Bioremediation of Chromium (VI) from Tannery Effluents using Selected Bacterial and Fungal Isolates and Agrowaste Adsorbents

By

Selvi . V

A thesis submitted to Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University) Coimbatore,

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Certificate

CERTIFICATE

This is to certify that the thesis entitled **"Bioremediation of Chromium (VI) from Tannery Effluents using Selected Bacterial and Fungal Isolates and Agrowaste Adsorbents"** *submitted to the Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, for the award of the degree of* ***Doctor of Philosophy in Biochemistry*** *is a record of original research work done by* ***Selvi. V*** *during the period of her study in the Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or similar title to any candidate of any University.*

G. P. Jeyanthi

27/11/2003

Signature of the Guide

Declaration

DECLARATION

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, under the supervision and guidance of **Dr. G.P. Jeyanthi, M.Sc., M.Phil, Ph.D.,** *Reader in Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore and it has not been submitted for the award of any Degree, Diploma, Associateship, Fellowship etc., of any other University or Institute.*

G. P. Jeyanthi. 27/11/2003

Supervisor

V. S. 27/11/03

Candidate

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Introduction

1.0 INTRODUCTION

Advances in science and technology since the industrial revolution, have enabled humans to exploit natural resources. The sudden introduction of xenobiotic chemicals results in accumulation of pollutants to problematic or even harmful levels (Shah and Thakur, 2002). Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment (Rajendran *et al.*, 2003). Metal pollution is the gift of industrial revolution, which has adversely affected the ecological balance causing serious health hazards. Problems of pollution by metals have aggravated for a variety of reasons, the most common being the unpredictability of mobility (Gupta, 2000).

Uncontrolled discharge of industrial effluents causes pollution of water by certain heavy metal ions. Consumption of such contaminated water leads to severe health problems. The metal ions may enter the food chain and get biomagnified through bioaccumulation, thereby increasing the magnitude of problem. In view of their high toxicity, environment mobility, non-biodegradability and stability, their removal becomes an absolute necessity (Vasudevan *et al.,* 2001; Gupta and Mohapatra, 2003).

It is well perceived that there is a permissible limit of each metal, above which they are generally toxic and some are even hazardous. Globally, in developed countries, pollution of the aquatic system is controlled by environmental protection acts and regulations enforced by environmental agencies. All effluents need to be assessed and require integrated pollution documentation before their final discharge (Gupta *et al.,* 2000).

The incidence of water pollution with loaded metal pollutants has reached such an alarming levels that environmentalists are finding it difficult to enforce control measures. All these heavy metals seriously interfere with

bioenvironmental processes, thereby posing a menace to the life on this planet (Gupta, 2000).

Due to rapid industrial development during the last two decades in India, the disposal of industrial effluents has become a serious problem. The tanning industry is one of the oldest cottage industries in India, which has now taken a predominant place in the country's economy (Dadhich *et al.,* 2002). Such remarkable expansion incidentally leads to extensive pollution of ground water and soil due to the release of sludge and effluent (Thangavel *et al.,* 2003).

This industry consumes huge amount of water and consequently generates large volume of wastewater. These are usually discharged to nearby river courses. The application of these industrial effluents to land has also been started during recent years as an alternative means of treatment and disposal.

There are about 3000 tanneries in India, mostly spreadover in TamilNadu, West Bengal, Uttar Pradesh, Maharashtra, Karnataka, Punjab and Rajasthan. Annually, about 80 million pieces of hides and 130 million pieces of skins are processed by the tanning industry of the country for manufacturing semi finished and finished leather. There are about 680 leather finishing units in our country producing about 1000 million square meter of finished leather per annum. The quantity of effluents discharged amounts to about 30 to 40 litres per kg of skin/hide processed and in the case of finishing units, the quantity is about 50 litres per kg of raw skin/ hide. A large number of tanneries (about 433) are located in TamilNadu and their contribution is about 70 percent in the total export of leather and leather products from the country (Chhonkar *et al.,* 2000).

Two types of tanning processes are mainly followed in India - vegetable tanning and chrome tanning. About 80% of tanneries are engaged in chrome tanning process. The leather industry discharges many toxic pollutants namely sulphides, phenolic compounds, chromium and other mineral salts, dyes and solvents. Chromium contributes a major share to the hazardous nature of tannery

effluents. The permissible limits set by several environmental agencies for total chromium in effluents is <2mg/l. This has necessitated the tanners to look for long and safe solution arising from the discharge of tannery effluent into the environment (Dadhich *et al.,* 2002).

In recent years, the extent of chromium pollution has been recognized and various chemical and biological remediation techniques which aim to reduce chromium and ultimately remove it from soils and waterways are being investigated. The solubility, bioavailability and toxicity of metal oxyanions are determined by their oxidation state. For chromium, a high oxidation state confers high solubility, reactivity and toxicity whereas a lower oxidation state has a much lower solubility and is kinetically inert (Smith and Gadd, 2000).

Chromium, the highly toxic heavy metal pollutant in the environment is known to interfere with biological system (Lakshmi and Sundaramoorthy, 2003). The potential toxicity of chromium depends strongly on its oxidation state. Hexavalent chromium is a well-known, highly carcinogenic and mutagenic agent, occupying an upper rank in the list of hazardous substances (Neves *et al.,* 2002). Cr(III) is a less toxic and less water-soluble form of chromium which plays an important role in the function of insulin hormone (Narin *et al.,* 2002). Chromium has long been known to be essential for proper lipid and carbohydrate metabolism in mammals. Chromium deficiency leads to symptoms associated with adult - onset diabetes and cardiovascular disease (Vincent, 1999).

Remediation of sites contaminated with toxic metals is particularly challenging. Unlike organic compounds, metals cannot be degraded and the cleanup usually requires their removal (Lasat, 2002).

Physico-chemical processes used for the removal of metals from aqueous solutions before they are disposed off, include chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, electrochemical treatment and evaporation (Kowshik and Nazareth, 1999). These

processes are expensive and not ecofriendly. Further, the major disadvantage with conventional treatment techniques is the production of sludge. As a result, an aquatic problem may change into solid disposal problem. In this light, biological materials have emerged as an ecofriendly and economic option (Gupta *et al.,* 2000).

The presence of chromium in the environment has selected microbial and plant-variants which are able to tolerate high levels of chromium compounds. The diverse chromium resistance mechanisms displayed by microorganisms and probably by plants, include biosorption, diminished accumulation, precipitation, reduction of Cr(VI) to Cr(III) and chromate efflux. Some of these systems have been proposed as potential biotechnological tools for the bioremediation of chromium pollution (Cervantes *et al.,* 2001).

The use of microorganisms to remove metals is an emerging technology and is gaining attention among environmental research communities. Microorganisms do not degrade metals but may immobilize them by sorption to cell components or exopolymers, or may catalyse metal precipitation or volatilization from polluted environments (Wang, 2001).

The metal uptake by microbial biomass takes place by two basic processes. The first is by living organisms, where the metal uptake is dependent on metabolic activity. The second process involves metal uptake by dead and living cells as a result of the chemical functional groups of the cell and in particular the cell wall (Bharani, 1999).

Sequestration and immobilisation of heavy metals, especially in the solutions of effluents and waste water, can be accomplished through biosorption, a passive process of metal uptake, using biomass. Biosorption is essentially a non-directed physicochemical complexation reaction between dissolved metal species and charged cellular components, that involves sorption and / or complexing of metals to living or dead cells. Also, insoluble metal species can be physically entrapped in the microbially produced extracellular matrix or

precipitated in bacterial or algal exudates (Kamaludeen *et al.*, 2003) Microorganisms such as fungi, bacteria and algae are the commonly used agents of biosorption (Kowshik and Nazareth, 1999).

Several isolated bacterial species were capable of catalysing the reduction of Cr(VI) to Cr(III), a less toxic and less water-soluble form of chromium, which could be used for bioremediation of Cr(VI) polluted environments (Viti *et al.,* 2003).

Even though microbial resistance to metals has been studied over the past 25 years, the last 10 years have been full of surprises and discoveries in this research. Furthermore, only limited information is available on the use of microorganisms for bioremediation purposes (Saxena *et al.,* 2000) and less information is available concerning the mechanisms of toxicity of chromium (Stohs *et al.,* 2000).

The application of activated carbon for the removal of heavy metals, colour, odour, taste and other organic compounds by adsorption process from the industrial wastewater is a common practice (Rao and Bhole, 2001). The high cost and difficulty in procuring commercial activated carbon paved the way to produce indigenous low cost activated carbons from agricultural wastes like coconut shell, rice husk, groundnut husk and tamarind nut (Rengaraj *et al.,* 2000).

Bearing in mind the ecotoxicological impact of chromium from tannery effluents, the present investigation has been designed with the following objectives:

1. To assess the physicochemical characteristics of the tannery effluent in order to device suitable treatment strategies for the removal of chromium

2. To isolate, select and characterise metal tolerant bacteria and fungi which can be used for the removal of Cr(VI) from the effluent

3. To identify the optimal experimental conditions and to identify the effect of selected external factors for the maximal and efficient remediation of Cr(VI) using selected bacterial and fungal isolates

4. To characterise the selected adsorbents prepared from agrowaste materials and to assess their Cr(VI) sorption capacity under various conditions

Based on the above objectives and aims, an attempt was made to undertake bioremediation of chromium load in a selected tannery industry effluent using bacterial and fungal isolates and selected agrowaste materials.

Review of Literature

2.0 REVIEW OF LITERATURE

The review of literature pertaining to the present investigation is organised and presented under the following headings:

2.1 HEAVY METAL POLLUTION

Environmental issues are of increasing concern and research is now being directed towards applying technology to ameliorate the effects of environmental

pollution (Vasudevan,1999). During the last few decades, extensive attention has been paid to the hazards arising from contamination of the environment by heavy metals (Kotrba *et al.,* 1999a).

Water pollution due to hazardous heavy metals has been a major global concern for environmentalists. The industrial, agricultural and municipal wastes are the key sources of these toxic metals in the wastewater (Elizabeth and Anuradha, 2000).

The consequences of massive water pollution in relation to human health are of great concern because two third of illness is reported to have been related to the water borne diseases through metal intoxication(Khurshid et al., 1998).

Water pollution caused by industrial wastes normally manifests itself with poisoning of aquatic life resulting in the reduction of the quantity and quality of fish and other aquatic life. Amongst the pollutants, the heavy metals received much attention owing to their profuse occurrence in industrial effluents (Khurshid *et al.,* 1998).

Heavy metals are emitted from the industries as liquids, solids and frequently as fine particles directed into the atmosphere and ultimately deposited on soil and water bodies. Indiscriminate dumping of industrial and municipal wastes is of great concern because of possible toxic impact of heavy metals on biological system (Sumathi *et al.,* 1999).

Emissions into the air constitute the greatest source of heavy metal pollution. Some of the prominent sources of atmospheric metal pollution are burning of fossil fuel to generate energy (V, Ni, Hg, Se, Sn), automobile exhaust (Pb), insecticides (As), manufacture of steel (Mn, Cr) and smelting (As, Cu, Zn), (Athar and Vohora, 1995).

Soil is an important subsystem of the terrestrial ecosystem. Disturbance of this system by toxic heavy metals may have profound influence on the

decomposition of organic matter, thereby affecting soil fertility. Soil enzymes play an important role in this process of decomposition and are often sensitive to heavy metal pollution (Devi *et al.,* 1995).

Pollution by heavy metals can be a much more serious problem than by organic substances because they are nonbiodegradable and persistent. The heavy metals are taken up by the primary producers of aquatic systems leading to biomagnification along the increasing trophic levels (Sultan and Fatima, 1999).

2.2 CHROMIUM POLLUTION AND ITS HAZARDS

2.2.1 Sources and occurrence of chromium pollution

Chromium is widely distributed in the environment, being found in air, water, soil and all biota. In trace amounts it is essential for human life (Losi *et al.,* 1994).

Chromium is one of the most widely used metals in industry, resulting in large quantities of this element being discharged into the environment. Both trivalent chromium and hexavalent chromium exist in various bodies of water in a variety of forms. Cr(VI) is very toxic and is carcinogenic and mutagenic. Cr(III), on the other hand, is much less toxic and significantly less soluble than Cr(VI) (Wang, 2001).

Major anthropogenic sources include metallurgical industries (ore refining and production of steel and alloys), chemical industries (pigment manufacturing, plating / metal finishing, corrosion inhibition, leather tanning, wood preservation, petroleum refining, textile manufacturing and pulp production) and combustion of coal and oil (Losi *et al.,* 1994; Smith and Gadd, 2000).

Naturally occurring chromium is almost always present in the trivalent state. Almost all the hexavalent chromium in the environment arises from human activities. In this oxidation state, chromium is relatively stable in air and pure water, but it is reduced to the trivalent state, when it comes into contact with

Recently, research on chromium toxicity has been heading towards studies on chromium compounds in intermediate oxidation states, mostly Cr(V) (Neves *et al.,* 2002).

The genotoxicity of Cr(V) and Cr(VI) increases according to the permeability characteristics of their complexes. The permeability of Cr(V) is equal to, if not greater than, that of Cr(VI) in terms of the total amount of chromium entering the cells. This suggests that Cr(V) is produced as a metabolic intermediate in the intracellular reduction of Cr(VI) (Codd, *et al.,* 2001; Vasant *et al.,* 2001; Ueno *et al.,* 2001).

Cr(VI) was identified as a potential carcinogen (Basketter *et al.,* 2001; De Flora, 2000). Cr(III) becomes toxic only in extremely high amounts (Krejpcio, 2001).

Industrial exposure to high doses of chromium can cause allergic dermatitis, skin ulcers and bronchogenic carcinoma. Because chromium is a potent sensitizer, external contacts with chromates and dichromates can induce allergic eczema in some people (Krejpcio, 2001).

In adult human subjects, the lethal oral dose is considered to be 50-70 mg soluble chromates per kilogram body weight. The clinical features of acute poisoning are vomiting, diarrhoea, haemorrhage and blood loss into the gastro intestinal tract, causing cardiovascular shock (Sharma *et al.,* 1978).

Chronic ulcers of the skin and acute irritative dermatitis have been consistently reported in workers exposed to chromium containing materials. Inhalation of Cr(VI) compounds causes marked irritation of the respiratory tract. Thus, ulceration and perforation of the nasal septum have occurred frequently in workers employed in the chromate using industries (Dayan and Paine, 2001). Cases of sinonasal cancer were reported by the International Agency for Research on Cancer possibly indicating a pattern of excess risk for these rare tumors (IARC, 1990).

Epidemiological studies of workers in the chromate production industry have consistently shown risks for lung cancer. Studies in experimental animals have confirmed Cr(VI) to be carcinogenic by inhalation but not by ingestion or skin contact (IARC, 1990).

The results of epidemiological studies carried out by Backyavathi and Kumar (1995), showed significant occurrence of various diseases due to occupational exposure to chromium in the following orders: Bronchitis > acute pharyngitis > gastritis > chrome hold ulcer > industrial dermatitis > eye irritation and corneal ulcer > nasal irritation and nasal perforation > injuries > acid burns asthma > pleurisy > fibrosis > tuberculosis.

Recent epidemiological studies have suggested that the chromate anion is a carcinogen, bronchogenic carcinoma being its principal effect (Chandra, 2001).

Consumer products such as Cr(III) tanned leather products also contain trace amounts of Cr(VI), which is formed by oxidation of Cr(III) during the tanning process. In a recent study of the Cr(VI) content of leather products bought on the Danish market, 35% of such articles had a Cr(VI) content above the detection limit of 3 ppm, ranging from 3.6 ppm to 14.7 ppm. Leachable Cr(III) was detected at levels of 430 - 980 ppm. Exposure to 10 ppm Cr(VI) alone elicited eczema in chromium sensitive patients. The eliciting capacity of Cr(III) has not been systematically investigated but, compared to Cr(VI), much higher concentrations were needed to elicit eczema (Hansen *et al.,* 2002). There are concerns that wearing chromium tanned leather gloves can provoke a lapse of hand dermatitis in chromium sensitive patients (Nygren and Wahlberg, 1998).

Chromium salts were identified as human contact allergens and caused contact dermatitis and hand eczema due to their presence in household bleach and consumer products (Basketter *et al.,* 2001).

Cr(VI) compounds are widely recognized as human carcinogens (Shin and Paik, 2000; Neves *et al.,* 2002; Basketter *et al.,* 2001; De Flora, 2000). Cr(III) becomes toxic only at extremely high amounts (Krejpcio, 2001).

Extensive studies *invitro* and in model systems indicate that the reactive intermediate, Cr(V), generated by cellular reduction of Cr(VI), is the likely candidate for the ultimate carcinogenic form of chromium compounds (Liu and Shi, 2001).

2.2.3 Effect of chromium on aquatic system

Having entered into the environment, heavy metals play a significant role in aquatic ecosystem thereby posing biological threat to public health. Thus to avoid health hazard, it is necessary to remove the heavy metals from wastewater before its disposal (Ingole and Bhole, 2000).

The heavy metals are taken up by the primary producers of aquatic systems leading to biomagnification along the increasing toxic levels (Sultan and Fatima, 1999). Extensive heavy metal pollution brings about changes in the nucleic acid contents of aquatic fauna, particularly fishes (Muley *et al.,* 2000).

The heavy metal contents of the aquatic animals originate from two routes of intake. The free ions and simple compounds dissolved in water are taken up directly through the epithelium of the skin, gills and alimentary canal while others, accumulated in food organisms are incorporated by nutrition. Plankters which serve as natural food of the fish species have a tendency to accumulate metals in their systems (Madamba and Pamulaklakin, 1994).

The level of pollutants detected in the tissues of organisms is the only direct measure of the proportion of the total toxicant delivery to biota and therefore indicates the fraction that is likely to enter and affect aquatic ecosystem. Carnivores at the top of the food chain such as birds and mammals including humans, obtain most of their pollutant burden from aquatic ecosystems

by way of their food especially fish. People who eat contaminated fish regularly, therefore, are most exposed to the risk of chronic poisoning (Yazdandoost and Katdare, 1999).

Sonawane *et al.* (2002) showed that high concentration of chromium in water was lethal to fish and corrosive to flesh. Chromium toxicity to aquatic organisms is primarily from exposure to dissolved rather than particulate sorbed toxicants (Gendusa *et al.,* 1993).

2.2.4 Effect of chromium on soil system

Heavy metal pollution of soils and aquifers is a serious and ongoing problem. Soil receives large quantities of metals from a variety of industrial wastes and municipal sewage sludge. One of the negative environmental impacts arising from the heavy metal pollution of soil is groundwater contamination. Rainwater percolating through the soil slowly extracts metals which eventually may reach aquifers. Acidification of the soil from acid rain accelerates this process (Arnfalk *et al.,* 1996; Gao *et al.,* 1997).

Soil is the primary recipient of the metallic pollutants and from the soil, they make their entry into the living beings easily (Gupta, 2002).

Chromium enters soil from natural processes and human activities. Although Cr(III) occurs naturally in the environment, Cr(VI) in the environment is usually related to anthropogenic activities. The ingestion of soil contaminated with chromium especially by children can have toxic consequences (Skowronski *et al.,* 2001).

Transformation of chromium in the soil is a very complex process due to the variety of its forms of occurrence and factors which could modify its chemical form. Chromium in soil and water usually occurs as trivalent or hexavalent ion. Occurrence of different chromium species is determined by chemical and physical properties of the soil (Wyszkowska *et al.,* 2001).

Cr(VI) leaching from contaminated soil into groundwater and surface water threatens water supplies and the environment (Tseng and Bielefeldt, 2002).

Soil enzymes play an important role in the process of decomposition and are often sensitive to heavy metal pollution. A study in Anantapur of Andhrapradesh groundnut field clearly indicated that chromium is toxic to the soil enzymes, amylase and invertase and was found to inhibit or delay recycling of nutrients (Devi *et al.,* 1995).

Addition of organic amendments (biosolid compost, farmyard manure, fish manure, horse manure, spent mushroom, pig manure and poultry manure) enhanced the rate of reduction of Cr(VI) to Cr(III) in the soil. There was a significant positive linear relationship between the extent of Cr(VI) reduction and the amount of dissolved organic carbon in the soil (Bolan *et al.,* 2003).

Although soil characteristics such as percentage of organic matter in soil, clay content, particle size and surface area can influence the bioavailability of metals, the most important properties of metals affecting their availability for absorption are their oxidation states, form of the minerals and the solubility of the metal compounds. The chemical and physical weathering environment of a contaminated soil will determine the long term fate of metals (Ruby *et al.,* 1999). Therefore, metal mobility, bioavailability, toxicity and the response of the metals to remedial action will be affected (Menzie *et al.,* 2000).

2.3 PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF CHROMIUM

Cr(III) is an essential nutrient that functions to help regulate insulin-mediated carbohydrate, lipid and protein metabolism (Lukaski, 1999), possibly as a part of a low molecular weight chromium binding substance (Vincent, 1999). In contrast Cr(VI) was identified as a potential carcinogen (Krejpcio, 2001).

Chromium (atomic number 24, relative atomic mass 51.996 occurs in each of the oxidation states from -2 to +6, but only the 0 (elemental metal form), +2, +3 and +6 states are common. Divalent chromium (+2) is unstable in most

compounds as it is easily oxidised to the trivalent form by air. Accordingly, only the trivalent Cr(III) and hexavalent Cr(VI) forms are important for human health. Valid generalisations of the biological effects of chromium in its elemental form cannot be made.

It is of great importance to realize that the +3 and +6 oxidation states have very different chemical and hence biological properties.

Soluble hexavalent chromium species are extremely toxic and exhibit mutagenic and carcinogenic effects on biological systems due to their strong oxidizing nature (McLean and Beveridge, 2001). In contrast, Cr(III) is the most stable form in biological systems and it doesnot penetrate biological membranes easily and it appears that the transport of specific chromium compounds is strictly regulated by the affected organism. Cr(III) ion has a strong tendency to form co-ordination compounds with a very slow reaction rate. This slow rate suggests that chromium exerts a structural function rather than an active site in an enzyme, which may explain that no chromium containing enzymes have been identified (Krejpcio, 2001).

2.4 PROCESSES INVOLVED IN TANNERY INDUSTRY

Tanning is the process of conversion of animal skins and hides to non-putrescible and tough material known as leather. This process converts the collagen, the major property of skin and hide, into a resistant condition. The tanning process also gives the necessary feel and physical characteristics of leather.

Leather processing involves - A. Pretanning steps B. Tanning C. Post tanning and D. Finishing steps

A. Pretanning operations (Beam house processing)

The dry matter of the skin is almost protein, reported to be about 85% collagen. The skin also contains lipids, albumin, carbohydrates and globulin in

minor amounts. In beam house processes, all the impurities are removed and collagen of the skin is made ready to absorb the tannin or chromium. The pretanning processes include :

a. Curing

A process which involves salting the hide. Hides can be cured in one of two ways :

- Wet - salting is done by salting the hide and then piling many skins together. They are then left to cure for a month, so that the salt can completely be absorbed into the skin.

- Brine - curing is more common than wet salting, as it is considered a faster and easier method. During brine curing, hides are positioned carefully in vats and smoothened with a mixture of salt and disinfectant. After 10 -16 hours, the skins are completely cured and are ready to move onto the next stage.

b. Soaking

Soaking in water for several hours to several days helps to remove the salt, dirt, debris, blood and excess animal fats. This also restores hides a hydrated 'natural' condition.

c. Liming

Hides are then immersed in a mixture of lime and water to loosen hair.

d. Dehairing and Defleshing

After liming, the hair and flesh are mechanically removed from the hide.

e. Scudding

In this process done by hand, hairs and fats which were missed by machine, are removed from the hide with a plastic tool or dull knife.

f. Deliming

This is done to remove the lime and alkaline chemicals present in the limed hide by treatment with either ammonium chloride or sulphate and washing again.

