

- The treatment of pulp plant effluent using soil column 1 (red soil) and 2 (sandy soil) showed that the colour of the effluent reduced considerably after passed though the columns. Column 1 (red soil) found to be superior than column 2 (sandy soil) in reducing the colour of the effluent. In addition, the pH and electrical conductivity of the treated effluent in this column was also seem to be lesser than untreated effluent till the passage of 1400th ml of effluent. About 95.85 per cent of the colour gets removed from the effluent at this level (1400th ml). Whereas beyond 1400 ml, the soil found to loose its buffering capacity (Fig. 5.1).

- The pH and electrical conductivity of the leachates did not exceed the critical limit prescribed by ISI, 1981. Till the passage of 1400th ml of effluent. In addition, the soil chemical and biological properties were also not altered very much at 30 cm depth where, the root zone of majority of the agricultural crops lies.

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