B. Tanning operations

There are two types of tanning :

1. Vegetable tanning and
2. Chrome tanning

Quantities and characteristics of effluents depend on the type of tanning. In some of the tanneries both the tanning processes are carried out.

1. Vegetable tanning

This type of tanning produces heavy leathers which are plumper, more easily tooled and embossed. They are also less affected by changes in humidity and body perspiration. The following are the sequential operations in vegetable tanning.

a. Tanning

The delimed skins and hides are soaked for 10 - 15 days in pits containing vegetable tan liquor which comprises vegetable substances (extracts of bark, wood and nut containing natural tannins) and also pyrogallol or catechol base.

b. Myrobing

The tanned materials are then soaked in myrobalan liquor.

c. Oiling

After myrobing, oil is applied liberally on the surfaces with suitable vegetable oil (pungam oil) which makes the leather soft, pliable and resistant to tearing. Incorporation of oil is also termed as stuffing.

d. Drying

The leather is dried and dyed.

2. Chrome tanning

It is carried out to prepare light and more resistant leather. It involves more operations than vegetable tanning, but the duration is much less.

a. Bating

Enzymes are added which cleanup the grain surface and further destroy any remaining hair roots and pigments. This makes the grain surface softer and cleaner.

b. Pickling

Water, sulphuric acid and salt are added to bring the skins to the proper pH for tanning. This acid environment makes the hides ready to accept the tanning chemicals. Pickled skins may be preserved at this stage.

c. Tanning

After pickling, they are tanned by soaking in chrome tan liquor containing chromium sulphate for 6 hours (pickling and tanning are done in the same drum). After tanning, sodium carbonate is added to the drum to fix the chrome. Then they are taken out of the drum and kept for 24 hours for complete absorption and fixation of chromium.

After tanning, the resulting product has a distinctive blue shade and is therefore known as wet blue. The hides or skins can be dried for further transport by pressurized rollers that will squeeze out excess moisture.

C. Post tanning operations

After tanning, some additional operations are required, which in general aim at leveling the hides and skins to give them a uniform thickness.

a. Splitting

The wet blue (which at this stage are fairly thick) are then split horizontally through their structure to produce two thinner pieces. The outer skin surface goes forward for processing as full grain leather, the underside is suede - sometimes also known as split leather.

b. Shaving

Any further correction of the thickness is done by shaving off any unwanted fleshy material.

c. Retanning

A second tanning process is carried out to prepare the leather for receiving the final colour (dyeing). Retanning removes free acids that are present in wet blue and makes the leather softer and firmer.

d. Dyeing

It is a process of colouring by means of soluble dyes. The leather is placed in rotating drums of dye. The dye is absorbed into the leather while the leather is tumbled in the drums. The dye penetrates the entire thickness of the hide. Therefore, even with years of use, the leather retains its colour over the entire surface.

e. Fat - liquoring

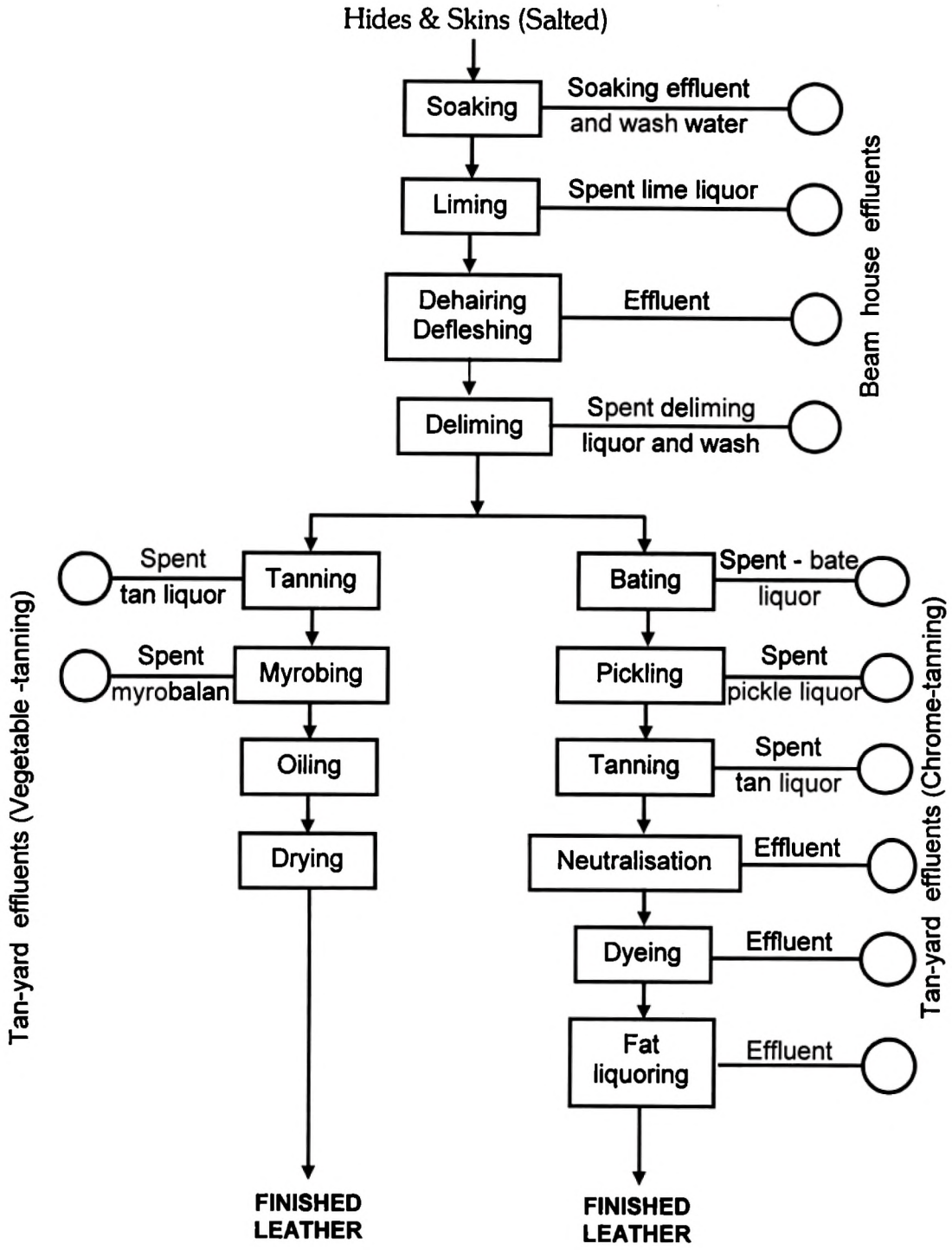
This process gives the final softness and flexibility to the tanned hide.

f. Rolling

The leathers are again passed through the pressurized rollers to remove the moisture and are then dried.

FIGURE 1

FLOW CHART OF TANNING (VEGETABLE AND CHROME) PROCESSES INDICATING VARIOUS EFFLUENT STREAMS



(Manivasakam, 1997; NLIDP, 2000; Mazumder *et al.*,1998).

PLATE 1

PRETANNING PROCESSES INVOLVED IN TANNERY INDUSTRY

Salting

Soaking

Liming

Dehairing

Defleshing

PLATE 2

TANNING PROCESSES INVOLVED IN TANNERY INDUSTRY

Pickling and basic chrome tanning

Basic chrome tanned leather

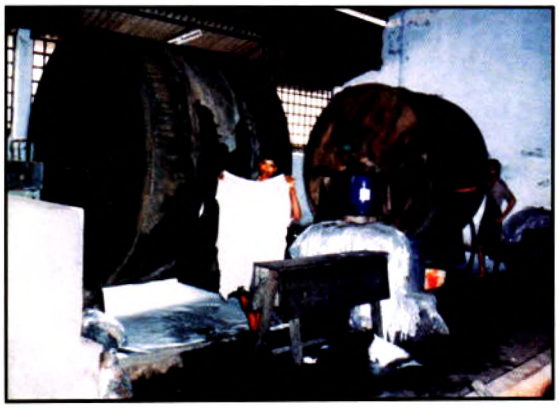
Wet blue samming

PLATE 3

POST TANNING PROCESSES INVOLVED IN TANNERY INDUSTRY

Splitting

Shaving



Retanning and dyeing

Leather after tanning

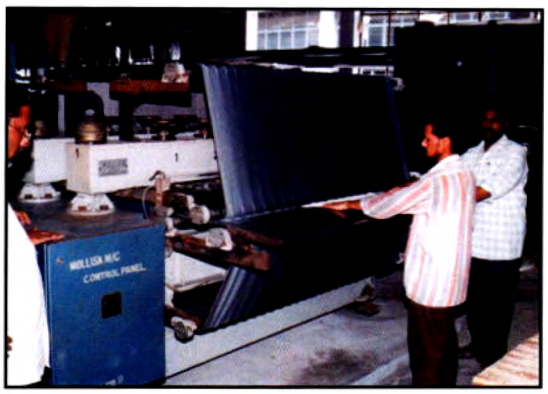
Rolling

Natural drying

PLATE 4

FINISHING PROCESSES INVOLVED IN TANNERY INDUSTRY

Spraying



Vibrations stacking

Ironing

Finished leather

D. Finishing

The specific steps in the finishing process are dependent on the requirements of the final product and vary for different leather. Waxes, pigments, dyes, glazes, oils and other solutions are also added to make the leather more appealing to the buyer.

The flow chart indicating all the processes described above is given in Figure 1. Plates 1 – 4 depict the same.

2.5 BIOREMEDIATION OF HEAVY METALS

In recent years, the quest for clean environment has stimulated interest in finding alternative methods of waste disposal, pollution removal and decontamination of toxic residues. The biological means of pollution removal from soil and water through biodegradation and bioremediation has been receiving new attention (Gunasekaran, 1999).

Bioremediation is the use of biological treatment systems to destroy or reduce the concentrations of hazardous wastes from a contaminated site (Rahman and Lakshmanaperumalsamy, 1999). It is defined as a strategy or process that uses microorganisms, plants or microbial or plant enzymes to detoxify contaminants in the environments. This concept includes biodegradation, which refers to the partial and sometimes total transformation or detoxification of contaminants by microorganisms and plants (Ramasamy and Thanga, 1999).

Bioremediation is more economical in many cases and acceptable than other physical and chemical processes because bioremediation results in complete removal of pollutant and its toxicity through metabolic reactions mediated by microorganisms (Gunasekaran, 1999).

Several issues concerning the ecological principles play an important role in bioremediation. The most important factors include substrate bioavailability,

oxygen concentration, temperature, pH, moisture, inorganic nutrients, organic substrates and toxins. For bioremediation, optimum conditions are obtained when the target contaminant is the growth limiting factor and all other environmental requirements are fulfilled (Vasudevan, 1999).

2.5.1 Criteria for bioremediation

To seriously consider bioremediation as a practical means of treatment, certain criteria must be met

- The organisms must have the necessary catabolic activity to degrade the contaminant at a reasonable rate to bring the concentration of the contaminant to a level that meets regulatory standards

- The target contaminant must be bioavailble (e.g. not sorbed as a sound residue)

- The contaminated site must have conditions conducive to microbial or plant growth or enzymatic activity

- The cost of bioremediation must be less than or at worst, no more expensive than other technologies that can also remove the contaminant.

The failure to meet any one of these criteria may cause rejection of a bioremediation approach (Ramasamy and Thanga, 1999).

2.5.2 Bioremeidation : Status and future demand

Bioremediation represents only one group of diverse clean up strategies. For it to prove commercially attractive and, hence, be employed extensively, it will need to provide cost - effective solutions for a broad range of difficult and demanding clean up technology. As far as meeting the criteria of both predictable performance and cost effectiveness, bioremediation is in its infancy. For it to

become a widespread commercial success, it must undergo an accelerated development based on scientific understanding rather than assumptions and good luck.

For the development of bioremedial processes to succeed commercially, it will be essential to link microbial ecology and microbial biochemistry, via microbial physiology, to biochemical and bioprocess engineering. Attention needs to be devoted to the integration of individual chemical transformations in the metabolism of microbial cells and to define the rate limiting steps in bioremediation. There is little value in enhancing the rates of microbially mediated reactions if the overall remediation process is controlled by physical limitations. Thus, consideration of microbial physiology by process engineers and of aspects of practical applications by cell and molecular biologists is essential. Only by integrating the expertise of these diverse disciplines workable and commercially viable bioremediation strategies will be devised (Rahman and Lakshmanaperumalsamy, 1999).

2.5.3 Bioremediation methods

These fall into two major categories. 1. Above ground treatment and 2. In situ bioremediation. Above ground treatment process includes land farming and solid phase bioremediation, bioreactors, biologically enhanced soil washing and composting. Among these, land farming and solid phase bioremediation have been used to treat contaminated soil more extensively than the other technologies.

In situ bioremediation deals with two forms of treatment processes. 1. By the manipulation of aqueous constituents and 2. By the stimulation of air movement (Bio venting). In the former, addition of nutrient supplements is preferred and in the latter active pumping of an aquifer for better control of infiltration and distribution of nutrients is employed (Vasudevan, 1999).

2.6 BIOREMEDIATION OF HEAVY METALS BY MICROORGANISMS

2.6.1 Selection of microorganisms

Successful bioremediation depends on the availability of appropriate microorganisms. Although it is generally agreed that more than 80% of the total microbial source awaits discovery, reactions mediated by both the known and the unknown (i.e. populations observed to be effective, but uncharacterised) portions of the resource are already employed in biotreatment and in bioremediation (Rahman and Lakshmanaperumalsamy, 1999).

Certain enrichment culture techniques could be employed to isolate microorganisms capable of degradation of xenobiotic compounds or decontamination of heavy metals. Under such conditions pollutant detoxifying organisms have a competitive advantage over the other organisms present in the environment.

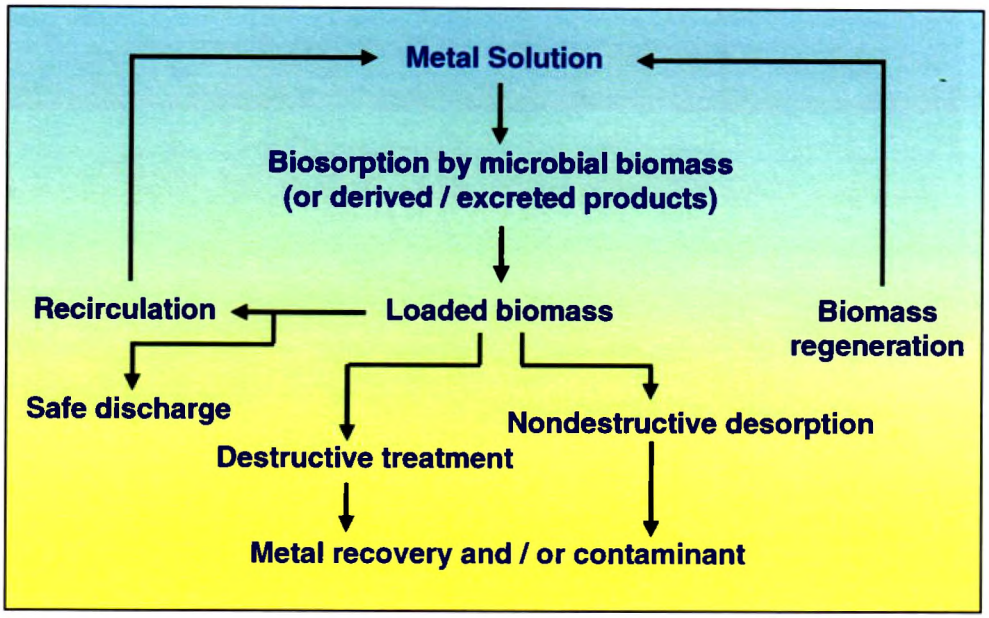
The microorganisms may be selected from the contaminated sites itself since the indigenous microflora could have been adapted to the contaminated environment (Gunasekaran, 1999).

2.6.2 Microbial metal removal

Bacteria (including actionmycetes), cyanobacteria, algae, fungi and yeasts are able to remove heavy metals and radionuclides from their external environment by means of physicochemical methods, e.g. adsorption, or by mechanisms dependent on metabolic activity, e.g. transport. Some physicochemical interactions may be indirectly dependent on metabolism via the synthesis of particular cell constituents or metabolites that may act as efficient metal - chelators or the creation of a particular micro environment in the vicinity of the cell that facilitates deposition and precipitation. Thus, living or dead microbial biomass is capable of metal accumulation as well as products produced by or derived from microbial cells as presented in Figure 2.

FIGURE 2

OUTLINE SCHEME OF METAL REMOVAL / RECOVERY FROM AQUEOUS SOLUTIONS BY MICROBIAL BIOMASS OR DERIVED PRODUCTS



(Gadd, 1990)

2.6.3 Metal removal by living cell systems

Many researchers have studied the use of live microbial systems for the purpose of remediating contaminated soils and waters (Atlas, 1995; Cervantes and Corona, 1994; Baker *et al.,* 1994; Saxena *et al.,* 2000; Saxena *et al.,* 2001; Muller *et al.,* 2001). These have received application in the decontamination of waste waters where metal concentrations may be below toxic levels. If living cells are used, there may be possibilities for removal of other pollutants from effluents. Both growing as well as nongrowing biomass can be used for metal uptake (Gadd, 1990).

In the process of continuously growing organisms for metal removal, it requires nutrients for the growth of organisms (Gunasekaran, 1999). Whenever studies are carried out using growing cells, the very low concentration of metal tolerated during growth leads to the difficulty in calculating the amount of metal

sequestered. But in case of metal tolerant organisms, the amount of metal sorbed during growth could be determined (Kowshik and Nazareth, 1999).

The metal accumulation of growing cells varied with the cell age. The maximum metal uptake took place during the lag period or the early stages of growth and declined as cultures reached a stationary phase (Bharani, 1999).

In addition to removal of metals from solution during growth, the grown cells can also sorb metals from solution. Especially where tolerance to metals during growth is fairly low, the grown cells can sorb the metals, at a higher concentration. In such instances, it is advantageous to use grown cells for metal sorption (Kowshik and Nazareth, 1999).

2.6.4 Metal removal by dead biomass

Bacteria, fungi, yeast and algae can remove heavy metals and radionuclides from aqueous solutions in substantial quantities (Kapoor and Viraraghavan, 1995).

The biosorptive capacity of dead cells may be greater, equivalent to or less than that of living cells. Systems using living cells are likely to be more sensitive to metal ion concentration (toxicity effects) and adverse operating conditions (pH and temperature). Furthermore, constant nutrient supply is required for systems using living cells (increased operating cost for waste streams devoid of nutrients) and recovery of metals and regeneration of biosorbent is more complicated for living cells. Also, the dead biomass can be procured from industrial sources as a waste product from established fermentation processes (Kapoor and Viraraghavan, 1995).

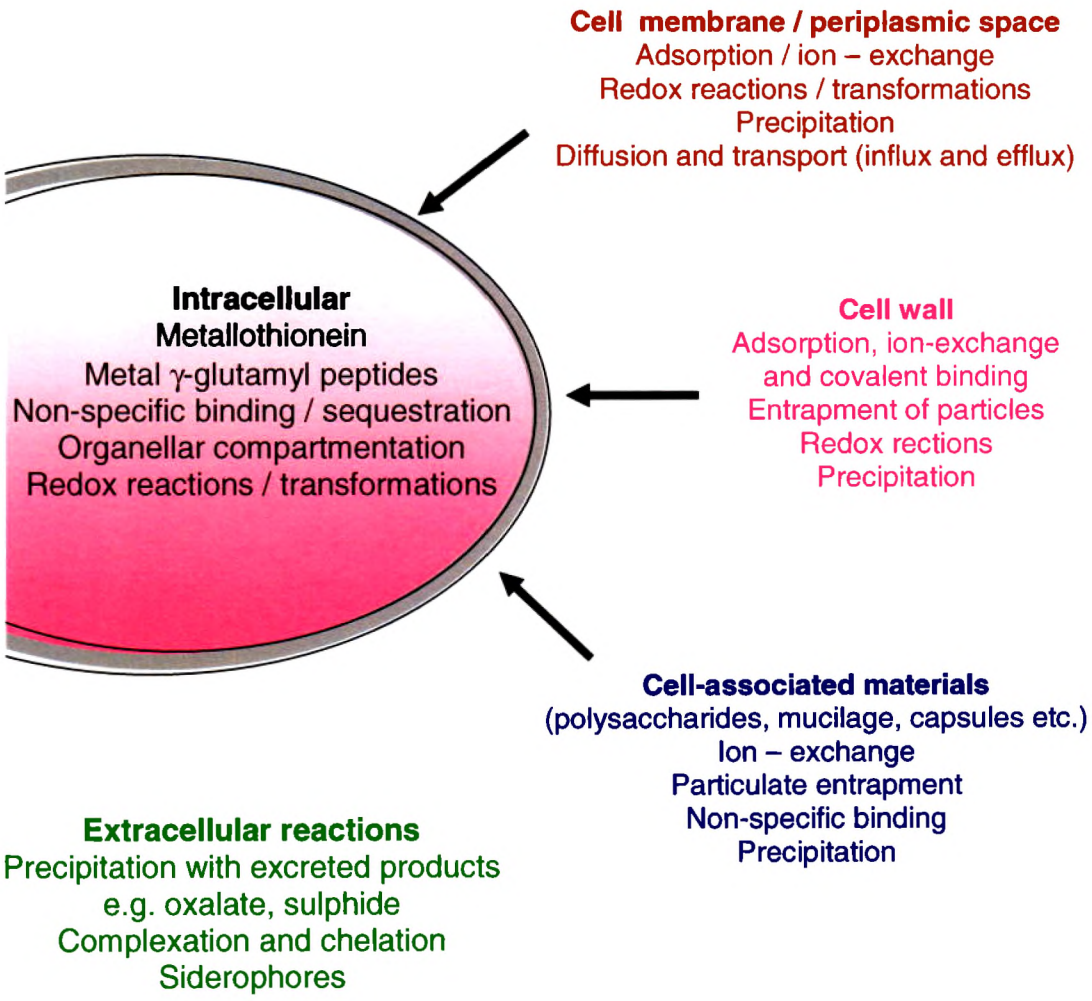
2.7 PROCESSES OF MICROBIAL METAL UPTAKE AND DETOXIFICATION OF TOXIC METALS

Once in the environment, metals may undergo transformation into various mobile forms and / or immobilization in an environmental sink. Biological activity

accounts for a large number of the environmental sinks of toxic metals, whether these are derived from natural or anthropogenic sources. Metal deposition by microorganisms is of great importance in biogeochemical cycles. Biotechnological approaches to the abatement of toxic metal pollution consist of selectively using and enhancing these natural processes to treat particular wastes. The processes by which microorganisms interact with toxic metals are very diverse and is represented in Figure 3.

FIGURE 3

PROCESSES CONTRIBUTING TO MICROBIAL UPTAKE AND DETOXIFICATION OF TOXIC METALS



(Gadd and White, 1993)

The processes by which microorganisms remove metals from solution are : (i) extracellular accumulation / precipitation; (ii) cell - surface sorption or complexation; (iii) intracellular accumulation (Muraleedharan *et al.,* 1991). Among these processes, process (i) may be facilitated by using viable microorganisms, process (ii) can occur with alive or dead microorganisms, while the process (iii) requires microbial activity. Metal uptake can also be carried out by purified biopolymers and other specialised molecules derived from microbial cells. These processes are not exclusive and several physicochemical and biological processes may be involved. In addition, metals can be removed from solids such as ores, sewage sludges and soils using microbial leaching (Gadd and White, 1993).

2.7.1 Metabolism independent biosorption

The term 'biosorption' is now frequently used to describe non-directed physicochemical interactions between metal species and microbial biomass (Gadd, 1990). Biosorption involves mechanisms like ion exchange, chelation and complexation, inorganic deposition via adsorption by physical forces and ion entrapment in inter and intra-fibrillar capillaries and spaces of structural polysaccharide network as a result of dilution through cell walls and membranes. Several active groups of cell constituents like acetamide group of chitin, structural polysaccharides of fungi, amine (amino and peptidoglycosides), sulfhydryl and carboxyl groups in protein, phosphodiester (teichoic acid), phosphate and hydroxyl polysaccharides participate in biosorption (Vasudevan *et al.,* 2001). The predominant mechanisms reported for the three types of microorganisms are:

1. Biosorption by fungi

Polysaccharides, in association with lipids and proteins, represent the main constituent of fungal cell wall. In filamentous fungi, the outer cell wall layers mainly contain neutral polysaccharides (glucans and mannans), while the inner layers contain more of glucosamines (chitin and chitosan) in a microfibrillar

structure. Ligands within these matrices include carboxylate, amino, phosphate, hydroxyl, sulphhydryl and other functional groups. Proteins are also found to be associated with metal binding (Vasudevan *et al.,* 2001). Many fungi and yeast have shown an excellent potential of metal biosorption, particulary the genera *Rhizopus, Aspergillus, Streptoverticillum* and *Saccharomyces* (Gupta *et al.,* 2000).

2. Biosorption by bacteria

The anionic nature of bacterial surface enables them to bind metal cations through electrostatic interactions (Shrivastava and Thakur, 2003; Vasudevan *et al.,* 2001). Because of their thickness and anionic character which is mainly due to peptidoglycan, teichoic acid and teichuronic acids, the cell walls of gram positive bacteria have high capacity for metal binding (Vasudevan *et al.,* 2001). Among bacteria, *Bacillus sp.* has been identified as having high potential for metal sequestration and has been used in commercial biosorbent preparation. Besides there are reports on the biosorption of metals using *Pseudomonas sp., E.coli* and *Staphylococcus sp.* (Saxena *et al.,* 2000; Gupta *et al.,* 2000).

3. Biosorption by algae

Special polysaccharides are present in the algal cell wall. The number and kind of binding sites depend on the chemical composition of the cell wall. It has been suggested that the polysaccharides of cell wall could provide amino and carboxyl groups as well as the sulphate. The amino, carboxyl group and the nitrogen and oxygen based moieties could also form coordinated bond with metal ions. Metal ion could also be electrostatically bonded to unprotonated carboxyl oxygen and sulphate. Covalent bonding between divalent cation and algal cell wall proteins has also been reported. Addition mechanisms such as entrapment of metal both in the form of insoluble microdeposits in the inter and intra-fibrillar capillaries and paracrystalline regions of polysaccharides and the binding to other biopolymers (RNA, polyphosphates) can contribute to the metal binding (Vasudevan *et al.,* 2001). Among photoautotrophs, marine algae became the

candidate of interest due to bulk availability to their biomass from water bodies. *Sargassum natans and Ascophyllum nodosum* in this group have shown very high biosorptive capacities for various metals. Besides marine algae, there are reports on binding of heavy metals to green algae, viz. *Chlorella sp.* and cyanobacteria (Gupta *et al.,* 2000).

2.7.2 Metabolism dependent intracellular accumulation

Metabolism dependent uptake of metal ion is usually a slower process than biosorption although greater amounts of metal may be accumulated by this means in some organisms, e.g. yeast. Transport of metal ions into microbial cells is inhibited by low temperatures, metabolic inhibitors and the absence of an energy source. Rates of uptake are influenced by the metabolic state of cells and the composition of the external medium (Gadd, 1990).

2.7.3 Intracellular localization and deposition

After uptake into cells, metals may be compartmentalised and / or converted to more innocuous forms by binding or precipitation. Dense intracellular deposits of uranium were observed in *Pseudomonas aeruginosa*, while other electron dense bodies, including polyphosphate, have been associated with intracellular metal accumulation in several bacteria, algae and fungi (Gadd, 1990).

Another common microbial response is the synthesis of intracellular metal binding proteins and these have been recorded in bacteria, cyanobacteria, algae, fungi and yeasts. Metallothioneins and other metal binding proteins, may have relevance to metal recovery since they can bind metals (Gadd, 1990).

A rapidly emerging research field involves bacterial surface expression of metal binding peptides for potential generation of noval biosorbents for removal of toxic metals from waste water. Also, recombinant DNA technology offers the possibility of improving the metal binding capacity of the bacteria

(Samuelson *et al.,* 2000). The expression of metal binding proteins were studied by many researchers on *E.coli* (Sousa *et al.,* 1996; Pazirandeh *et al.,* 1998; Kotrba *et al.,* 1999a; Kotrba *et al.,* 1999b).

2.8 METAL REMOVAL BY ADSORPTION

Adsorption plays an increasingly important role in the removal of specific target compounds or classes of compounds from water and waste water (Rathi and Puranik, 2002).

Adsorption is the exchange of material at the interface between two immiscible phases in contact with one another. This process is being mostly employed for the removal of dissolved substances from water and wastewaters (Kannan and Vanangamudi, 1991).

Removal of heavy metals from metal bearing waste water is usually achieved by physicochemical processes before discharging the effluents into natural water-body systems. Physicochemical processes in use for heavy metal removal from waste water include precipitation, coagulation, a reduction process, ion exchange, membrane processes (such as ultrafiltration, electrodialysis and reverse osmosis) and adsorption. High costs, process complexity and low removal efficiency of membrane processes have limited their use in heavy metal removal (Bharani, 1999). Most of the other methods very often present problems as secondary polluting effects (reagents employed in strong excess) or high operational costs (Siddiqui *et al.,* 1999).

Studies on the treatment of effluent bearing heavy metals have revealed adsorption to be highly effective, cheap and an easy method among the physicochemical treatment processes (Jasuja *et al.,* 1997). Activated carbon being the first choice is a valuable sorbing medium. However it suffers from disadvantage of its high manufacturing and regeneration cost (Verma and Rehal, 1996).

A search for a low cost and easily available adsorbent had led to the investigation of materials of agricultural and biological origin along with industrial by products, as potential metal sorbents (Bharani, 1999).

Siddiqui *et al.* (1999) showed that the phosphate treated saw- dust could successfully be used for the separation of Cr(VI) from Zn^{2+}, Ni^{2+} and Cu^{2+} and their removal and recovery from electroplating waste water.

The report on the use of thin layer chromatography (TLC) and column chromatographic techniques as a tool for studying the sorption capacity of teak leaves powder towards heavy metal cations has been documented by Ajmal *et al.* (2001).

An adsorbent CACMM2, extracted from a cactus was studied for the adsorption of Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+} and Zn^{2+} from aqueous solution and it was found to remove more than 83% of chromate in a freshly prepared and exhausted chromate commercial solution (Morales *et al.,* 2001).

The removal and recovery of Cr(III) from aqueous solutions with a spheroidal cellulose adsorbent containing the carboxyl anionic group was investigated by Liu *et al.* (2001). The adsorption process follows both the Freundlich and Langmuir adsorption isotherms and has been found to be endothermic. The adsorption mechanism is explained on the basis of complexation and ion exchange, between which the complexation adsorption is predominant.

The use of unconventional materials in this field has been examined by several workers. Interesting results have been reported with surface modified saw dust carbon for the removal of arsenic (Raji and Anirudhan, 1999), sorption of Pb(II) on chemically modified saw dust (Raji *et al.,* 1997), flyash for the removal of Ni (Kannan, 1991), removal of chromium using chitosan and chitin and leaf materials (Vasanthy and Lakshmanaperumalsamy, 1993), adsorption of

heavy metal cations from aqueous solutions by wool fibers (Balkose and Baltacioglu, 1992), copper and zinc uptake by bone mineral (Panda, 1995), coconut husk carbon for the removal of arsenic (Manju *et al.,* 1998) and uranium biosorption by dried roots of Eicchornia crassipes (Bhainsa and D'souza, 2001).

It has also been shown that peat (McKay and Porter, 1997; Sharma and Forster, 1995), rice husk carbon (Srinivasan *et al.,* 1998), teak leaves carbon (Singh and Lal, 1992) sunflower plant dry powder (Bhalke *et al.,* 1999), bark substrate (Khangan *et al.,* 1996) and an activated carbon prepared from coconut tree saw dust (Selvi *et al.,* 2001) can be used in the same way to extract metals from solution.

Experimental Procedure

3.0 EXPERIMENTAL PROCEDURE

Chromium in higher load causes an important environmental health hazard. Chromium is a major contaminant of tannery effluent and its discharge into the environment poses serious health hazards (Turick *et al.,* 1996). Even the presence of relatively low concentration of Cr(VI) in waste waters can significantly reduce the efficacy of biological sewage treatment (Vankova *et al.,* 1999).

Several physical and chemical methods are available for removing metal ions from industrial effluents. Some disadvantages of these methods include high operating costs, the requirement for preliminary treatment steps and the difficulty of treating the solid waste subsequently generated.

Researches in recent years indicated that many microorganisms can accumulate large concentration of metals. Biosorption of heavy metals by microbial cells has been recognised as potential alternative to existing technologies for removing heavy metals from wastes (Ramteke, 2000).

In the present study, chromium tolerant bacterial and fungal species were isolated from the tannery effluent and sludge samples, characterised and analysed for their efficiency in the uptake of chromium under various experimental conditions. Besides these, several adsorbents prepared from agricultural wastes were tried for the removal of chromium from the contaminated samples. The study was conducted in five phases :

PHASE I

3.1 CHARACTERISATION OF THE SELECTED TANNERY EFFLUENT SAMPLE

PHASE II

3.2 ISOLATION AND CHARACTERISATION OF THE BACTERIA AND FUNGI FROM THE TANNERY EFFLUENT AND SLUDGE SAMPLES

PHASE III

3.3 CHROMIUM UPTAKE STUDIES USING BACTERIAL ISOLATES

PHASE IV

3.4 CHROMIUM UPTAKE STUDIES USING FUNGAL ISOLATES

PHASE V

3.5 CHROMIUM UPTAKE STUDIES USING SELECTED AGROWASTE ADSORBENTS

PHASE I

3.1 CHARACTERISATION OF THE SELECTED TANNERY EFFLUENT SAMPLE

The increased rate of human population and rapid industrialisation in India have created problems of disposal of waste products which are indiscriminately discharged in the nearby river, reservoirs, lakes and tanks and even in the adjoining fields with almost no pretreatment (Sivakumar *et al.*,1999). Tannery effluent and sludge appear to be one of the major sources of pollution throughout India. The effluent contains toxic chemicals which are deposited on the soil causing adverse effect on human health and crop production (Vadivoo and Vijayalakshmi,1998). Hence, for assessing the pollution load, an attempt has been made in the present study to estimate the physicochemical characteristics of the tannery effluent. Sludge samples were used for the isolation of metal tolerant bacteria and fungi. Since the study was focussed on the removal of Cr(VI) from tannery effluents, the sludge was not characterised. Plates 5 shows the storage tanks containing the sludge and the effluent.

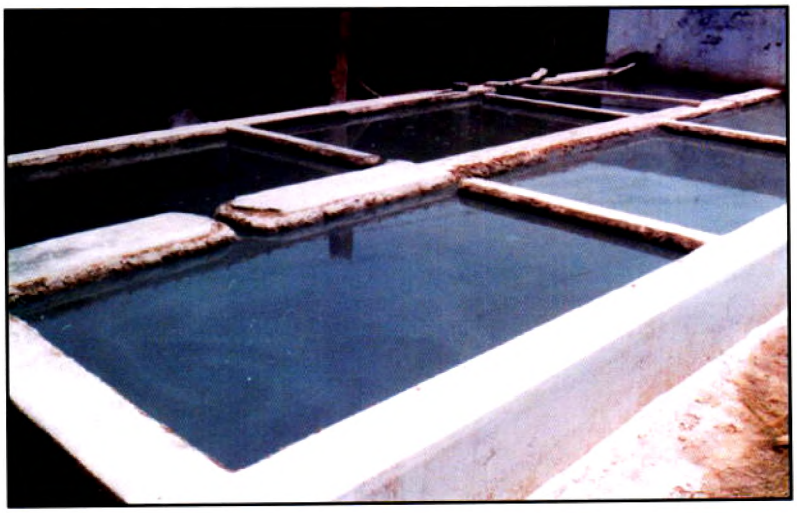
3.1.1 Collection of the tannery effluent samples

About five litres of the untreated tannery effluent samples were collected from the discharge point from a tannery industry at Erode. The effluents were collected in polythene containers. Sludge was collected at a depth of about 30 cm from areas, where the effluent has been discharged over years.

PLATE 5

STORAGE TANKS FOR TANNERY SLUDGE AND EFFLUENT

Sludge



Effluents

3.1.2 Analysis of the physicochemical characteristics of the tannery effluent

The tannery industry is engaged in the treatment of raw pelts of animals to make them suitable and durable for various industrial purposes. Raw hide is limed, unhaired, degreased and pickled before turning into flattering leather. These pre-tanning operations are followed by tanning, dyeing and finishing. It was observed that effluents from liming, tanning and dyeing operations contributed to 90% of the pollution load of the combined effluent (Radha *et al.,* 1995). The leather processing requires large quantities of chemicals like sodium chloride, chromium sulphate, calcium salts, ammonium salts, sodium sulphide, acids, alkalies, fat liquor and organic dyes (Chhonkar *et al.,* 2000). Hence the possible contaminants expected in the tannery effluent are sodium, magnesium, tannin, chromium, chlorides, suspended particles and total dissolved salts.

The effluent samples collected were analysed for selected physicochemical characteristics. The parameters analysed, their methods of analysis, references and the appendices in which these methods are elaborated are given in Table 1.

PHASE II

3.2 ISOLATION AND CHARACTERISATION OF THE BACTERIA AND FUNGI FROM THE TANNERY EFFLUENT AND SLUDGE SAMPLES

Microorganisms play important roles in the environmental fate of toxic metals with a multiplicity of mechanisms effecting transformations between soluble and insoluble forms. These mechanisms are integral components of natural biogeochemical cycles and are of potential for both insitu and exsitu bioremedial treatment processes for solid and liquid wastes (Gadd, 2000). Microorganisms such as fungi, bacteria and algae are the commonly used agents for biosorption of metals (Kowshik and Nazareth, 1999). It has been shown that bioremediation by microorganisms is economically and environmentally superior to chemical treatment (Shakibaie *et al.,* 1999).

TABLE 1

PHYSICOCHEMICAL CHARACTERISTICS OF THE TANNERY EFFLUENT ANALYSED IN THE STUDY AND THEIR METHODS OF ANALYSIS

CHARACTERISTICS	METHOD OF ANALYSIS	REFERENCE	APPENDIX NO.
Colour	Visual	-	-
pH	Using digital pH meter	-	-
Electrical Conductivity	Using conductivity bridge	-	-
Turbidity	Visual	-	-
Total Suspended Solids	Filtration method	APHA, 1998	1
Total Dissolved Solids	- do -	-do-	2
Biochemical Oxygen Demand	Winkler's iodometric method	APHA, 1989	3
Chemical Oxygen Demand	Open reflux titrimetric method	APHA, 1998	4
Chlorides	Titrimetric method	Vogel, 1964	5
Sulphates	Turbidimetric method	APHA, 1998	6
Ammoniacal nitrogen	Titrimetric method		7
Oil and Grease	Soxhlet extraction method		8
Sulphides	Iodometric method		9
Sodium and Potassium	Flame photometric method		10
Calcium	EDTA Titrimetric method		11
Magnesium	By calculation		12
Phenolic compounds	Direct photometric method		13
Total chromium, copper, zinc and iron	Atomic absorption spectrophotometric method		14
Hexavalent chromium	Colorimetric method		15

3.2.1 BACTERIA

3.2.1.1 Isolation of bacteria

100g sludge sample was withdrawn and dispersed in 100 ml of tannery effluent. It was stirred well and homogenised in a shaker. From this, 10g of the sample was withdrawn and dispersed in 100 ml of sterile distilled water and stirred well. The sample was serially diluted till 10^{-4} dilutions were reached. 1.0 ml of the sample was withdrawn and transferred to sterile petriplates. Nutrient agar medium supplemented with 50 mg/l of Cr(VI) was poured onto the petridish and then incubated at 37°C for 48 hours, till colonies of bacteria were developed on the petriplate. The individual colonies were selected and transferred to nutrient agar slants.

Culture medium

Nutrient agar medium was used as the culture medium and all experiments were carried out in this medium. The composition of the nutrient agar media is given below :

Peptone	-	5g
Beef extract	-	5g
NaCl	-	5g
Distilled water	-	1000ml
pH	-	7.0 ± 0.1

For nutrient broth agar alone was omitted.

3.2.1.2 Screening bacterial species for chromium tolerance

The isolated bacterial species were inoculated into the petriplates containing medium amended with increasing concentrations of Cr(VI) ranging from 50 to 250 mg/l. The four bacterial species which were highly tolerant to chromium were isolated and later identified as *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.*

3.2.I.3 Identification of Bacteria

The isolates of bacteria were identified according to Bergey's manual of determinative bacteriology (1984) based on their morphological, cultural and biochemical characteristics following the procedures of Aneja (1996) and Kannan (1996) as given in Appendix - I7.

3.2.1.4 Maintenance of bacterial cultures

The stock cultures were maintained on nutrient agar slants. The organisms were first inoculated in agar slants and incubated for 24 hours and then stored at 5°C in a refrigerator. The cultures were periodically transferred to a fresh agar slant at intervals of 5 months.

3.2.1.5 Assessment of the growth pattern of bacterial isolates

The growth curve of bacterial isolates was determined spectrophotometerically at 600 nm at different incubation periods namely 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 hours following the procedure of Bhattacharya (1986) as given in Appendix - 18.

3.2.1.6 Growth conditions

Cultures were grown at 37°C with shaking at 200 rpm. The experiments were conducted in 250 ml conical flasks containing 100 ml of nutrient broth. For chromium uptake studies, 2% v/v of exponential growing cultures were used (Saxena *et al.,* 2000).

3.2.2 FUNGI

3.2.2.1 Enrichment technique

100 g of sludge sample and 100 ml of tannery effluent were taken in 100 ml beaker. 5.0g of dextrose was added as the nutrient followed by the addition of 50 ml of sterile distilled water. It was covered and incubated for 15 days at room temperature.

3.2.2.2 Isolation of fungi

After enrichment for 15 days, a quantity of 5g sludge sample was withdrawn and dispersed in 100 ml sterile distilled water. It was stirred well and homogenised in a shaker. The sample was serially diluted till 10^{-4} dilutions were reached. 1.0 ml of the sample from each dilution was withdrawn and transferred to sterile petriplates. Potato dextrose agar medium was poured onto the petridish and then incubated at room temperature for 7 days. 1.0 ml of 10 g/l streptomycin sulphate was added to prevent bacterial contaminations. The individual colonies of fungi developed on the petriplate were selected and transferred to potato dextrose agar slants and maintained at room temperature.

3.2.2.3 Culture medium

The isolates of fungi were cultured in potato dextrose agar medium.

Composition of potato dextrose agar (PDA) medium

Agar agar	-	15g
Potato (peeled)	-	250g
Dextrose	-	20g
Distilled water	-	1000 ml
pH	-	6.0

3.2.2.4 Identification of fungal isolates

Fungal hyphae, spores and fruiting structures were mounted on a clean glass slide with lactophenol cotton blue and a cover slip was placed on the mountant. Thus the preparation was observed under the microscope and the isolates of fungi were identified as *Aspergillus terreus, Cladosporium sp. and Colletotrichum sp.* (Gilman, 1967; Aneja, 1996; Freeman and Katan, 1997).

Preparation of lacto phenol cotton blue

Phenol crystals	:	20.0 g
Lactic acid	:	20.0 g
Glycerol	:	40.0 g
Distilled water	:	20.0 ml
Cotton blue dye	:	0.05 g

The distilled water was taken in a beaker and phenol crystals were added to this and warmed in a water bath till the latter dissolved completely. Lactic acid and glycerol followed by cotton blue dye were added to this (Lactic acid is to maintain acid pH; phenol is to dissolve aniline dye; cotton blue is to stain the fungi and glycerol is to provide moisture to the preparation).

3.2.2.5 Screening fungal isolates for chromium tolerance

Potato dextrose agar medium was taken and various concentrations of the stock solutions ranging from 10 to 200 mg/l were added to each petridish before adding the medium, so that the level of heavy metal in each petridish would be in the order of 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 200 mg and 300 mg/l. 20 ml of the medium was added, mixed and allowed to solidify. The fungal cultures were inoculated in the petriplate containing the solidified medium and incubated. Suitable controls were maintained. The highest concentration of heavy metal in which the fungi were able to grow was taken as the tolerance level of chromium for fungus.

3.2.2.6 Maintenance of fungal cultures

The stock cultures were maintained on potato dextorse agar slants. The organisms were first inoculated in agar slants and incubated for a short period and then stored at 5°C in a refrigerator. The cultures were periodically transferred to fresh agar slants at intervals of 3 months.

PHASE III

3.3 CHROMIUM UPTAKE STUDIES USING BACTERIAL ISOLATES

Bioremediation has emerged as an inexpensive solution for heavy metal removal. Microorganisms including bacteria, algae, fungi and yeasts can accumulate heavy metals and related compounds. Both living and dead cell of bacteria, as well as derived or excreted products eg. cell walls,

pigments and polysaccharides are all capable of metal removal from solution (Gupta *et al.,* 2000). Hence it is of interest to study the feasibility of using microbial biomass in place of conventional adsorbents for the removal of heavy metals from the effluents.

Bacterial cells used in this investigation have been cultured in the absence of chromium in order to obtain quantitative estimates of the uptake characteristics of bacterial isolates.

Two experiments were performed. The first was designed to provide quantitative data on the bioaccumulation of Cr(VI) by four bacterial isolates in standard nutrient broth medium. In the other experiment, the ability of the bacteria to accumulate Cr(VI) from the tannery effluents was determined.

3.3.1 Preparation of synthetic chromium solution

28.3g potassium dichromate was dissolved in 100 ml of sterile distilled water so as to give 100 mg of Cr(VI) per ml. The synthetic chromium solution is used for uptake studies using selected bacteria, fungi and agrowaste adsorbents in order to optimise the experimental conditions and to assess the bioremediation effieiency of these agents in chromium uptake.

3.3.2 Chromium uptake experiments with standard medium

For experiments with standard medium, 250 ml Erlenmeyer flasks were prepared with 100 ml sterile nutrient broth. Various volumes of chromium stock solution were added to each flask to give initial Cr(VI) concentrations ranging from 20 to 100 mg/l and 2% v/v exponential growing culture was then added.

All flasks were incubated at 37°C on an orbital shaker at 200 rpm. Samples were removed at the end of the incubation period of 96 hours, centrifuged and supernatants analysed for the residual chromium concentration. The dry weight of the bacterial cells were determined by isolating the cells by

centrifugation at 4000 rpm for 10 minutes at 4°C, washed twice with sterile distilled water and dried at 105°C until constant weight.

Assessment of the influence of various factors on the uptake of chromium

3.3.2.1 Cell age

In order to investigate the influence of cell age on the uptake characteristics of the four selected bacterial isolates, 250 ml Erlenmeyer flasks were prepared with 100 ml of sterile nutrient broth amended with 100 mg/l concentration of Cr(VI). 2% v/v growing cultures were added at the end of 5, 10, 15, 20 and 24 hours of the growth period. All the flasks were incubated at 37°C in an orbital shaker at 200 rpm. Samples were removed at the end of 24 hours, centrifuged and supernatants analysed for the residual chromium concentration.

3.3.2.2 Metal ion concentration

250 ml Erlenmeyer flasks were prepared with 100 ml of sterile nutrient broth. Various volumes of Cr(VI) stock solution were added to each flask to give initial Cr(VI) concentrations ranging from 20 to 100 mg/l and 2% v/v exponential growing culture was then added and the experiments were carried out as mentioned earlier.

3.3.2.3 Tannic acid

In order to determine the influence of tannic acid on chromium uptake, various concentrations (0.01, 0.05, 0.1, 0.5 and 1% w/v) of it was added to the nutrient broth amended with 100 mg/l Cr(VI) prior to the addition of cells and the uptake was monitored as detailed earlier in section 3.3.2.

3.3.2.4 Sodium chloride

To study the effect of sodium chloride on the uptake of chromium, the salt was added aseptically (1, 2, 3, 4 and 5% w/v) to the nutrient broth amended with

100 mg/l Cr(VI) prior to the addition of cells as mentioned earlier and the uptake was determined at regular intervals of time.

3.3.2.5 Metabolic state

A comparison of chromium uptake among dead, metabolically inhibited and resting cells was carried out. 100 mg of bacterial cells were drawn from nutrient broth after 24 hours incubation, pelleted by centrifugation, washed and exposed to sodium azide ($1x10^{-4}M$) for 20 minutes. These cells were washed and used as metabolically inhibited cells for chromium uptake study. Dead cells were prepared by treating 100 mg exponentially growing cells with formaldehyde (10% solution w/v) for 10 minutes. A viable count was made to ensure that the cells were dead. Cells without any treatment served as resting cells. The different categories of cells were suspended in 100 mg/l chromium solution aseptically and chromium uptake was determined at various incubation periods namely 6, 12, 18, 24, 48, 72 and 96 hours.

3.3.2.6 Incubation period

250 ml Erlenmeyer flasks were prepared with 100 ml of sterile nutrient broth contaminated with 100 mg/l Cr(VI) and 2% v/v of exponential growing culture was then added. All the flasks were incubated at 37°C on an orbital shaker at 200 rpm. Samples were removed at 6, 12, 18, 24, 48, 72 and 96 hours. Centrifuged and supernatants were analysed for the residual chromium concentration. Analysis of Cr(VI) and Cr(III) in the supernatant of the culture medium were carried out by colorimetric methods as given in Appendix -15 and 16.

Determination of intracellular chromium

Intracellular chromium was determined for the confirmation of bioaccumulation. The biomass was obtained after 96 hours incubation in 100 ml nutrient broth alone and nutrient broth containing 100 mg/l of chromium. Cells

were isolated by centrifugation at 4000g for 10 minutes at 4°C, washed twice and dried at 105°C until constant weight. The dried biomass was digested with 5 ml nitric acid and brought to the final volume of 10 ml. Total chromium was determined by atomic absorption spectrophotometry and calculated against standard solutions of chromium.

3.3.3 Chromium uptake experiments with tannery effluents

For experiments with tannery effluent, 90 ml of tannery effluent was inoculated with 2% v/v exponential growing culture of bacterial isolates and 10 ml of sterile nutrient broth. Samples were removed after 6, 12, 18, 24, 72 and 96 hours incubation and the chromium concentration was determined.

PHASE IV

3.4 CHROMIUM UPTAKE STUDIES USING FUNGAL ISOLATES

A large number of microorganisms belonging to various groups, viz., bacteria, fungi, yeast, cyanobacteria and algae have been reported to bind a variety of heavy metals to different extents. Among the microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall materials which shows excellent metal binding properties.

Fungi, including yeasts, have received attention in connection with metal biosorption, also because waste fungal biomass arises as a by product from several industrial fermentations (Gupta *et al.,* 2000).

3.4.1 Chromium uptake by living cells

3.4.1.1 Assessment of the effect of different dilutions of the tannery effluent on chromium uptake and growth by selected fungal isolates

The tannery effluent was diluted in the ratio 1:25, 1:50, 1:75 and 1:100. 100 ml quantities of diluted samples were taken in 250ml Erlenmeyer flasks and sterilized. 1% carbon source in the form of glucose was added. Each flask was

inoculated with 5 mm diameter disc taken from the periphery of 7 day old culture plate. The flasks were then incubated at 28°C for 7 days after which the produced mycelial mats were collected by filtration. They were washed several times with distilled water and then dried in oven at 75°C till constant weight. The total chromium present in the filtrate was determined to find out the efficiency of selected fungal isolates in the removal of chromium from the tannery effluents.

3.4.1.2 Assessment of the effect of temperature on chromium uptake and growth by selected fungal isolates

100 ml quantities of diluted synthetic solution were taken in 250 ml Erlenmeyer flasks and then sterilised. 1% carbon source in the form of glucose was added. Each flask was inoculated with 5 mm diameter disc taken from the periphery of 7 day old culture plate. The flasks were then incubated at varying temperatures (20°C, 25°C, 30°C, 35°C and 40°C) for 7 days and filtered. The growth was measured in terms of biomass. Total chromium content was determined in the filtrate.

3.4.1.3 Assessment of pH on chromium uptake and growth by selected fungal isolates

To check the pH optima for the biosorption process, 5 mm diameter disc taken from the periphery of 7 day old culture plate was placed in 250 ml Erlenmeyer flasks containing 100 ml quantities of stertile diluted synthetic solution with 1 % glucose with pH of 3.0, 4.0, 5.0, 6.0 and 7.0. The flasks were then incubated at 28°C for 7 days and filtered. The growth was measured in terms of biomass. Total chromium content was determined in the filtrate.

3.4.2 Chromium uptake by dead cells

3.4.2.1 Preparation of the biomass

Fungal cells for the uptake studies were obtained by culturing in liquid medium at 28°C for 70 hours. The cells were separated from the broth by filtration, washed with deionized water, resuspended and washed again. The biomass was dried for 10 hours at 60°C. The product was ground in a mortar and pestle.

3.4.2.2 Assessment of the influence of various factors on chromium uptake

Series of flasks were prepared with known volumes of serial dilutions of standard chromium solution. The pH was adjusted with 1N HNO_3 and 1N NaOH. Weighed quantities of the dried biomass were added and the flasks were agitated at 200 rpm at 28°C for 18 hours. The biomass was removed by filtration through a 0.45 μm millipore membrane filter and the filtrates were analysed for total chromium concentrations using atomic absorption spectrophotometer.

The fungal biosorption studies were carried out under varying experimental conditions namely pH (3.0, 4.0, 5.0, 6.0 and 7.0), intital metal concentration (10, 25, 50, 75 and 100 mg/l), biomass concentration (1.0, 2.0, 3.0 and 4.0 g/100 ml) and temperature (20°C, 25°C, 30°C and 35°C).

3.4.3 Studies on the effect of chromium concentration on fungal metabolism

3.4.3.1 Culturing of fungal isolates in media amended with different concentrations of chromium

250 ml Erlenmeyer flasks were prepared with 100 ml of sterile liquid medium. Various volumes of stock solutions of chromium were added to each flask to give initial chromium concentration values ranging from 10 to 100 mg/l. Each flask was inoculated with 5 mm diameter disc taken from the periphery of 7 day old culture plate. The flasks were then incubated at 28°C for 7 days after which the produced mycelial mats were collected by filtration and washed several times with distilled water, then dried in oven at 75°C till constant weight.

3.4.3.2 Analysis of mycelia to study the effect of different concentrations of chromium on fungal metabolism

The harvested fungal mats were thoroughly washed several times with distilled water, then dried in an oven at 75°C till constant weight to estimate the amount of biomass. The biomass was analysed for selected biochemical parameters namely protein, phosphorus, DNA, RNA and total carbohydrates.

The details of the methods of analysis of these parameters, the references for the methodology and the appendices in which they are given are presented in Table 2.

TABLE 2

BIOCHEMICAL PARAMETERS ANALYSED IN THE BIOMASS AND CELL WALL OF SELECTED FUNGAL ISOLATES

Biochemical parameter	Method of analysis	Reference	Appendix No.
Total Carbohydrates	Colorimetric method	Hodge and Hofreiter, 1962	19
Protein	-do-	Lowry *et al.*, 1951	20
Phosphorus	-do-	Fiske and Subbarow , 1925	21
*DNA isolation	Precipitation method	Marmur, 1961	22
*DNA estimation	Colorimetric method	Giles and Meyer, 1965	23
*RNA isolation	Precipitation method	Brawerman, 1974	24
*RNA estimation	Colorimetric method	Lin and Schjeide, 1969	25

* Analysed only in the biomass

3.4.4 Studies on the effect of chromium on cell wall composition of fungal isolates

3.4.4.1 Isolation of cell wall

The fungal isolates were cultured in media amended with different concentrations of chromium as detailed earlier in section 3.4.3.1. After 7 days of culture, the mycelia were collected by filtration, washed extensively with water and disrupted in a 50mM Tris-HCl, pH 7.5 buffer containing 50mM EDTA and 1mM phenyl methyl sulfonyl fluoride in a homogeniser in the presence of 1 mm diameter glass beads at 4°C. The disrupted mycelial suspension was centrifuged

(8000 x g for 10 minutes) and the cell wall pellet was washed thrice with the same buffer dried at room temperature for 16 hours (Fontaine *et al.,* 2000).

3.4.4.2 Analysis of the cell wall of fungal isolates to study the effect of chromium on cell wall composition

The cell walls isolated from the three selected fungal isolates treated with different concentrations of chromium were analysed for total carbohydrates, protein and phosphorus. The details of analysis of these biochemical parameters are shown in Table 2.

PHASE V

3.5 CHROMIUM UPTAKE STUDIES USING SELECTED AGROWASTE ADSORBENTS

Adsorption is one of the most useful techniques and activated carbons have been suggested as adsorbents for the treatment of industrial wastewaters. Commercially available activated carbons are expensive. Therefore there is a need to produce low cost activated carbons that can be applied effectively for pollution control (Singh and Srivastava, 2000). The production of low cost activated carbons from agricultural wastes like coconut shell, rice husk, groundnut husk and tamarind nut can act as alternates to commercial activated carbons (Rengaraj *et al.,* 2000).

3.5.1 Selection of agrowaste adsorbents for the removal of chromium

Sulphonated and nitrated coconut shell carbon, coir pith carbon, saw dust carbon, modified sugarcane bagasse and groundnut shell carbon were used as adsorbents in the present study.

3.5.2 Preparation of the adsorbents

3.5.2.1 Nitrated and sulphonated coconut shell carbon

Coconut shells, important kitchen wastes were dried in the sun for a week. All the outer fibres were removed and the inside of the shells were cleaned and

dried. The shells were burnt in a pit without allowing too much access of air allowed to smoulder. The resulting carbonaceous matter was ground to an average particle size of 0.35 mm. 5 g of coconut shells gave about 1.3g of charcoal.

Nitrated coconut shell carbon was prepared by treating the powdered carbon with 0.75 N nitric acid overnight. The carbon was washed repeatedly till there was no more acid water and then was air dried. The carbon was termed as nitrated carbon.

In the same way as above, another portion of coconut shell carbon was treated with 0.75N sulphuric acid and the final washed and dried carbon was called sulphonated carbon (Krupadam and Rao, 1997). Nitration and sulphonation were done to enhance adsorption.

3.5.2.2 Coirpith carbon

Coirpith was collected from a local coir factory and washed with distilled water repeatedly to remove the water soluble impurities and surface adhered particles and then dried at 100°C to get rid of moisture and other volatile impurities. A pseudo activated carbon was prepared by treating 20g of coir pith with 20ml of concentrated sulphuric acid. It was then heated at 150°C for about 12 hours. The carbonised material was washed with distilled water to remove sulphate ions and dried at 100°C. It was sieved to get - 80 +230 mesh size particles.

3.5.2.3 Saw dust carbon, modified sugarcane bagasee and groundnut shell carbon

The saw dust, sugarcane bagasse and groundnut shells were collected and dried. They were ground in order to increase the surface area and sieved to -80 +30 mesh size. 2.0 g of each powder was treated with 20 ml of 0.2 N sulphuric acid and 5 ml of 39% formaldehyde and the whole mixture was stirred for 6 hours at 50°C and then filtered. The residue was washed several times with distilled water and dried at 50°C in an electric oven.

3.5.3 Characteristics of the adsorbents

The physical characteristics of the adsorbents analysed included bulk density, moisture, ash content and volatile matter. They were analysed as per standard methods given in Appendix - 26.

3.5.4 Batch studies with synthetic chromium solution and tannery effluent

Batch adsorption studies were carried out using 250 ml stoppered conical flasks containing 100 ml of synthetic chromium solution (100mg/l) with 2.0 g of each adsorbent. The initial pH of the solution was adjusted using 0.1 M NaOH and 0.1 M HCl. The flasks were shaken for 3 hours at 30°C in a temperature controlled shaking machine. Appropriate control flasks without adsorbent were also maintained. After equilibrium period, the contents of the flasks were filtered using Whatman No. 4 filter paper and the amount of chromium present in the filtrate was determined by diphenyl carbazide method (APHA, 1998) as given in Appendix - 15.

These adsorption studies were carried out with synthetic chromium solution under varying experimental conditions namely pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0), adsorbent dose (0.5 g, 1.0 g 1.5 g and 2.0 g), initial chromium concentration (20, 40, 60, 80 and 100 mg/l) and contact time (30, 60, 90, 120 and 150 minutes).

The adsorption studies were also carried out with undiluted tannery effluent under optimum pH (4.0), adsorbent dose (20g/l) and contact time (3 hours) with the selected agrowaste adsorbents.

3.5.5 Adsorption isotherm

The results of the adsorption of Cr(VI) on selected agrowaste adsorbents under varying experimental conditions were studied using adsorption isotherm. There exists a close relationship between the adsorbate concentration and the amount unadsorbed at a constant temperature. It is generally given the name adsorption isotherm. The study of adsorption isotherm in any adsorption

Results and Discussion

process is helpful in determining the adsorption capacity of the selected material under varying experimental conditions and thus helps in selecting the adsorbent for the removal of any metal ion. Mathematically it is represented by the empirical relationships known as Freundlich and Langmuir adsorption isotherms (Bhalke *et al.*,1999).

The Freundlich adsorption isotherm is given as follows :

$$\log\frac{x}{m} = \log K + \frac{1}{n}\log C_e$$

where, x/m is the amount of Cr(VI) adsorbed at equilibrium per unit weight of adsorbent (mg/g), C_e is the equilibrium concentration (mg/l) of sorbate, K is a constant which is a measure of the adsorption capacity and $\frac{1}{n}$ is a measure of adsorption intensity (Siddiqui *et al.*, 1999).

The Langmuir adsorption isotherm is in the form :

$$\frac{1}{x/m} = \frac{1}{q_o} + \left(\frac{1}{q_o b}\right)\frac{1}{C_e}$$

where, C_e is the equilibrium concentration (mg/l) of sorbate and x/m is the amount of Cr(VI) adsorbed at equilibrium per unit weight of adsorbent (mg/g). q_o and b are Langmuir constants related to the adsorption capacity and equilibrium constant respectively (Swamy *et al.*, 1998).

4.0 RESULTS AND DISCUSSION

The present study was aimed to develop processes for the removal of chromium from tannery effluent using bacteria and fungi isolated from tannery sludge and adsorbents prepared from agricultural and industrial wastes.

Four species of bacteria namely *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.* were isolated from the tannery effluent and tannery sludge samples and their efficiency in the uptake and bioaccumulation of chromium under various conditions was assessed. Similarly, three species of fungal isolates namely *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* were studied for their chromium uptake capacities. The adsorbents namely nitrated and sulphonated coconut shell carbons, saw dust carbon, coir pith carbon, modified sugarcane bagasse and groundnut shell carbon were also studied for their chromium sorption capacities under different conditions.

The results of the study are discussed under the following headings:

PHASE I

4.1 CHARACTERISATION OF TANNERY EFFLUENT

4.1.1 Physical characteristics of the tannery effluent

4.1.2 Chemical characteristics of the tannery effluent

4.1.3 Heavy metal contents in the tannery effluent

PHASE II

4.2 CHARACTERISATION OF SELECTED BACTERIAL AND FUNGAL ISOLATES

4.2.1 Morphological and biochemical characterisation of bacterial isolates

4.2.2 Growth pattern of selected bacterial isolates

4.2.3 Screening of selected bacterial isolates for Cr(VI) tolerance

4.2.4 Morphological characteristics of selected fungal isolates

4.2.5. Screening of selected fungal isolates for Cr(VI) tolerance

PHASE III

4.3 UPTAKE AND BIOACCUMULATION STUDIES OF CHROMIUM USING BACTERIAL ISOLATES

4.3.1 Influence of cell age on Cr(VI) uptake

4.3.2 Effect of metal ion concentration on Cr(VI) uptake

4.3.3 Effect of tannic acid on Cr(VI) uptake

4.3.4 Effect of sodium chloride on Cr(VI) uptake

4.3.5 Comparison of Cr(VI) uptake by resting, dead and metabolically inhibited cells

4.3.6 Bioaccumulation and Cr(VI) reduction

4.3.7 Treatment of tannery effluents using the selected bacterial isolates

PHASE IV

4.4 CHROMIUM UPTAKE STUDIES USING SELECTED ISOLATES OF FUNGI

4.4.1 Chromium uptake by growing cells

4.4.2 Chromium uptake by dead cells

4.4.3 Effect of chromium on metabolic activity and cell wall composition of the selected fungal isolates

PHASE V

4.5 CHROMIUM UPTAKE STUDIES USING SELECTED AGROWASTE ADSORBENTS

4.5.1 Characteristics of the selected agrowaste adsorbents

4.5.2 Effect of pH on the removal of Cr(VI) using selected agrowaste adsorbents

4.5.3 Effect of adsorbent dose on the removal of Cr(VI) using selected agrowaste adsorbents

4.5.4 Effect of initial Cr(VI) concentration and contact time in the removal of Cr(VI) using selected agrowaste adsorbents

4.5.5 Adsorption isotherm

4.5.6 Adsorption of Cr(VI) from tannery effluent using the selected agrowaste adsorbents

PHASE I

4.1 CHARACTERISATION OF TANNERY EFFLUENT

Tanneries consume a huge amount of water and only a small fraction of the same is used by the products and lost by evaporation. The rest finds its way into the water courses as wastewater. Pollution potential of these wastes arises from high concentration of organic and inorganic solids and also some of the chemicals used during the tanning. Hence, it is necessary to characterise the physicochemical properties of tannery effluent in order to assess the toxicity caused by this industry (Mariappan and Rajan, 2002).

4.1.1 Physical characteristics of the tannery effluent

The results of the physical parameters analysed in tannery effluent samples are given in Table 3.

TABLE 3

PHYSICAL CHARACTERISTICS OF THE TANNERY EFFLUENT

Parameters	Effluent sample	# IS tolerance limit IS - 2490-1981
Colour	Deep brown	NM
Odour	Pungent	NM
Turbidity	Turbid	NM
pH	7.30	5.5 - 9.0
Electrical conductivity (μmhos/cm)	10340	NM
Total suspended solids (mg/l)	576	100
Total dissolved solids (mg/l)	8336	2100

Values are mean of triplicates.
NM - Not mentioned.
- Tolerance limits for industrial effluents discharged into inland surface waters prescribed by the Bureau of Indian Standards.

Colour, odour and turbidity

The tannery effluent was found to have strong colour and unpleasant odour and it was turbid.

Observation of colour is the simplest test to determine the effectiveness of treatment of an effluent. It serves as a guide in deciding the quantity of chemicals to be used for the removal of colour and ensures economical treatment (Manivasakam, 1997). Industrial wastes usually have their characteristic odours due to the presence of a large variety of contaminants. Chlorine, added for disinfection of waters, may combine with certain impurities like phenolic compounds to produce highly offensive odours (Goel, 1997). The strong colour and unpleasant odour of tannery effluent was also reported by Dadhich *et al.* (2002) and Mariappan and Rajan (2002). The colour of the effluent might be due to the use of chemicals in tanning and dyeing processes.

Turbidity is yet another physical parameter which influences the quality of water. Turbidity in water might be due to the presence of finely divided organic and inorganic matter in suspended and colloidal forms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 1998). The turbidity of the tannery effluent might be due to the discharge of large amounts of solids that include chlorides, calcium, magnesium, sulphates and sulphides.

pH

pH of the tannery effluent (7.3) was found to be within the tolerance limit of 5.5 - 9.0, as prescribed by the Bureau of Indian Standards for the discharge of wastewater into inland surface waters.

pH is a measure of hydrogen ion concentration or more precisely the hydrogen ion activity. pH is one of the most important parameters, as it indicates instantaneously the acid or alkaline condition of an effluent. Further, all the treatment processes are based on pH values only (Manivasakam, 1997).

The pH of similar tannery effluent samples was reported to be between 7.5 to 10 (Dadhich *et al.*, 2002; Sujatha and Gupta, 1996; Verma and Verma, 1995).

Sujatha and Gupta (1996) reported that pH at the different tanning processes were changing variably. It was found to be high at liming process (12.75) and least at chrome tanning process (3.0). However in the present study, pH was recorded in the combined effluent sample collected at the site of discharge.

Electrical conductivity (EC)

Electrical conductivity is the ability of a substance to conduct the electrical current. Water becomes a conductor of electrical current when substances are dissolved in it and the conductivity is proportional to the amount of dissolved substances which act as conductors (Michael, 1984).

The electrical conductivity recorded in tannery effluent was 10340 μmhos/cm which indicates that tannery effluents have more ionic substances. Many researchers have recorded the EC in tannery effluent samples between 5800-14000 μmhos/cm (Chandra, 2001; Chhonkar *et al.*, 2000; Mariappan and Rajan, 2002).

Total suspended solids (TSS)

The observations made in the present study showed that the level of total suspended solids in the tannery effluent (576 mg/l) was much above the limits prescribed by the Bureau of Indian Standards (100 mg/l) for the discharge of effluents into the inland surface waters.

The undissolved matter present in water and wastewater is usually referred to as suspended solids. It is one of the valuable parameters in judging the pollution potential of an effluent, pollution load on receiving streams

and also to decide the efficiency of treatment units. The main effect of the suspended matter in aquatic systems is the limiting of light by increasing turbidity (Abel, 1989).

Thorat and Wagh (2000) reported a high TSS (3150 mg/l) in raw tannery effluent samples. Similarly, Radha *et al.* (1995) and Prakash (2001) have indicated the TSS of 12,960 mg/l and 7700 mg/l respectively in similar tannery effluent samples.

Total dissolved solids (TDS)

The amount of total dissolved solids present in tannery effluent was found to be 8336 mg/l which is much greater than the tolerance limits (2100 mg/l) prescribed by the Bureau of Indian Standards.

Determination of dissolved solids particularly represents the inorganic mineral matter content (Manivasakam, 1997). The effluents from chrome tanning (as in the present study) generally contain much higher concentrations of dissolved salts and suspended solids compared to those from vegetable tanning (Chhonkar *et al.*, 2000). Dadhich *et al.* (2002) reported a high level of total dissolved solids (29000 mg/l) in water samples near tannery industries.

Sujatha and Gupta (1996) have reported the value of 4000 mg/l of dissolved soilds in composite tannery effluent eventhough the effluent from other processes namely soaking, liming, deliming, picking, chrome tanning, dying and fat liquoring were very high between 5200 to 76800 mg/l.

4.1.2 Chemical characteristics of the tannery effluent

Table 4 shows the levels of the chemical parameters studied in tannery effluent.

TABLE 4

CHEMICAL CHARACTERISTICS OF THE TANNERY EFFLUENT

Parameter (mg/l)	Effluent sample	# IS tolerance limit IS - 2490-1981
Biochemical oxygen demand	1200	30
Chemical oxygen demand	2055	250
Anions		
Chlorides	2458	1000
Sulphates	2743	1000
Sulphides	8.0	2.0
Cations		
Calcium	86.4	NM
Magnesium	40.2	NM
Sodium	106	NM
Potassium	256.8	NM
Ammoniacal Nitrogen	88.7	50
Oil and grease	8	10
Phenolic compounds	0.457	1.0

Values are mean of triplicates
NM - Not mentioned
- Tolerance limits for industrial effluents discharged into inland surface waters prescribed by the Bureau of Indian Standards

Biochemical oxygen demand (BOD)

It is evident from the table that the mean BOD level in the tannery effluent was 40 times more than the tolerance limit.

BOD is taken as an indirect measure of water quality. It is infact a measure of the amount of oxygen required by microbes while stabilising decomposable organic matter (Sharma, 1997), Thus, the BOD values can be used as a measure of waste strength and also as an indicator of the degree of pollution. The BOD values are very useful in process design and loading calculation as well as the measure of efficiency of water treatment plants. The BOD value is also useful in stream pollution control management and in evaluating the self purificatiion capacity of a waterbody (Agrawal, 1999).

A number of workers have reported high levels of BOD in tannery effluents between 600-1200 mg/l (Radha *et al.*, 1995; Thorat and Wagh, 2000; Kandasamy and Santhaguru, 1994; Chhonkar *et al.*, 2000; Prakash. 2001).

Chemical oxygen demand (COD)

The chemical oxygen demand in the tannery effluent was found to be 2055 mg/l which is much above the permissible limits (250mg/l) set by Bureau of Indian Standards for the discharge of effluents in inland surface waters.

COD is often used as a measurement of pollutants in wastewater and natural waters. Chemical oxygen demand is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions (APHA, 1998).

In industrial practice, wastewater obtained is likely to contain a number of organic as well as inorganic substances including products, intermediates, byproducts and unreacted raw materials. The detailed analysis of such wastewater is complex primarily because of multivariants. It was therefore considered appropriate to take COD as the measure of all the organic solutes present in the wastewater sample. Considering the parameters specified by the statutory agencies for the treatment and disposal of wastewater, it is felt that COD and colour reflect the practical aspects of wastewater treatment on the industrial scale (Rathi and Puranik, 2002).

A high COD in tannery effluents of 4300 mg/l, 6740 mg/l, 792 mg/l and 21,000 mg/l were reported by Chandra (2001), Prakash (2001), Dadhich *et al.* (2002) and Thorat and Wagh (2000) respectively.

Anions - chlorides, sulphates and sulphides

Table 4 indicates the level of chlorides, sulphates and sulphides presented in the tannery effluent.

The concentration of chlorides were high (2458 mg/l) above the tolerance limit (1000 mg/l). Generally, chloride at low concentration is not harmful. However, fruit trees were reported to be affected due to chloride toxicity (Sujatha and Gupta, 1996).

Dadhich *et al.* (2002) have reported that the concentration of chloride was high (221- 405 mg/l) in river samples where the tannery effluents are discharged. The underground water samples had the chloride concentration of 295, 310, 272 and 240 mg/l respectively at various distances from the tannery units exceeding the limits of 250 mg/l.

Tannery effluent had excessive chloride content and when it was discharged into a river, it adversely affected the quality of water. Discharge of tannery effluents on land adversely affected ground water due to the presence of chlorides, chromium, boron and arsenic (Dadhich *et al.*, 2002).

High chloride content in similar effluents were reported by Mariappan and Rajan (2002), Dadhich *et al.* (2002) and Verma and Verma (1995) which were 9743, 590 and 2500 mg/l respectively.

Sulphate is yet another parameter that has shown its contamination reporting 2743 mg/l as against 1000mg/l given by Indian Standards for its discharge into the inland surface water. A high amount of sulphates (1122 mg/l) was reported by Thorat and Wagh (2000), in a similar tannery effluent.

The concentration of sulphides was found to be high (8.0 mg/l) which also exceeded the tolerance limit (2.0 mg/l) prescribed by the Bureau of Indian Standards. The high concentration of sulphide in the effluent might be due to the liming process where sodium sulphide (for 4 cubic meter float - 75 kg sodium sulphide and 60 kg lime) is used. Hydrated lime increases the pH of the solution which leads to swelling of skins and subsequently loosening of all unwanted hairs (Mazumder *et al.*, 1998). The soluble sulphides are considered to be highly toxic to plants and soil microorganisms (Dadhich *et al.*, 2002).

Cations - calcium, magnesium, sodium and potassium

The levels of calcium and magnesium (86.4 and 40.2 mg/l) recorded in the tannery effluent samples were less than the reports of Dadhich *et al.* (2002) (360 and 120 mg/l) in a similar tannery effluents. The tannery effluent contains fairly good amount of calcium and magnesium because lime is used for loosening the hair (Thorat and Wagh, 2000).

In the present study, sodium and potassium levels (106 and 256.8 mg/l respectively) in the tannery effluent were comparable to those given by Mariappan and Rajan (2002) (1280 and 40 mg/l respectively) in a similar tannery effluent.

Ammoniacal nitrogen

It is evident from Table 4 that the mean ammoniacal nitrogen level in the tannery effluent (88.7 mg/l) was higher than the tolerance limit (50 mg/l) prescribed by Indian Standards Institution.

In polluted waters, ammonia generally arises from the aerobic and anaerobic decomposition of the nitrogenous organic matter. It is yet another parameter to assess the degree of pollution (Manivasakam, 1997).

Oil and grease

It is seen from the values presented in Table 4 that the oil and grease contents in the tannery effluent (8 mg/l) was slightly less than the permissible limit (10 mg/l).

Oil is a natural substance, extracted usually from the upper strata of the earth and commonly called petroleum. Even though there is no comprehensive definition for oil, the oily substances have several properties in common namely lighter than water, immiscible with water, flammable, spread on water forming slick and generally liquid at room temperature (Goel, 1997). In industries, oil is applied as lubricating medium in the machineries.

In effluents collected from similar tannery industries, Mariappan and Rajan (2002) have reported the level of oil and grease (0.016 mg/l) much lesser than the permissible limit whereas Thorat and Wagh (2000) reported a much higher level (12.0 mg/l) above the tolerance limit

Phenolic compounds

The phenolic compounds in the tannery effluent (0.457 mg/l) was lower than the tolerance limit (1.0 mg/l) prescribed by Bureau of Indian Standards for the discharge of effluents into the inland surface waters.

Phenolic compounds, collectively referred as phenols are hydroxy derivatives of benzene or its condensed nuclei. They impart a characteristic objectionable odour to receiving waters. They are well known disinfectants and hence they resist biological activity and are toxic to fish (Manivasakam, 1997). Chandra (2001) observed that the phenolic compounds in a similar tannery effluent (25 mg/l) was much above the tolerance limit.

4.1.3 Heavy metal contents in the tannery effluent

Table 5 depicts the mean level of total chromium, hexavalent chromium, copper, iron and zinc in the tannery effluent samples.

TABLE 5

HEAVY METAL CONTENTS IN THE TANNERY EFFLUENT

Parameter (mg/l)	Effluent sample	# IS tolerance limit IS-2490-1981
Total chromium	38.2	2.0
Hexavalent chromium	12.6	0.1
Copper	24.7	3.0
Iron	56.9	NM
Zinc	2.0	5

Values are mean of triplicates
NM - Not mentioned
- Tolerance limits for industrial effluents discharged into inland surface waters prescribed by the Bureau of Indian Standards.

Tanning industries, along with other inorganic constituents, contain appreciable amount of heavy metals which pollute both water and soil, the two important components of the environment (Sharma *et al.*, 1996).

Total and hexavalent chromium

It is observed from the table that the total chromium and hexavalent chromium contents of 38.2 and 12.6 mg/l respectively were present in the tannery effluent samples whereas the tolerance limits prescribed by Bureau of Indian Standards for the discharge of effluents into inland surface waters are 2.0 and 0.1 mg/l respectively. Hence the removal of 94.7% of total chromium and 99.2% of hexavalent chromium has to be perfomed before discharging such effluents.

Chandra (2001) observed the presence of 154 mg/l of total chromium in an effluent collected from disposal site of a similar tannery industry. Radha *et al.* (1995) have reported that the sectional effluent from chrome tanning section consisted of 500 mg/l of chromium.

The analysis of tannery effluents for the presence of chromium was carried out by many other researchers and the values were reported to be 2.8 mg/l (Verma and Verma, 1995), 0.4 mg/l (Sharma *et al.*, 1996) and 2.0 mg/l (Thorat and Wagh, 2000).

Copper, iron and zinc

The levels of copper (24.7 mg/l) in the tannery effluent was much higher than the tolerance limit (3.0 mg/l). The iron content in the tannery effluent was found to be 56.9 mg/l. The level of zinc was less (2.0 mg/l) as compared to the tolerance limit (5.0 mg/l).

Chandra (2001) has recorded the iron and copper contents to be 27.3 and 0.64 mg/l respectively in a similar tannery effluent.

Toxic metal ions present in industrial effluent lead to considerable deterioration of ecosystem. The proper effluent treatment approach has to be realised for the separation and the recovery of such metal ions from the wastewater (Gupta and Anjum, 2002).

PHASE II

4.2 CHARACTERISATION OF SELECTED BACTERIAL AND FUNGAL ISOLATES

Bacteria and fungi which were tolerant to chromium were isolated from the tannery effluent and sludge samples for the bioremediation of chromium from tannery effluents.

4.2.1 Morphological and biochemical characterisation of bacterial isolates

The morphological and biochemical characteristics of selected bacterial isolates are depicted in Table 6.

TABLE 6

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE SELECTED BACTERIAL ISOLATES

Parameter	Characteristics			
	Isolate 1	**Isolate 2**	**Isolate 3**	**Isolate 4**
Shape (simple staining)	Rod	rod	rod	spherical
Size (μm)	1.5-3.0 μm	1.0-3.0 μm	1-1.6 μm	1.0 μm
Colour	white	white	white	white
Motility	motile	motile	motile	non motile
Gram staining	negative	negative	positive	positive
Carbohydrate fermentation test				
Lactose	-	-	-	A
Dextrose	-	AG	A	A
Sucrose	-	-	A	A
Indole test	-	+	-	-
Methyl red test	-	+	-	+
Voges proskaur test	-	-	+	+
Citrate test	+	-	-	-
Nitrate test	+	+	+	+
Urease test	-	+	-	-
Catalase test	+	+	-	+
Oxidase test	-	-	+	-
Isolate identified as	*Pseudo - monas sp.*	*Proteus sp.*	*Bacillus sp.*	*Staphylo- coccus sp.*

+ = Positive A = Acid
- = Negative AG = Acid and Gas

From the table it is seen that isolate 1 was a gram negative, rod shaped and motile bacterium with white colonies on nutrient agar medium. The size of the bacterium was found to be between 1.5 and 3.0 μm. The biochemical characterisation of isolate 1 showed negative results for carbohydrate

fermentation, indole production, methyl red, voges proskaur, urease and oxidase tests. However, citrate, nitrate and catalase tests were positive. Based on the morphology and biochemical tests, isolate 1 was identified at the genus level as *Pseudomonas sp.*

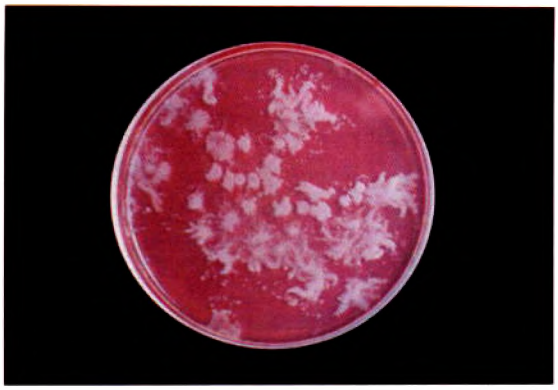
Isolate 2 was found to be a rod shaped, gram negative and motile bacterium with the size ranging from 1.0 to 3.0 μm. White colonies were produced on nutrient agar medium. The biochemical characterisation of isolate 2 showed negative result for voges proskaur, citrate and oxidase tests whereas indole, methyl red, nitrate, urease and catalase tests were positive. In the carbohydrate fermentation test, the isolate 2 produced both acid and gas with dextrose but no reaction was noticed with lactose and sucrose as substrates. Based on these morphological and biochemical tests, isolate 2 was identified as *Proteus sp.*

It is evident from the table that isolate 3 was a rod shaped, gram positive motile bacterium with the size ranging from 1 to 1.6 μm. White colonies were produced on nutrient agar medium. Biochemical characterisation indicated a negative result for indole, methyl red, citrate, urease and catalase whereas positive tests were noticed with voges proskaur, nitrate and oxidase tests. The isolate 3 was capable of fermenting both dextrose and sucrose with the production of acid whereas lactose was not fermented. Based on these morphological and biochemical tests, the isolate 3 was identified as *Bacillus sp*

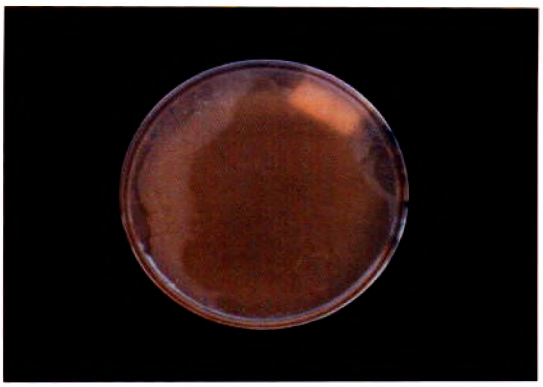
The morphological features of isolate 4 revealed that it was a spherical shaped, gram positive, non motile bacterium of size 1.0 μm. White colonies were formed on nutrient agar medium. The biochemical characterisation of isolate 4 showed negative results for indole, citrate, urease and oxidase tests. However, methyl red, voges proskaur, nitrate and catalase tests were positive. Based on the morphological and biochemical tests, the isolate 4 was identified as *Staphylococcus sp.* Plate 6 shows the selected bacterial isolates.

PLATE 6

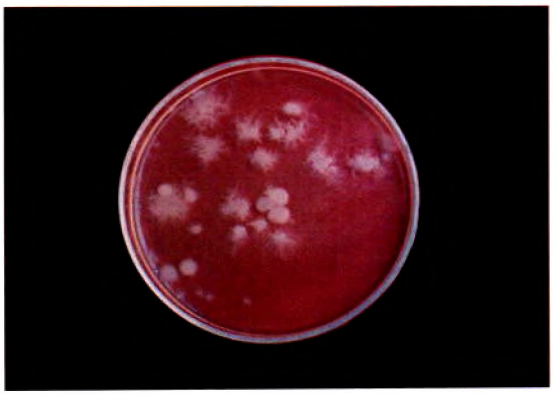
SELECTED BACTERIAL SPECIES ISOLATED FROM TANNERY EFFLUENT AND SLUDGE SAMPLES



Pseudomonas sp.



Proteus sp.

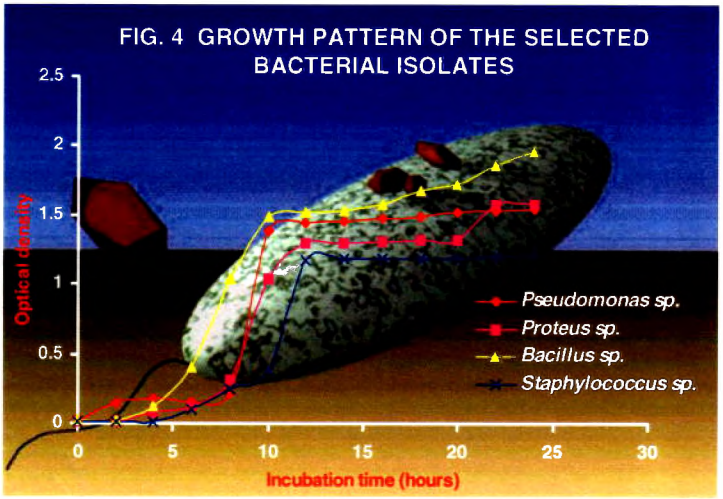


Bacillus sp.

Staphylococcus sp.

4.2.2 Growth pattern of selected bacterial isolates

The growth pattern of selected bacterial isolates was assessed in order to find out the duration of exponential growth phase of each culture. The data are depicted in Figure 4.



It was evidenced from the figure that the duration of exponential growth phase was 8 -10 hours for *Pseudomonas sp., Proteus sp.* and *Bacillus sp.* The exponential growth phase for *Staphylococcus sp.* was found to be between 10 and 12 hours.

4.2.3 Screening of selected bacterial isolates for Cr(VI) tolerance

In order to assess the tolerance level of selected bacterial isolates for Cr(VI), the bacterial isolates were inoculated in a medium amended with different concentrations of Cr(VI) and were observed for their growth.

The growth of the selected bacterial isolates recorded with different concentrations of Cr(VI) permitting growth is presented in Table 7.

From the table, it was evident that Cr(VI) upto 200 mg/l permitted the growth of *Pseudomonas sp.,* upto 160 mg/l permitted the growth of *Proteus sp.,* upto 300 mg/l permitted the growth of *Bacillus sp,* and upto 400 mg/l permitted the growth of *Staphylococcus sp.* Therefore, among all the isolates of bacteria, the *Staphylococcus sp.* was found to be tolerant to the highest level of Cr(VI) of 400 mg/l, which was followed by *Bacillus sp., Pseudomonas sp.* and *Proteus sp.*

TABLE 7

GROWTH OF SELECTED BACTERIAL ISOLATES IN VARIOUS CONCENTRATIONS OF Cr(VI)

Concentration of Cr(VI) mg/l	*Pseudomonas sp.*	*Proteus sp.*	*Bacillus sp.*	*Staphylococcus sp.*
20	+	+	+	+
40	+	+	+	+
60	+	+	+	+
80	+	+	+	+
100	+	+	+	+
120	+	+	+	+
140	+	+	+	+
160	+	+	+	+
180	+	-	+	+
200	+	-	+	+
300	-	-	+	+
400	-	-	-	+
500	-	-	-	-

\+ - Growth
\- - No growth

Nair and Krishnamurthi (1991) demonstrated that the *Pseudomonas aerugenosa* isolated from tannery effluent could tolerate 80 to 100 ppm of trivalent chromium in liquid cultures and upto 100 to 200 ppm of hexavalent chromium in plate count agar. A chromate resistant bacterium *Bacillus circulans* was isolated by Srinath *et al.*(2002) which was able to accumulate 35.5 mg Cr g^{-1} dry weight. Viti *et al.* (2003) isolated and characterised a *Pseudomonas sp.* from chromium contaminated soil which was resistant to high concentrations of chromate.

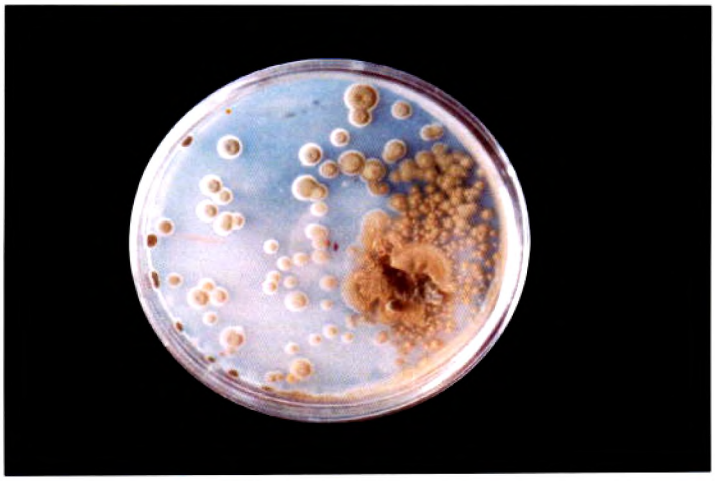
4.2.4 Morphological characteristics of selected fungal isolates

Three selected fungal isolates were identified as *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* (Plate 7) based on the observations of

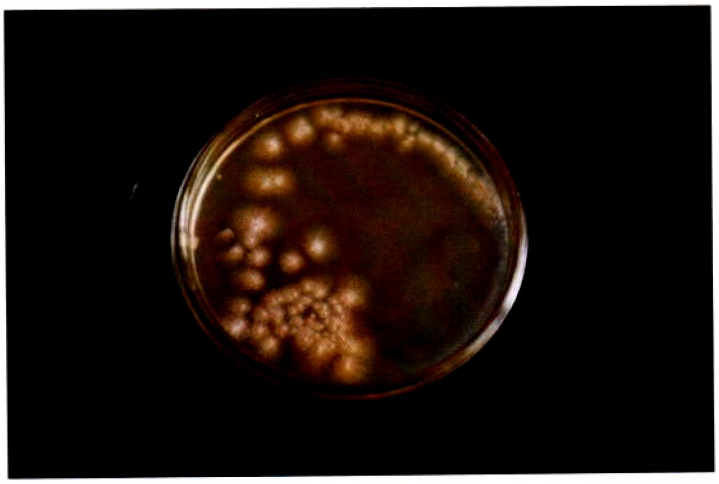
PLATE 7

SELECTED FUNGAL SPECIES ISOLATED FROM TANNERY EFFLUENT AND SLUDGE SAMPLES

Aspergillus terreus



Cladosporium sp.



Colletotrichum sp.

fungal growth on the slide culture, plate culture and the stained preparation (lactophenol cotton blue microscopic mount) and based on the details of sporulating structures observed microscopically under low and high power objectives. The interpretation drawn from the observations are detailed below:

Aspergillus terreus

The surface of the colonies on potato dextrose agar was found to be pinkish - cinnamon (puff) colour which turned to deeper brown shades on aging of the cultures and the reverse side of the plate was white. The vesicles were small (10-16μ) and dome-shaped. Phialides were found to be biseriate. Proximal (primary) phialides were shorter than distal (secondary) phialides. Conidia were smooth, elliptical and formed as long chains. The conidiophores were short, smooth and colourless. Cleistothecia were not found.

Cladosporium sp.

Colonies were olive brown on potato dextrose agar medium, large and thick vegetative hyphae, conidiophores and conidia were equally pigmented. Conidiophores were more or less distinct from the vegetative hyphae, erect, straight, branched and floccose. Conidia were one celled, smooth globose and ovate. The conidia were closest to the conidiophore and where the chains branched, were shield shaped.

Colletotrichum sp.

Pinkish white colonies were seen on potato dextrose agar medium. Conidiophores were pink, numerous, oval in shape with mycelia projected around its surface. Sclerotia were usually abundant, setose, spherical and were often confluent. Conidia were straight, fusiform, attenuated at the ends. Appressoria were common, clavate and brown.

4.2.5 Screening of selected fungal isolates for Cr(VI) tolerance

The growth of the selected fungal isolates recorded with various Cr(VI) concentrations permitting growth is presented in Table 8.

TABLE 8

GROWTH OF SELECTED FUNGAL ISOLATES IN VARIOUS CONCENTRATIONS OF Cr(VI)

Concentration mg/l	*Aspergillus terreus*	*Cladosporium sp.*	*Colletotrichum sp.*
20	+	+	+
40	+	+	+
60	+	+	+
80	+	+	+
100	+	+	+
120	+	+	+
140	+	+	+
160	+	+	+
180	-	-	+
200	-	-	+
300	-	-	+
400	-	-	-
500	-	-	-

\+ - Growth
\- - No growth

From the table, it was evident that concentration of Cr(VI) upto 160 mg/l permitted the growth of two isolates namely *Aspergillus terreus* and *Cladosporium sp.* during 7 days of incubation and both of them failed to grow after this concentration. *Colletotrichum sp.* was unaffected upto 300 mg/l and no growth was observed after this concentration.

Baldi *et al.* (1990) showed that the *Candida sp.* isolated from a sewage treatment plant receiving wastes from tannery industries in Italy was highly resistant to Cr(VI) growing at Cr(VI) concentrations upto 500 mg/l.

Ismail *et al.* (1991) showed that the fungi *Aspergillus terreus* could tolerate 1000mg/l of zinc whereas *Cladosporium herbarum* could tolerate only 100 mg/l of zinc. Lokesha and Somashekar (1991) demonstrated that *Rhizopus arrhizus* could tolerate 1000 mg/l of chromium.

PHASE III

4.3 UPTAKE AND BIOACCUMULATION STUDIES OF CHROMIUM USING BACTERIAL ISOLATES

There is a great interest in using microbiological systems for extraction of metal ions from wastewaters and it is accepted that much more impetus must be exercised over treatment of environmental wastes. It has been shown that bioremediation by microorganisms is economically and environmentally superior to chemical treatment and will be one of the solutions for pollution abatement (Shakibaie *et al.,* 1999).

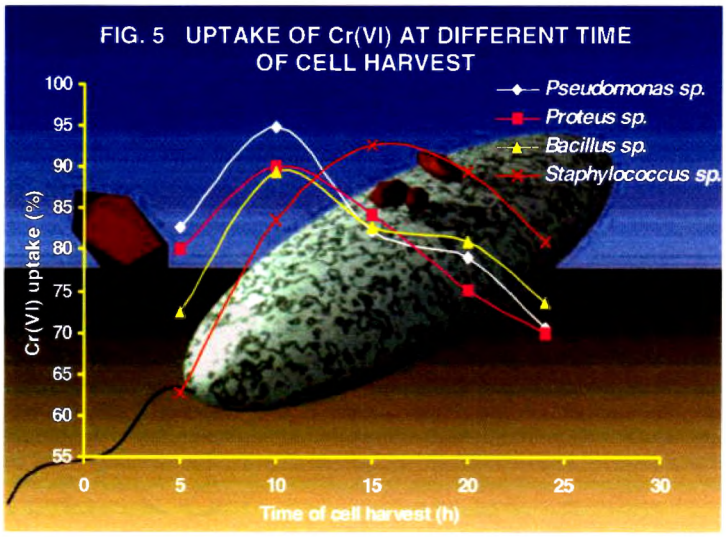
Several isolated bacterial species were found to be capable of catalysing the reduction of Cr(VI) to Cr(III), a less toxic and less water soluble form of chromium which could be used for bioremediation of Cr(VI) polluted environments (Viti *et al.,* 2003).

In the present study, selected bacterial isolates namely *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.* were analysed for their efficiency in the removal of Cr(VI) from synthetic solution (sections 4.3.1 to 4.3.6) and tannery effluent (section 4.3.7) under various conditions.

4.3.1 Influence of cell age on Cr(VI) uptake

The influence of cell age on Cr(VI) uptake by the selected bacterial isolates was studied and the results obtained are presented in Figure 5.

The bacterial isolates used in the study were cultured in a basal medium without chromium and harvested at different periods and used as inoculum.



The bacterial isolates of *Pseudomonas sp.*, *Proteus sp.* and *Bacillus sp.* harvested at the end of 10 hours of growth attained their maximum potential to accumulate Cr(VI). *Staphylococcus sp.* showed a maximum uptake of Cr(VI) when the cells used as inoculum were harvested at the end of 15 hours of growth. From this study it became evident that the ability of the cells to accumulate Cr(VI) was strongly influenced by culture age.

Galli *et al.* (1985) reported higher permeability of the cells in the early phase of growth which might account for maximum uptake of Cr(VI) in the early phase of growth. In aged bacterial cells 50% reduction in muramic acid, glucosamine, diamino pimelic acid, glycine, alanine, glutamic acid and glucose have been reported by Nair and Krishnamurthi (1991) due to autolysis. The cell surface of all microorganisms is negatively charged owing to the presence of various anionic structures. This gives bacteria the ability to bind metal cations (Lovely *et al.*, 1991). Therefore, reduction in the uptake of chromium by aged cultures might be due to loss of certain cell constituents involved in metal binding.

4.3.2 Effect of metal ion concentration on Cr(VI) uptake

Metal binding by bacteria has been shown to be profoundly influenced by the concentration of the metal (Shuttleworth and Unz, 1993).

An attempt was made in the present study to find out the influence of varying initial metal concentrations on the uptake of Cr(VI) by selected bacterial isolates and thereby altering their growth. The results are depicted in Table 9 and 10.

TABLE 9

PERCENTAGE UPTAKE OF Cr(VI) BY THE SELECTED BACTERIAL ISOLATES AT DIFFERENT INITIAL CONCENTRATIONS OF CHROMIUM

Initial concentration of Cr(VI) (mg/l)	*Pseudomonas sp.*		*Proteus sp.*		*Bacillus sp.*		*Staphylococcus sp.*	
	Cr(VI) uptake (mg/l)	% removal of Cr(VI)	Cr(VI) uptake (mg/l)	% removal of Cr(VI)	Cr(VI) uptake (mg/l)	% removal of Cr(VI)	Cr(VI) uptake (mg/l)	% removal of Cr(VI)
Control	-	-	-	-	-	-	-	-
20	19.6	98.00	19.26	96.30	18.93	94.65	19.10	95.50
40	39.10	97.75	38.20	95.50	37.53	93.83	37.73	94.33
60	58.43	97.38	56.80	94.66	55.63	92.72	56.76	94.60
80	76.10	95.13	73.63	92.03	73.56	91.95	74.30	92.87
100	94.13	94.13	90.20	90.20	89.43	89.43	91.60	91.60
LSD (5%) Conc Vs Uptake	**0.96**							

Values are mean of three replicates
Incubation period - 96 hours
Incubation temperature - 37°C
pH - 7.0

TABLE 10

GROWTH OF THE SELECTED BACTERIAL ISOLATES AT DIFFERENT INITIAL CONCENTRATIONS OF CHROMIUM

Initial concentration of Cr(VI) (mg/l)	Growth in terms of biomass (g dry wt/l)			
	Pseudomonas sp.	*Proteus sp.*	*Bacillus sp.*	*Staphylococcus sp.*
Control	6.02	6.67	6.20	7.83
20	5.76	6.07	5.98	7.24
40	5.09	5.45	5.54	6.76
60	4.51	4.65	5.07	6.13
80	4.15	4.20	5.54	5.81
100	3.78	3.90	4.08	5.11
LSD (5%) Conc Vs Growth	**0.34**			

Values are mean of three replicates
Concentration of Cr(VI) in nutrient broth - 100 mg/l
Incubation period - 96 hours
Incubation temperature - 37°C
pH - 7.0

With *Pseudomonas sp.* no significant difference was observed in the percentage uptake of Cr(VI) from 20 to 60 mg/l of initial Cr(VI) concentration and thereafter a significant decrease was observed with increasing metal ion concentrations. A similar trend was observed with *Proteus sp., Bacillus sp.* and *Staphylococcus sp.* where they showed a significant decrease in the percentage uptake of Cr(VI) at higher concentration range (80 and 100 mg/l).

Under identical conditions, the maximum percentage removal of Cr(VI) by the selected bacterial isolates was in the following decreasing order : *Pseudomonas sp.* (98%), *Proteus sp.* (96.3%), *Staphylococcus sp.* (95.5%) and *Bacillus Sp.* (94.65%).

All the isolates showed a decreased growth in terms of the biomass with increased initial concentration of Cr(VI). At the initial concentration of 20 mg/l of Cr(VI), only *Bacillus sp.* were consistent in their growth when compared with that

of the controls. Thereafter the growth was gradually decreased with increasing initial metal concentration. Similar trend was noticed in other metal removal systems. Hemalatha *et al.* (1997) in their studies with *Bacillus sp* HL-1 and *Pseudomonas sp*. HL-2 showed maximum uptake percentage of Cr(VI) at lower initial metal concentrations. Konopka *et al.* (1999) observed that the microbial biomass was decreased as the concentration of lead increased.

Bridge *et al.* (1999) had reported that the microorganisms released a diverse range of specific and nonspecific metal binding compounds in response to high levels of toxic metals, which can ameliorate the presence of toxic metals and mediate uptake.

It became evident from the study that there was an inverse relationship between chromium uptake and initial metal concentration and between growth and initial metal concentration among all the isolates.

4.3.3 Effect of tannic acid on Cr(VI) uptake

Tannic acid is a major contaminant present in the tannery waste water. To find out the influence of tannic acid on Cr(VI) uptake, the present investigation was carried out with varying tannic acid concentration in the synthetic solution and the data are depicted in Table 11.

An inverse relation between Cr(VI) uptake and tannic acid concentration was observed in all the isolated bacteria. At 0.01% tannic acid concentration, the results were consistent when compared to the corresponding controls in all the selected isolates of bacteria except *Staphylococcus sp.* Thereafter Cr(VI) uptake was decreased with increasing concentration of tannic acid.

The reduction in the uptake percentage of Cr(VI) with increasing concentration of tannic acid might be due to the binding of tannic acid to the

cationic sites in the cell wall proteins of bacteria as opined by Nair and Krishnamurthi (1991).

TABLE 11

PERCENTAGE UPTAKE OF Cr(VI) BY THE SELECTED BACTERIAL ISOLATES FROM A STANDARD MEDIUM AMENDED WITH DIFFERENT CONCENTRATIONS OF TANNIC ACID

Concentration of tannic acid (%)	*Pseudomonas sp.*	*Proteus sp.*	*Bacillus sp.*	*Staphylococcus sp.*
0.00	94.13	90.20	89.43	91.6
0.01	94.03	89.93	89.07	91.00
0.05	92.30	87.37	86.23	89.06
0.10	89.37	85.33	83.90	87.16
0.50	87.26	83.10	81.20	85.13
1.00	83.06	79.10	77.06	82.47
LSD (5%) Conc Vs Uptake	**0.39**			

Values are mean of three replicates
Concentration of Cr(VI) in nutrient broth - 100 mg/l
Incubation period - 96 hours
Incubation temperature - 37°C
pH - 7.0

4.3.4 Effect of sodium chloride on Cr(VI) uptake

The influence of sodium chloride on Cr(VI) uptake by the selected bacterial species was studied and the results obtained are presented in Table 12.

All the selected bacterial isolates except *Proteus sp.* recorded increased uptake of Cr(VI) with 1% sodium chloride compared to the respective controls. The uptake of Cr(VI) was decreased with further increase in the concentration of sodium chloride.

As the concentration of sodium chloride was increased, it was reasonable to expect a build up of osmotic potential. The uptake of Cr(VI) by the microorganisms seems to be more markedly influenced by the salinity of the medium (Nair and Krishnamurthi, 1991). The influence of salinity on zinc uptake

by microorganisms was observed by Babich and Stotzky (1978), who showed a decrease in zinc uptake by *E.coli, Pseudomonas aeruginosa* and *Bacillus cereus* in presence of sodium chloride.

TABLE 12

PERCENTAGE UPTAKE OF Cr(VI) BY THE SELECTED BACTERIAL ISOLATES FROM A STANDARD MEDIUM AMENDED WITH DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE

Concentration of sodium chloride (%)	*Pseudomonas sp.*	*Proteus sp.*	*Bacillus sp.*	*Staphylococcus sp.*
0.0	94.13	92.40	89.43	91.6
1.0	96.03	92.10	90.46	93.07
2.0	93.1	94.43	92.47	92.1
3.0	92.5	91.26	90.70	91.53
4.0	91.33	89.23	89.37	90.17
5.0	90.00	88.5	87.50	89.26
LSD (5%) Conc Vs Uptake	**0.56**			

Values are mean of three replicates
Concentration of Cr(VI) in nutrient broth - 100 mg/l
Incubation period - 96 hours
Incubation temperature - 37°C
pH - 7.0

4.3.5 Comparison of Cr(VI) uptake by resting, dead and metabolically inhibited cells

A comparative study of Cr(VI) uptake by resting, dead and metabolically inhibited cells at varying incubation periods was carried out to find out the possibility of the involvement of metabolic processes in the uptake of Cr(VI) by selected species of bacteria. The results are presented in Table 13.

In *Pseudomonas sp.* the resting cells showed an increased Cr(VI) uptake with increasing incubation period upto 48 hours and thereafter no significant increase was observed with further increase in the time of incubation.

TABLE 13

Cr(VI) UPTAKE FROM CHROMIUM SOLUTION BY RESTING, DEAD AND METABOLICALLY INHIBITED CELLS OF SELECTED BACTERIAL ISOLATES AT VARIOUS INCUBATION PERIODS (%)

Incubation time (h)	*Pseudomonas sp.*			*Proteus sp.*			*Bacillus sp.*			*Staphylococcus sp.*		
	Resting cells	Dead cells	Metabolically inhibited cells	Resting cells	Dead cells	Metabolically inhibited cells	Resting cells	Dead cells	Metabolically inhibited cells	Resting cells	Dead cells	Metabolically inhibited cells
0	-	-	-	-	-	-	-	-	-	-	-	-
6	22.13	53.93	22.03	33.13	68.07	32.5	41.00	72.20	40.13	24.00	52.43	26.37
12	52.5	73.37	53.96	58.20	83.27	56.17	62.33	92.33	59.17	49.10	75.37	51.20
18	72.77	88.13	73.37	81.43	98.00	80.07	83.96	96.9	81.26	72.10	89.33	75.10
24	97.23	98.13	98.13	97.23	98.23	97.06	96.00	98.03	95.16	96.23	97.63	97.10
48	98.06	98.13	98.17	97.43	98.43	97.50	96.27	98.27	96.13	97.17	98.03	97.43
72	98.20	98.76	98.23	97.43	98.67	97.63	96.40	98.36	96.33	97.43	98.33	97.50
96	98.20	98.80	98.43	97.63	98.7	97.63	96.56	99.00	96.57	97.60	98.47	97.63
LSD (5%) Time Vs Uptake	**0.57**			**0.41**			**0.56**			**0.57**		

Values are mean of three replicates
Incubation period - 96 hours
Incubation temperature - 37°C
pH - 7.0

However, the dead cells and metabolically inhibited cells of *Pseudomonas sp.* showed an increased percentage uptake of Cr(VI) upto the incubation period of 24 hours and thereafter no significant increase in the percentage uptake of Cr(VI) was observed with increasing incubation period.

Resting and metabolically inhibited cells of *Proteus sp.* recorded an increased percentage uptake of Cr(VI) upto 24 hours and thereafter further increase in the incubation period resulted in a nonsignificant variation in the removal of Cr(VI) whereas dead cells showed the percentage increase in Cr(VI) uptake upto 18 hours and then no significant increase was observed with increasing incubation period.

A significant increase in the percentage uptake of Cr(VI) was observed among resting and dead cells of *Bacillus sp.* upto 24 hours of incubation period whereas metabolically inhibited cells showed a significant increase upto 48 hours of incubation period. Thereafter no significant increase in Cr(VI) was observed with further increase in the Cr(VI) uptake.

Among the resting and dead cells of *Staphylococcus sp.,* a significant increase in the percentage uptake Cr(VI) was observed upto 48 hours of incubation whereas metabolically inhibited cells of *Staphylococcus sp.* showed the increase in the percentage uptake of Cr(VI) upto 24 hours of incubation period. No further increase in the percentage uptake of Cr(VI) was observed with further increase in the incubation period.

Gadd (1990) indicated that although living and dead cells are capable of metal accumulation, there might be differences in the mechanisms involved in either case, depending on the extent of metabolic dependence and in several bacteria, maximum uptake of Cr(VI) was found to be by metabolism independent biosorption.

4.3.6 Bioaccumulation and Cr(VI) reduction

Since the initial report of chromate and dichromate reduction from industrial wastewater by *Pseudomonas dechromaticans* by Romanenko and Korenkov (1977), the list of microbial strains reported to accumulate, resist and / or reduce hexavalent chromium either aerobically or anaerobically has grown. This list later included additional strains of *Pseudomonas* (Horitsu *et al.,* 1987; Cervantes *et al.,* 1990; De Leo and Ehrlich, 1994; McLean and Beveridge, 2001), *Enterobacter* (Wang *et al.,* 1989), *Alcaligenes* (Nies *et al.,* 1989). *Escherichia, Micrococcus* and *Bacillus* (Gvozdyak *et al.,* 1986; Fujie *et al.,* 1994, Shen and Wang, 1994) as well as mixed culture populations (Fude *et al.,* 1994; Turick *et al.,* 1996, Bader *et al.,* 1999).

Reduction of Cr(VI) to nontoxic Cr(III) using microorganisms is one of the most effective methods to degrade toxic Cr(VI), which is a widespread and toxic pollutant in industrial waste water.

The reduction of Cr(VI) to Cr(III) by selected bacterial isolates was observed at various incubation periods and the results are shown in Table 14.

It is evident from the table that all the four isolates were able to reduce Cr(VI) to Cr(III). Cr(III) was first measurable after 12 hours growth in the medium where *Pseudomonas sp.* was grown. The appearance of Cr(III) in the medium was noticed after 48 hours of growth of both *Proteus sp.* and *Bacillus sp.* and 24 hours of growth of *Staphylococcus sp.*

All the four bacterial isolates have recorded a sharp decline in the level of Cr(VI) in the medium only after 12 hours of incubation time. The subsequent increase in the rate of removal might relate to an increase in growth rate during the log phase of the organisms as opined by Saxena *et al.* (2000). Ramteke (2000), observed an initial rapid uptake of Ni(II) by *Pseudomonas stutzer* who accounted this for the biosorption of metal ion on the cell wall, which is a fast reaction.

TABLE 14

STATUS OF Cr(VI) AND Cr (III) IN A STANDARD MEDIUM WITH 100 mg/l Cr(VI) AFTER TREATMENT WITH THE SELECTED BACTERIAL ISOLATES AT DIFFERENT INCUBATION TIME

Incubation time (h)	Residual chromium in culture medium (mg/l)							
	Pseudomonas sp.		*Proteus sp.*		*Bacillus sp.*		*Staphylococcus sp.*	
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)
0	100	-	100	-	100	-	100	-
6	88.1	-	91.3	-	89.2	-	86.1	-
12	58.2	0.9	63.8	-	68.2	-	69.1	-
18	47.0	1.2	53.4	-	59.0	-	58.2	-
24	36.5	2.6	42.1	-	49.2	-	47.5	1.8
48	18.6	3.5	38.5	0.7	37.1	1.1	21.5	3.7
72	9.0	4.4	22.6	1.6	19.5	2.8	5.5	6.8
96	1.6	5.5	8.2	2.0	7.3	7.3	1.2	8.0

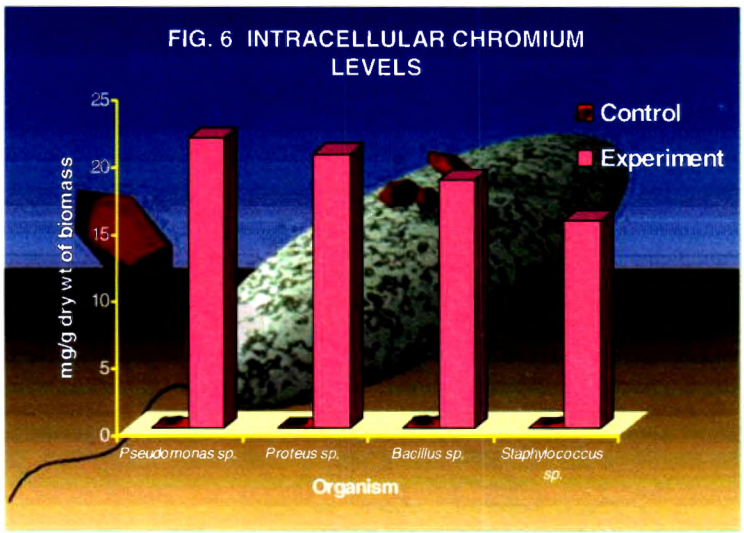
Values are mean of three replicates
Incubation temperature - 37°C
pH - 7.0

As Cr(VI) is a known toxin and mutagen (Codina *et al.*,1995), it is probable that intracellular chromium accumulates only in the form of Cr(III). The appearance of Cr(III) in a culture medium would therefore be due to its release from the cells.

Cr(VI) resistant strains were found to reduce Cr(VI) to Cr(III) under anaerobic conditions. This was attributed to the use of chromate as an electron acceptor by the organism concerned (*Enterobacter sp. ; Pseudomonas sp.*) as reported by Fujie *et al.* (1994), Wang *et al.* (1990), Horitsu *et al.* (1987), Ishibashi *et al.* (1990) and Lovely and Philips (1994).

The reduction takes place on the cell surface, forming insoluble chromium hydroxide in the external medium. Chromate is a strong oxidant and intracellular chromate is undoubtedly toxic for bacterial cells. Chromate reduction on the cell surface and the formation of insoluble chromium hydroxide, seemed to favour protection of the cells from the toxicity of Cr(VI) (Rapoport and Muter, 1995).

In the present study, after 98 hours incubation, the Cr(III) level had reached 5.5%, 2.0%, 4.3% and 8.0% of the initial Cr(VI) content in the medium containing *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.* respectively.



The bioaccumulation of total chromium was confirmed by atomic absorption analysis of the biomass and the results are shown in Figure 6. The dry biomass of selected bacterial isolates obtained after 96 hours growth in a medium amended with 100 mg/l Cr(VI) contained 21.63, 20.48, 18.55 and 15.55 mg

chromium / g cells of *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.* respectively.

Various microbial species, mainly *Pseudomonas*, have been shown to be relatively efficient in the bioaccumulation of uranium, copper, lead and other metal ions from polluted effluents (Lovely *et al.,* 1991). The enhanced bioaccumulation of heavy metal ions by bacterial cells through metal binding peptides was reported by Kotrba *et al.* (1999b) who worked on hybrid Lam B proteins of *E.coli.*

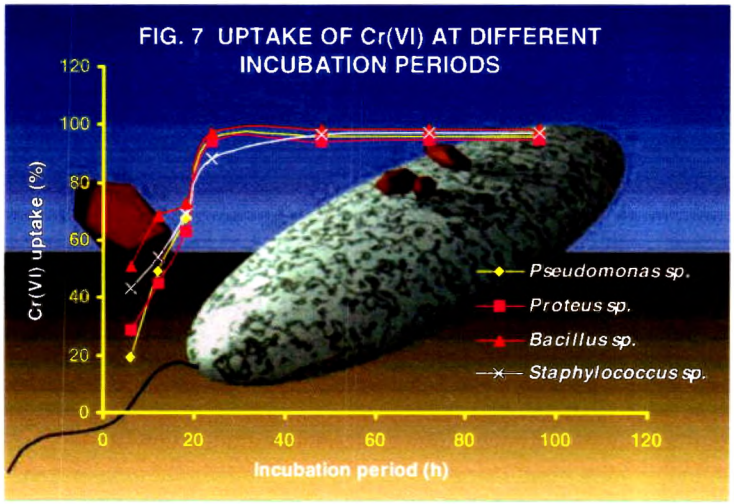
Smith and Gadd (2000) showed that 88% of the total chromium was removed from solution by sulphate reducing bacterial biofilms. About 80% of the total chromium was precipitated out of solution with bacterial biofilm retaining less than 10% of the chromium. *Bacillus circulans* and *Bacillus megaterium* were able to bioaccumulate 34.5 and 32.0 mg chromium / g dry weight respectively in 24 hours when the initial concentration was 50 mg Cr(VI) / l (Srinath *et al.,* 2002).

4.3.7 Treatment of tannery effluents using the selected bacterial isolates

Metal remediation strategies using microorganisms can minimise the bioavailability and biotoxicity of heavy metals. Low cost and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison to physicochemical methods for heavy metal removal (Rajendran *et al.*,2003). Hence, the selected bacterial isolates were studied for their efficiency in the removal of chromium from tannery effluents.

The selected bacterial isolates were used to treat the tannery effluent containing an initial concentration of 34 mg/l of Cr(VI). A 2% (v/v) of culture from exponential growth phase was added to the effluent and samples were removed for analysis after different incubation periods. The results of this study are presented in Figure 7.

All the four isolates of bacteria showed a steady increase in the percentage uptake of Cr(VI) with increase in the time of incubation from 6 to 24 hours and thereafter no significant difference in the percentage uptake of Cr(VI) was observed with further increase in the incubation period.



Maximum removal of Cr(VI) was obtained with *Bacillus sp.* (98.45%) which was followed by *Staphylococcus sp.,* (97.21%), *Pseudomonas sp.* (96.24%) and *Proteus sp.* (94.92%). Saxena *et al.* (2000) had reported the removal of about 90% of Cr(VI) from the tannery effluent by *Staphylococcus cohnii.*

Microbes can significantly affect the distribution of metals in the environment, since they have developed means to use them for their benefit. This clearly holds promise for effective, economical and eco-friendly metal bioremediation technology for industrial exploitation and pollution free environment (Rajendran *et al.*, 2003)

PHASE IV

4.4 CHROMIUM UPTAKE STUDIES USING SELECTED ISOLATES OF FUNGI

Among the microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall material, which shows excellent metal binding properties. Many fungi and yeasts have shown an excellent potential for metal biosorption (Gupta *et al.,* 2000).

The metal uptake by fungal biomass takes place by two basic processes. The first is by living organisms where the metal uptake is dependent on the metabolic activity. The second process involves metal uptake by dead and living cells as a result of the chemical functional groups of the cell and, in particular, the cell wall. It should be noted that the bioremediation by the second process may also be involved during the metabolism dependent metal uptake of growing cells (Kapoor and Viraraghavan, 1995).

Thus, in the present study, the uptake of Cr(VI) by both growing cells and non-living biomass of three selected fungal isolates namely *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* was examined under selected conditions. These fungal isolates were selected based on their increased Cr(VI) tolerance and the feasibility of the use of these isolates for the detoxification of Cr(VI) in the tannery effluent was assessed.

4.4.1 Chromium uptake by growing cells

Effect of dilution of the tannery effluent on Cr(VI) uptake and growth

The efficiency of Cr(VI) uptake by three selected fungal isolates namely *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* was assessed 7 days after inoculation of the fungal species in graded dilutions (undiluted, 1:25, 1:50, 1:75 and 1:100) of the tannery effluent. The results obtained are presented in Table 15.

All the three fungal isolates showed an increasing trend in Cr(VI) uptake with increasing dilution. Among the three fungal isolates, *Colletotrichum sp.* recorded a maximum uptake of 91.20% of Cr(VI) from tannery effluent at the highest dilution of 1:100. It was followed by *Aspergillus terreus* (89.30%) and *Cladosporium sp.* (75.17%) in the same dilution. Dias *et al.* (2002) reported that a maximal chromium uptake values of 96.5 mg/g was attained in a culture medium containing 100% of effluent stream supplemented with 1% glucose, after 6 days of incubation by *Aspergillus terreus* UFMG - F01.

TABLE 15

Cr(VI) UPTAKE BY THE THREE SELECTED FUNGAL ISOLATES AND THEIR GROWTH AT DIFFERENT DILUTIONS OF THE TANNERY EFFLUENT

Dilution of the tannery effluent	Cr(VI) uptake (%)			Growth in terms of biomass (g dry wt/l)		
	Aspergillus terreus	*Cladosporium sp.*	*Colletotrichum sp.*	*Aspergillus terreus*	*Cladosporium sp.*	*Colletotrichum sp.*
Undiluted	46.20	39.09	73.07	0.446	0.543	0.433
1 : 25	57.24	48.03	78.12	0.823	1.636	1.246
1 : 50	58.42	49.19	78.72	0.953	1.693	1.3
1 : 75	64.17	51.37	88.17	3.623	1.76	2.51
1 : 100	89.30	75.17	91.20	6.453	2.543	4.65
LSD (5%)	**0.40**			**0.05**		

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

The growth in terms of biomass was also significantly increased with increasing dilution with all the three fungal isolates. The results also showed that the amount of Cr(VI) removed from tannery effluent was directly proportional to the mycelial biomass produced. The maximum biomass was obtained with *Aspergillus terreus* (6.453 g dry wt/l) at the end of 7 days incubation in 1:100 diluted effluent sample which was followed by *Colletotrichum sp.* (4.65 g dry wt/l) and *Cladosporium sp.* (2.543 g dry wt/l). It was seen from the studies of Kowshik and Nazareth (1999) that there was an increase in the removal of metal salts (lead, manganese, iron and copper) with increase in the growth of culture and the metal uptake by mycelial cells was found to be both cell bound as well as intracellular accumulation.

Effect of temperature on Cr(VI) uptake and growth

The rate and extent of accumulation of heavy metals were found to be dependent on environmental parameters, such as pH, temperature and interference by certain anions and cations (Gupta *et al.,* 2000). Thus, the uptake experiments were conducted to evaluate the potential of three selected fungal isolates in the removal of Cr(VI) from synthetic solution and to assess their growth at varying temperatures (20°C, 25°C, 30°C, 35°C and 40°C). The results obtained are presented Table 16.

It was observed that all the fungal isolates were efficient in the removal of Cr(VI) from solution over a wide range of temperatures between 25 and 35°C. Below 30°C the uptake of Cr(VI) was less with all the three fungal isolates. The optimum temperature for Cr(VI) uptake and the growth with *Aspergillus terreus* was found to be 40°C whereas in *Cladosporium sp.*, it was found to be 30°C. *Colletotrichum sp.* recorded an optimum temperature of 35°C for its growth and Cr(VI) uptake.

TABLE 16

Cr(VI) UPTAKE BY THE THREE SELECTED FUNGAL ISOLATES AND THEIR GROWTH AT DIFFERENT TEMPERATURES

Temperature (°C)	Cr(VI) uptake (%)			Growth in terms of biomass (g dry wt/l)		
	Aspergillus terreus	*Cladosporium sp.*	*Colletotrichum sp.*	*Aspergillus terreus*	*Cladosporium sp.*	*Colletotrichum sp.*
20	62.45	42.38	51.25	1.476	1.327	0.137
25	73.40	63.13	73.26	2.443	2.09	1.336
30	89.11	87.14	93.15	4.106	4.26	4.24
35	91.20	82.24	96.35	5.823	4.11	5.073
40	92.12	72.37	92.24	5.97	3.427	4.18
LSD (5%)	**0.32**			**0.15**		

Values are mean of three replicates
Incubation period - 7 days
Concentration of Cr(VI) - 100 mg/l
pH - 6.0

Among the three selected fungal isolates, *Colletotrichum sp.* was found to be the best in Cr(VI) uptake (96.35%) whereas *Aspergillus terreus* and *Cladosporium sp.* recorded 92.12% and 87.14% respectively at their corresponding optimal temperatures. Growth in terms of biomass also was found to be directly proportional to the metal uptake with all the selected fungal isolates. Wang (2001) observed in his study that the optimal pH and temperature for the biological reduction of chromium coincided with the optimal growth conditions of cells. The activities of the microorganisms seemed to attain a peak when pH and temperature were controlled in the optimum ranges.

The transport of metal ions into microbial cells is inhibited by low temperatures, metabolic inhibitors and absence of an energy source as opined by Gadd (1990). Strandberg *et al.* (1981) detected an increase in the rate of uranium uptake by *Saccharomyces cerevisiae* with an increase in temperature between 20 and 50°C. Rapoport and Muter (1995) showed in their studies with yeast that the kinetics of sorption process was dependent on the temperature of incubation, the preferred temperature for sorption being 30 and 45°C.

Effect of pH on Cr(VI) uptake and growth

The biosorption capacities of both living and nonliving microbial biomass for heavy metal ions were found to be strongly dependent on pH of the solution. pH seemed to greatly affect the uptake of metal ions by fungi (Kapoor and Viraraghavan, 1995). Thus, Cr(VI) uptake experiments were carried out with the three selected fungal isolates at different pH and the results obtained are given in Table 17.

The maximum uptake of Cr(VI) was observed at pH 5.0 with *Aspergillus terreus* and *Cladosporium sp.,* whereas *Colletotrichum sp.,* showed a maximum uptake of Cr(VI) at pH 6.0. Among the three fungal isolates, *Colletotrichum sp.,* was found to be the best in the removal of Cr(VI) (96.14%) compared to *Aspergillus terreus* (91.19%) and *Cladosporium sp.,* (87.13%) at their optimum

TABLE 17

Cr(VI) UPTAKE BY THE THREE SELECTED FUNGAL ISOLATES AND THEIR GROWTH AT DIFFERENT pH

pH	Cr(VI) uptake (%)			Growth in terms of biomass (g dry wt/l)		
	Aspergillus terreus	*Cladosporium sp.*	*Colletotrichum sp.*	*Aspergillus terreus*	*Cladosporium sp.*	*Colletotrichum sp.*
3.0	70.31	64.27	73.27	2.37	2.21	1.82
4.0	82.11	72.14	87.15	4.12	3.60	2.83
5.0	91.19	87.13	88.21	5.71	4.27	2.94
6.0	81.08	73.24	96.14	4.06	3.67	5.27
7.0	73.33	64.16	72.19	2.57	2.23	1.77
LSD (5%)	**0.06**			**0.06**		

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
Concentration of Cr(VI) - 100 mg/l

pH. Growth in terms of biomass was also found to coincide with the Cr(VI) uptake in all the selected fungal isolates. Gadd (1990) suggested that low external pH generally decreased the rate and extent of metal biosorption.

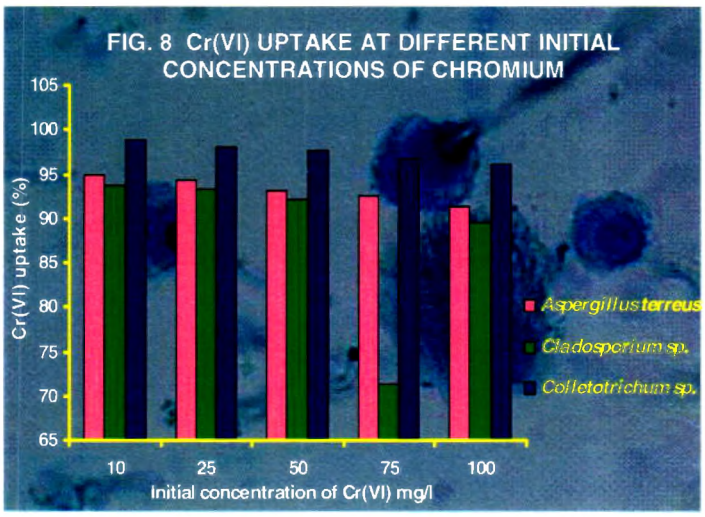
Due to the ion exchange nature of metal biosorption, copper(II) uptake was recorded to be strongly affected by the solution pH and optimum adsorption pH was determined as 5.0 for all the live and treated *Candida sp.,* from the experiments of Aksu and Donmez (2001).

The different pH binding profiles for heavy metal ions could be due to the nature of the chemical interactions of each metal with microbial cells and are related to the isoelectric point of the cell. At pH values above the isoelectric point, there is a net negative charge on the cells and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such as to promote reaction with the metal cations. As the pH is lowered, however, the overall surface charge on the cells becomes positive, which will inhibit the approach of positively charged metal cations (Sag and Kutsal, 1996).

4.4.2 Chromium uptake by dead cells

Effect of metal ion concentration on Cr(VI) uptake

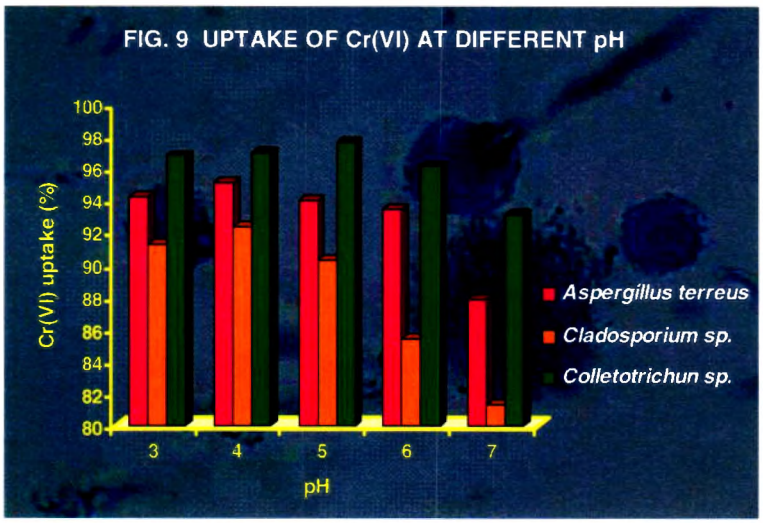
Since the extent of biosorption was found to be dependent on metal ion concentration (Iqbal and Saeed, 2002), the Cr(VI) uptake studies with the selected three fungal isolates were carried out at different initial concentrations of



chromium in the synthetic solution in order to find the optimum concentration for the metal uptake. The results are depicted in Figure 8.

It was observed from the results that with an increase in the initial concentration of metal in the solution, Cr(VI) uptake was decreased with the dead biomass of all the three selected fungal isolates. The dead biomass of *Colletotrichum sp.* was found to remove greatest percentage of Cr(VI) (98.9%) at the initial concentration of 10 mg/l which was followed by *Aspergillus sp.* (95%) and *Cladosporium sp.* (93.8%) respectively with the same initial Cr(VI) concentration. In contrary, Aksu and Donmez (2001) showed that at the optimal conditions, metal ion uptake increased with increasing initial copper (III) concentration upto 30 mg/l with the dead biomass of *Candida sp.* Bai and Abraham (2001) recorded a higher adsorption percentage at lower initial concentrations of chromium ions with biomass of *Rhizopus nigricans.*

Effect of pH on Cr(VI) uptake



The biosorption capacities of both living and nonliving biomass for heavy metal ions were strongly dependent on pH of the solution (Kapoor and Viraraghavan, 1995). Thus the effect of pH on Cr(VI) uptake from the synthetic solution was studied with the selected fungal isolates. The results are dipicted in Figure 9.

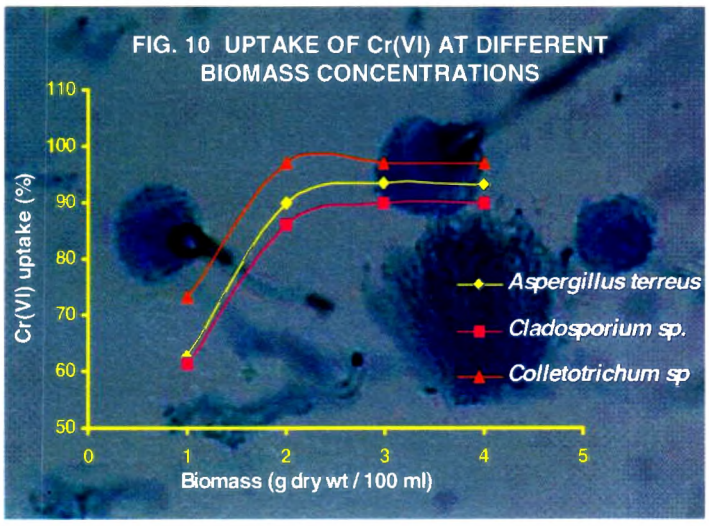
It was observed that the dead biomass of all the three selected fungal isolates were able to adsorb metal ions over a wide range of pH and the maximum uptake of Cr(VI) was found to occur between pH 4 and 5.

Aspergillus terreus and *Cladosporium sp.* recorded an optimum pH of 4.0 where the Cr(VI) uptake was found to be 95.08% and 92.40% respectively. *Colletotrichum sp.*, recorded a maximal Cr(VI) uptake of 97.64% at the optimum pH of 5.0. Golab and Orlowska (1991) showed in their studies that the removal of UO_2^{2+} and Pb(II) from aqueous solution by *Streptomyces sp.* was pH dependent especially in the case of UO_2^{2+} with maximum uptake in the pH range of 5 to 6. Fourest *et al.* (1994) have observed the maximum sorption capacity of dead biomass of *Rhizopus arrhizus* for lead at pH 7.0 while silver uptake was weakly affected. Uranium removal of penicillium was more or less the same in the pH range of 2.5 - 9.5. This showed that there were pH dependent variations in the behaviour of fungal cell wall surfaces (Kapoor and Viraraghavan, 1995).

Bai and Abraham (2001) reported that the powdered biomass of *Rhizopus nigricans* showed an optimum pH of 2.0 for biosorption of Cr(VI). They attributed this to the predominance of anionic species of chromium ($HCrO_4^-$, $Cr_2O_7^{2-}$, $Cr_4O_{13}^{2-}$ and $Cr_2O_{10}^{2-}$) at this low pH, which could easily interact with the positively charged cell wall ligands. Suhasini *et al.* (1999) reported that the maximum cobalt removal by Rhizopus sp. based biosorbent PFB_1 was obtained at pH 7.0. They however observed an average biosorption of 85% in the pH range 2 to 8.

Effect of biomass concentration on Cr(VI) uptake

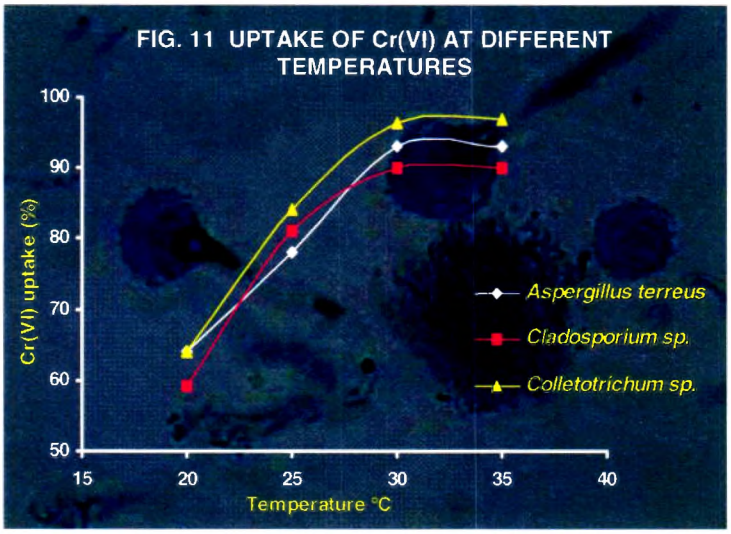
The results of various dosages of biomass of the selected fungal isolates on the uptake of Cr(VI) from the synthetic solution are presented in Figure 10.



It was observed that the percentage removal of Cr(VI) was increased with increase in the biomass of all the selected fungal

isolates (upto 3.0 g /100ml) indicating increased availability of the active sites for biosorption. Within the range of biomass dosage tested, an equilibrium was reached between dissolved and solid bound sorbate after the dose of 3.0 g/100ml. The *Colletotrichum sp.* recorded a maximum Cr(VI) uptake of 97.18% which was followed by *Aspergillus terreus* (93.26%) and *Cladosporium sp.* (90.15%) at equilibrium biomass dosage. Bai and Abraham (2001) reported an optimum dosage of 0.5% (w/v) biomass of *Rhizopus nigricans* for the removal of Cr(VI) and at this dosage, 75% of the ions were removed within 30 minutes of contact and maximum removal was obtained after 8 hours. The optimum biomass concentration for waste biomass from beverage distillery was determined as 2.0 g/l and under optimised conditions, the uptake of chromium was found to be 38 mg/g after 3 hours. This corresponded to a reduction of chromium concentration of approximately 94% (Dias *et al.*, 2001).

Effect of temperature on Cr(VI) uptake



Chromium uptake by the dead biomass of the three selected fungal isolates at different temperatures was studied and the results are shown in Figure 11.

It was shown that the uptake of Cr(VI) by the dead biomass of all the selected fungal isolates was increased with increasing temperature upto 30°C and no further increase in Cr(VI) uptake was noticed above this temperature.

Bai and Abraham (2001) have reported higher chromium removal efficiency by *Rhizopus nigricans* biomass at temperature above 30°C and decrease in sorption at a higher temperature of 50°C. They attributed this

decrease to the possible damage caused to the active binding sites in the biomass. In contrary, Suhasini *et al.* (1999) observed a decrease in cobalt sorption with increase in temperature from 30°C to 45°C by the *Rhizopus sp.* waste biosorbent. Similar reports of decrease in silver sorption by *Saccharomyces cerevisae* from 4°C to 55°C has been reported by Singleton and Simmons (1996).

Simple physical sorption is generally exothermic. However this cannot be extrapolated to the phenomenon of biosorption. The first and foremost reason is that, biosorption basically involves an exchange of two metals. Thus, the overall reaction can be either endo or exothermic. The energy liberated due to the binding of one ion is compensated by absorption of the same by the ion released. Secondly, apart from ion-exchange, the phenomenon involves complex formation as well. As biosorption occurs in a very narrow temperature range, temperature effects are only of secondary importance (Gupta and Mohapatra, 2003)

The use of dead biomass has an additional advantage in the bioremediation in highly variable conditions and toxic metallic contaminations prevalent in many ways, preclude the use of living organisms and necessitate the utilisation of non living systems for metal removal (Maruti and Akhtar, 1995). The dead biomass can also be regenerated and reused for sequestering more metal ions (Kowshik and Nazareth, 1999).

4.4.3 Effect of chromium on metabolic activity and cell wall composition of the selected fungal isolates

The tolerance of fungi to a variety of heavy metals and the use of microorganisms and / or microbial biomass in the removal and recovery of heavy metals from aqueous solutions, as well as radioactive materials and other toxic pollutants from wastewater and industrial effluents have been investigated by several workers (Gadd and Griffiths, 1980; Osman and Elwy, 1989; Gadd and White, 1993; Arica *et al.,* 2001). The biopolymers of fungal cell walls have been reported to be useful in the removal of heavy metals (Muzzarelli, 1985).

Cr(VI) is more toxic to bacteria and fungi than the less soluble Cr(III). Hexavalent chromium is a strong oxidant and this property is likely to be related to its toxicity for most organisms. Biological membranes are impermeable to Cr(III) but Cr(VI) can penetrate through and may be reduced in the mitochondria, nucleic acid and cytoplasm to Cr(III) which readily forms insoluble chromium hydroxides at pH 7.5. Cr(III) which may be generated inside the cell is suggested to bind to the protein and interact with nucleic acids (Rapoport and Muter, 1995). The literature pertaining to the study of heavy metal toxicity on the metabolism of fungi are limited.

Thus, an attempt was made to find out the effect of metal toxicity on fungal metabolism, the development of resistance to heavy metals in fungi and on the changes in the chemical composition of the cell wall of the selected fungal isolates.

Effect of Cr(VI) concentration on the growth of fungi

Growth in terms of biomass was assayed after culturing the selected fungal isolates for 7 days in a medium amended with various concentrations of Cr(VI).

The values shown in Table 18 reveal the mycelial dry weight of the fungi in liquid medium at different Cr(VI) concentrations. The growth yield (mycelial dry weight) of *Aspergillus terreus* decreased significantly at 5% level with increasing concentrations of Cr(VI) from 25 to 100 mg/l.

The growth yield of *Cladosporium sp.*, and *Collectotrichum sp.,* was also found to be decreased significantly when compared with the control at different concentrations of Cr(VI). This might be attributed to the inhibition of biological activities of the fungus as reported with *Candida albicans* by Ross (1982).

TABLE 18

GROWTH OF SELECTED FUNGAL ISOLATES AT DIFFERENT INITIAL CONCENTRATIONS OF CHROMIUM

Concentration of Cr(VI) (mg/l)	*Aspergillus terreus* (g dry wt /l)	*Cladosporium sp.* (g dry wt /l)	*Colletotrichum sp.* (g dry wt /l)
Control	11.18	11.07	6.73
10	10.85	9.95	6.25
25	9.98	8.63	5.84
50	8.23	7.19	5.24
75	6.14	5.31	4.99
100	5.91	4.23	4.75
LSD (5%) Conc Vs Growth	**0.49**	**0.30**	**0.19**

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

Effect of Cr(VI) on total carbohydrate content of the mycelia and cell walls

The carbohydrate content in mycelia and cell walls of the three selected fungal isolates at different Cr(VI) concentrations are given in Table 19. It was observed that the total carbohydrate content in the mycelia of all the selected fungal isolates was significantly increased when compared to their respective controls. This might be due to the accumulation of carbohydrates in the mycelial mats which seemed to be a mechanism for the detoxification of metals by fungi. Ismail *et al.*(1991) noted that application of nickel or zinc to *Chlorella sp.* accelerated the total carbonhydrate accumulation at all concentrations. In contrast, in the present study, the carbohydrate content of cell wall of all the selected fungal isolates was found to be decreased significantly with increasing concentration of Cr(VI). Venkateshwerlu and Stotzky (1986) reported that the cell wall of copper containing culture of *Cunninghamella blakesleeana* contained less hexosamine and alkali soluble neutral sugar than the controls.

TABLE 19

TOTAL CARBOHYDRATE CONTENT IN MYCELIA (mg/100 ml dry weight) AND CELL WALLS (μmole/mg dry cell wall) OF THE SELECTED FUNGAL ISOLATES AT DIFFERENT CONCENTRATIONS OF CHROMIUM

Concentration of Cr(VI) (mg/l)	*Aspergillus terreus*		*Cladosporium sp.*		*Colletotrichum sp.*	
	Mycelia	Cell wall	Mycelia	Cell wall	Mycelia	Cell wall
Control	22.55	0.25	27.09	0.52	24.17	0.45
10	22.71	0.18	27.64	0.32	24.41	0.35
25	23.44	0.15	28.41	0.27	25.05	0.22
50	24.61	0.14	29.21	0.20	25.93	0.18
75	25.03	0.11	28.63	0.19	26.40	0.10
100	26.15	0.09	26.36	0.14	27.75	0.09
LSD (5%) Conc. Vs Carbohydrate content	**0.12**	**0.03**	**0.19**	**0.04**	**0.12**	**0.03**

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

Effect of Cr(VI) on total protein content of the mycelia and cell walls

The protein contents of the mycelia and cell wall of the selected fungal isolates at different Cr(VI) concentrations are given in Table 20.

It was observed that the protein content of the mycelia of *Aspergillus terreus* was significantly increased with increasing concentration of Cr(VI) whereas a significant decrease was noticed in the mycelia of *Colletotrichum sp.*

TABLE 20

TOTAL PROTEIN CONTENT IN MYCELIA (mg/100 ml dry weight) AND CELL WALLS (μmole/mg dry cell wall) OF THE SELECTED FUNGAL ISOLATES AT DIFFERENT CONCENTRATIONS OF CHROMIUM

Concentration of Cr(VI) (mg/l)	*Aspergillus terreus*		*Cladosporium sp.*		*Colletotrichum sp.*	
	Mycelia	Cell wall	Mycelia	Cell wall	Mycelia	Cell wall
Control	0.38	0.37	0.28	0.29	0.29	0.19
10	0.57	0.63	0.29	0.47	0.21	0.36
25	0 .61	0.65	0.30	0.61	0.19	0.42
50	0.64	0.69	0.28	0.66	0.18	0.45
75	0.57	0.73	0.23	0.66	0.17	0.56
100	0.38	0.80	0.22	0.69	0.13	0.61
LSD (5%) Conc. Vs Protein content	**0.06**	**0.02**	**0.05**	**0.03**	**0.04**	**0.04**

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

There was no significant difference in the protein content of *Cladosporium sp.*, up to a concentration of 50 mg/l and thereafter a significant decrease was noticed with increasing concentrations. Metwally and Abou-Zeid (1996) suggested that protein production was directly affected by the type and concentration of the metal. They showed that protein synthesis by *Aspergillus ochraceus* was found to be increased relative to the control with increasing lead concentration upto 600 ppm. In contrary, there was a general decrease in protein synthesis with increasing manganese concentration. Sinha *et al.* (2002) have reported a decrease in protein concentration with increase in chromium concentrations in the plants *Najas indica* cham. *Vallisneria spiralis* L. and *Alernanthera sessilis* R. Br. These plants were found to be effective in the removal of chromium from synthetic chromium solution and tannery effluent. The inhibitory effect of metal on protein content at higher concentrations might be due

to phosphate deficiency as a result of its precipitation, membrane damage, change in permeability and/or selective inhibition of enzyme synthesis (Ziochevskaya and Rukhadze, 1968, Cole, 1977). Khovrychev *et al.* (1980) suggested that protein synthesis is most sensitive to toxic compounds among other metabolic processes and its inhibition leads to the death of cells.

In the present study, protein contents in the cell walls were found to be significantly increased in all the selected fungal isolates, when compared with their controls. Venkateshwerlu and Stotzky (1986) showed that blue cell walls (copper treated) of *Cunninghamella blakesleeana* contained more protein than the control which might be one of the reasons for the high accumulation of copper, which would have complexed with the NH_2 groups of the protein.

Effect of Cr(VI) on the phosphorus content of the mycelia and cell walls

The phosphorus contents in both the mycelia and cell wall of the selected fungal isolates exposed to various concentrations of Cr(VI) are presented in Table 21.

It was observed from the results that there was no significant change in the phosphorus content in the mycelia of all selected fungal isolates at lower concentrations. Cr(VI) treatment at elevated concentrations resulted in significant decrease in the accumulation of phosphorus as compared with their controls. This might be due to increased catabolic activity as suggested by Ismail *et al.* (1991).

In contrast, the phosphorus content in the cell wall of all the selected fungal isolates was found to be increased with increasing metal ion concentration as compared with their controls. Similar results were obtained from the studies of Ismail *et al.* (1991) who found that the copper treated *Aspergillus terreus, Clasosporium herbarum* and *Fusarium oxysporum* were coupled with significant inhibition of phosphorus accumulation. Venkateshwerlu and Stotzky (1986) found that the phosphorus content of the cell wall of *Cunninghamella blackesleeana* exposed to copper was two fold higher than in the control cell walls.

TABLE 21

TOTAL PHOSPHORUS CONTENT IN MYCELIA (mg/100 ml dry weight) AND CELL WALLS (µmole/mg dry cell wall) OF THE SELECTED FUNGAL ISOLATES AT DIFFERENT CONCENTRATIONS OF CHROMIUM

Concentration of Cr(VI) (mg/l)	*Aspergillus terreus*		*Cladosporium sp.*		*Colletotrichum sp.*	
	Mycelia	Cell wall	Mycelia	Cell wall	Mycelia	Cell wall
Control	0.35	0.12	0.13	0.23	0.27	0.19
10	0.31	0.17	0.14	0.29	0.24	0.22
25	0.36	0.18	0.14	0.39	0.24	0.28
50	0.40	0.21	0.13	0.41	0.25	0.30
75	0.32	0.24	0.11	0.45	0.19	0.35
100	0.26	0.27	0.07	0.47	0.13	0.39
LSD (5%) Conc. Vs Phosphorus content	**0.05**	**0.03**	**0.03**	**0.03**	**0.04**	**0.02**

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

Effect of Cr(VI) on nucleic acids content in the mycelia

The RNA and DNA in the biomass of *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* are given in Table 22. Cr(VI) treatment, at all concentrations, recorded a significant increase in the cellular RNA and no significant change in DNA at lower concentrations in *Aspergillus terreus*. However, at higher concentrations, between 75 to 100 mg/l of Cr(VI), a significant decrease in DNA was observed.

A significant increase in the RNA content was observed in *Cladosporium sp.* at lower concentrations. The DNA content was found to be increased at the lowest initial concentration of Cr(VI) and a significant decrease was noticed between all the concentrations of Cr(VI). A significant decrease in DNA was

observed at the highest concentration of Cr(VI) (100 mg/l) when compared with control.

TABLE 22

TOTAL NUCLEIC ACIDS CONTENT IN MYCELIA (mg/100 ml dry weight) OF THE SELECTED FUNGAL ISOLATES AT DIFFERENT CONCENTRATIONS OF CHROMIUM

Concentration of Cr(VI) (mg/l)	*Aspergillus terreus*		*Cladosporium sp.*		*Colletotrichum sp.*	
	RNA	DNA	RNA	DNA	RNA	DNA
Control	0.28	0.32	0.36	0.29	0.41	0.27
10	0.44	0.30	0.48	0.33	0.55	0.20
25	0.44	0.30	0.46	0.31	0.49	0.19
50	0.46	0.29	0.39	0.28	0.39	0.17
75	0.53	0.26	0.36	0.25	0.36	0.15
100	0.65	0.21	0.33	0.21	0.33	0.14
LSD (5%) Conc Vs Nucleic acids content	**0.03**	**0.02**	**0.03**	**0.02**	**0.04**	**0.03**

Values are mean of three replicates
Incubation period - 7 days
Incubation temperature - 28°C
pH - 6.0

Colletotrichum sp. recorded a significant increase in RNA content at the lower concentrations of Cr(VI) which was followed by a gradual decrease at higher concentrations when compared with control. A significant decrease in DNA with increasing concentrations was observed with *Colletotrichum sp.* when compared with control.

The decrease in RNA and DNA contents at higher concentrations might be due to the higher toxicity of Cr(VI). Stearns *et al.* (1994) observed the DNA damage in various cell culture systems and animal tissues *invivo* by Cr(VI).

These results provide evidence for the resistance of selected fungal isolates at lower concentrations and the toxic effects at higher concentrations of Cr(VI).

PHASE V

4.5 CHROMIUM UPTAKE STUDIES USING SELECTED AGROWASTE ADSORBENTS

The most commonly used procedures for removing metals from industrial wastes are chemical reduction, precipitation and ion exchange methods. However, these procedures have significant disadvantages, such as incomplete metal removal, high reagent or energy requirements or the generation of toxic sludge or other waste products that require safe disposal. Therefore, there is a need for the development of low-cost, easily available materials which can adsorb hexavalent chromium economically (Sharma and Forster,1995; Panswad *et al.,* 2001; Murugesan and Vasanthy, 2003).

In the present work, easily available low cost agrowaste materials were modified and used as adsorbents for the removal of Cr(VI) from synthetic solution under various conditions and in the tannery effluent.

4.5.1 Characteristics of the selected agrowaste adsorbents

The characteristics of the adsorbents prepared from the selected agrowaste materials are presented in Table 23. It was observed that the nitrated and sulphonated coconut shell carbons had higher bulk density (0.59 and 0.58 g/cc respectively) than the other adsorbents. The bulk density of the selected agrowaste adsorbents ranged between 0.20 and 0.59 g/cc. Swamy *et al.* (1998) reported 0.27 g/cc bulk density for bagasse fly ash and 0.61 g/cc bulk density for activated carbon (commercial grade). Srinivasan *et al.* (1998) reported 0.36 g/cc bulk density for rice husk carbon.

The moisture content of the selected agrowaste adsorbents was in the range of 6.7 to 15.05%. Modified sugarcane bagasse recorded the highest

moisture percentage whereas nitrated coconut shell carbon registered the lowest moisture percentage.

TABLE 23

CHARACTERISTICS OF SELECTED AGROWASTE ADSORBENTS

Adsorbent	Bulk density (g/cc)	Moisture content (%)	Ash content (%)	Volatile matter (%)
Nitrated coconut shell carbon	0.59	6.70	8.42	17.10
Sulphonated coconut shell carbon	0.58	6.80	8.92	16.87
Coir pith carbon	0.20	14.44	18.62	20.25
Saw dust carbon	0.49	12.02	16.28	19.13
Modified sugarcane bagasse	0.22	15.05	27.0	25.25
Groundnut shell carbon	0.30	12.10	22.16	27.95

The ash content of the selected agrowaste adsorbents was found to be in the range of 8.42 to 27.0%. The ash contents of nitrated and sulphonated coconut shell carbons (8.42 and 8.92% respectively) were similar to that of commercial grade activated carbon (8.78%) as reported by Swamy *et al.* (1998). He also reported the ash content of 30.98% for bagasse flyash and 2.65% for laboratory grade activated carbon.

The volatile matter of the selected agrowaste adsorbents was in the range of 16.87 to 27.95%. The sulphonated coconut shell carbon was found to possess the lowest percentage of volatile matter whereas the highest percentage was recorded in groundnut shell carbon. According to Swamy *et al.* (1998), the volatile matter in bagasse flyash, laboratory grade activated carbon and commercial grade activated carbon were 29.96, 7.74 and 16.08% respectively.

4.5.2 Effect of pH on the removal of Cr(VI) using selected agrowaste adsorbents

The influence of pH of the synthetic solution on the extent of adsorption of Cr(VI) is shown in Table 24.

TABLE 24

PERCENTAGE ADSORPTION OF Cr(VI) ON SELECTED AGROWASTE ADSORBENTS WITH VARYING pH

pH	Nitrated coconut shell carbon	Sulphonated coconut shell carbon	Coirpith carbon	Sawdust carbon	Modified sugarcane bagasse	Groundnut shell carbon
4.0	86.77	84.81	75.14	78.97	68.13	79.13
4.5	86.01	83.29	72.12	74.08	64.99	75.99
5.0	80.38	78.42	67.11	68.05	60.67	70.07
5.5	78.36	72.89	63.89	65.94	57.19	66.23
6.0	71.15	71.10	59.96	62.10	55.46	62.11
6.5	64.81	69.20	56.18	57.97	52.87	59.12
7.0	61.15	64.12	51.11	54.59	49.12	57.25
L.S.D. (5%) pH Vs Adsorption	**0.52**	**0.60**	**0.40**	**0.37**	**0.24**	**0.24**

Values are mean of three replicates
Temperature - 30°C
Contact time - 3 hours
Adsorbent dose - 20 g/l

The percentage adsorption of Cr(VI) from synthetic solution was found to be increased significantly with decreasing pH from 7.0 to 4.0 with all the selected agrowaste adsorbents. At pH 4.0, the percentage removal of Cr(VI) was maximum with nitrated coconut shell carbon (86.77%) which was followed by sulphonated coconut shell carbon (84.81%), groundnut shell carbon (79.13%), saw dust carbon (78.97%), coir pith carbon (75.14%) and modified sugarcane bagasse (68.13%).

The maximum adsorption at low pH values may be attributed to the large number of H^+ ions present at low pH values which might neutralise the negatively charged surface of adsorbents or convert a neutral group to a positively charged group thereby reducing the hindrance to the diffusion of dichromate ions. The enhancement of the adsorption of Cr(VI) species might be due to a decrease in the forces of repulsion between the adsorbent and adsorbate (Kannan and Vanangamudi,1991). Singh *et al.* (1994) opined that the higher sorption at lower pH might be due to chloro-chromate complex anion (CrO_3Cl) formation in the presence of hydrochloric acid.

At higher pH, the reduction in adsorption may be possibly due to the abundance of OH^- ions creating increased hindrance to diffusion of dichromate ions (Jasuja *et al.,* 1997).

Siddiqui *et al.* (1999) reported that the adsorption of Cr(VI), Zn (II), Cu(II) and Ni (II) is highly pH dependent. The maximum adsorption of Zn (II), Ni (II) and Cu(II) on phosphate treated saw dust was observed between pH 7.0 to 8.0, whereas the percentage adsorption of Cr (VI) was increased as the pH was decreased. 50% of Cr(VI) removal was reported at neutral pH and 100% removal was obtained at pH 2.0. Although maximum adsorption of Cr(VI) takes place in highly acidic solution (pH 2.0), it will not be practicable to reduce pH of solutions to such a low level because it will involve greater consumption of acid first and alkali later in order to neutralise the effluent before its discharge (Jasuja *et al.,* 1997). Verma and Rehal (1996) showed that pH is a dominant solution parameter controlling adsorption. The solution pH affects the chemistry of the metals, the activity of the functional groups in the adsorbent as well as the competition of metallic ions for the binding site. The optimum pH for adsorption of Cr(VI) by *Albizia lebbeck* was 2.0. The metal removal efficiency was 94.3% (Verma and Rehal, 1996).

Selvi *et al.* (2001) reported that the adsorption of Cr(VI) on the activated carbon prepared from coconut tree saw dust was pH dependent and maximum removal was observed in the acidic pH range.

Ajmal *et al.* (2001) reported that the adsorption of Cr(VI) was maximum between pH 4.0 and 6.0 on teak leaves.

From the present investigation it is clear that, the nitrated coconut shell carbon has a greater adsorption potential for Cr(VI) among all other adsorbents at pH 4.0.

4.5.3 Effect of adsorbent dose on the removal of Cr(VI) using selected agrowaste adsorbents

The effect of adsorbent dose was studied by varying the amount of adsorbent dose (0.5, 1.0, 1.5 and 2.0g/100ml) and performing the adsorption studies at pH 4.0. The data obtained are depicted in Table 25.

TABLE 25

PERCENTAGE ADSORPTION OF Cr(VI) ON SELECTED AGROWASTE ADSORBENTS WITH VARYING ADSORBENT DOSE

Absorbent dose (g/100ml)	Nitrated coconut shell carbon	Sulphonated coconut shell carbon	Coirpith carbon	Sawdust carbon	Modified sugarcane bagasse	Groundnut shell carbon
0.5	40.12	36.88	32.63	33.34	36.16	30.73
1.0	60.16	58.14	46.80	52.18	48.69	40.10
1.5	78.20	75.95	69.25	60.21	59.14	52.22
2.0	86.94	84.50	76.03	78.93	70.00	66.06
L.S.D. (5%) Dose Vs Adsorption	**0.27**	**0.26**	**0.27**	**0.54**	**0.28**	**0.19**

Values are mean of three replicates
Temperature - 30°C
Contact time - 3 hours
pH - 4.0

The results obtained in the present study indicate that the amount of Cr(VI) adsorbed and hence the percent removal of Cr(VI) by all the selected

agrowaste adsorbents was significantly increased with increase in the adsorbent dose in the synthetic solution at pH 4.0. This is because at the higher dose of adsorbent, due to an increased surface area, more number of adsorption sites are available resulting in higher Cr(VI) removal (Manju and Anirudhan, 1997).

In the present study, the nitrated and sulphonated coconut shell carbons were found to be efficient among other adsorbants in the removal of Cr(VI) from solution. At the adsorbent dose of 2.0 g/100 ml, the nitrated coconut shell carbon adsorbed 86.94% Cr(VI) which was followed by sulphonated coconut shell carbon (84.5%), saw dust carbon (78.93%), coirpith carbon (76.03%), modified sugarcane bagasse (70%) and groundnut shell carbon (66.06%).

Kannan and Vanangamudi (1991) demonstrated that the percentage adsorption of Cr(VI) at constant pH increased with increase in the dose of lignite coal.

It was inferred from the reports of Siddiqui *et al.* (1999) that by increasing the adsorbent dose of phosphate treated saw dust from 0.2 to 3.0 g, the removal efficiency of Zn(II), Ni(II), Cu(II) and Cr(VI) increased but adsorption density decreased. The decrease in adsorption density might be attributed to the fact that some of the adsorption sites remain unsaturated during the adsorption process, whereas the number of available adsorption sites increases by increasing the adsorbent doses and that might result in the increase in the efficiency of removal.

Khangan *et al.* (1996) showed that the uptake of Cu^{2+} ion initially increased with increasing weight of the adsorbent (*Aegle marmelos* bark substrate) from 0.5 g to 2.0 g beyond which it almost remained constant. Jasuja *et al.* (1997) demonstrated that the removal of Cr(VI) increased with increasing dose of *Ablesmoschus esculentus*. However, further increase of the substrate resulted in marginal increase in Cr(VI) removal.

4.5.4 Effect of initial Cr(VI) concentration and contact time in the removal of Cr(VI) by selected agrowaste adsorbents

The effect of initial Cr(VI) concentration and contact time on the sorption potential of selected agrowaste adsorbents namely nitrated coconut shell carbon, sulphonated coconut shell carbon, coirpith carbon, saw dust carbon, modified sugarcane bagasse and groundnut shell carbon were investigated at pH 4.0 with the adsorbent dose of 2.0g. The results of the study are depicted in Tables 26, 27 and 28.

The adsorption efficiency (percentage removal of Cr(VI)) of all the selected agrowaste adsorbents were significantly decreased with increasing Cr(VI) concentrations from 20 to 100 mg/l at all the selected contact times (30, 60, 90, 120 and 150 minutes).

The decrease in the percent removal of Cr(VI) with an increase in the initial Cr(VI) concentration at any given contact time might probably be due to the fact that for a fixed adsorbent dose, the total available adsorption sites are limited thereby adsorbing almost the same amount of Cr(VI), causing a decrease in the percent removal of Cr(VI) corresponding to an increase in the initial sorbate concentration.

Singh and Tiwari (1997) reported a similar trend of metal adsorption in different initial concentrations.

Jasuja *et al.* (1997) showed that there was a decrease in the percentage adsorption with rise in initial concentration of Cr(VI). It shows that the uptake is highly dependent on the initial concentration of the metal ions in the solution. The higher uptake at low concentration can be attributed to the availability of more parking spaces (ie. active centres) on the surface of the adsorbent for lesser number of adsorbate ions. Similar results were observed by Liu *et al.* (2001) that the removal of Cr(III) from aqueous solutions with a spheroidal cellulose adsorbent was found to be time, pH and initial concentration of Cr(III) dependent.

TABLE 26

PERCENTAGE ADSORPTION OF Cr(VI) ON NITRATED AND SULPHONATED COCONUT SHELL CARBONS AT DIFFERENT CONTACT TIME WITH VARYING INITIAL SORBATE CONCENTRATIONS

Initial concentration of Cr(VI) (mg/l)	Nitrated coconut shell carbon					Sulphonated coconut shell carbon				
	Contact time (minutes)									
	30	60	90	120	150	30	60	90	120	150
20	51.15	74.55	87.23	92.05	93.36	41.15	65.53	84.61	90.66	91.36
40	49.18	70.30	86.69	91.74	93.00	40.00	60.2	80.25	89.29	89.50
60	43.98	68.44	83.37	90.16	90.44	37.19	50.81	79.22	87.86	88.22
80	38.74	52.61	80.08	88.54	88.62	31.44	40.45	77.46	85.70	86.50
100	33.98	49.54	79.02	86.77	87.06	28.50	38.97	75.98	84.70	84.90
L.S.D. (5%) Conc Vs Time	**0.85**					**0.68**				

Values are mean of three replicates
Temperature - 30°C
pH - 4.0

TABLE 27

PERCENTAGE ADSORPTION OF Cr(VI) ON COIRPITH CARBON AND SAWDUST CARBON AT DIFFERENT CONTACT TIME WITH VARYING INITIAL SORBATE CONCENTRATIONS

Initial concentration of Cr(VI) (mg/l)	Coirpith carbon					Saw dust carbon				
	Contact time (minutes)									
	30	60	90	120	150	30	60	90	120	150
20	38.03	68.42	78.00	87.92	88.33	50.92	70.57	88.77	90.67	90.50
40	35.76	60.18	72.50	86.10	86.28	48.52	69.85	87.42	87.70	88.20
60	34.90	58.41	70.39	82.93	82.97	46.41	67.00	84.12	84.91	85.14
80	31.95	56.37	69.16	80.53	80.67	42.37	65.14	81.29	82.37	82.46
100	28.03	50.03	67.14	76.11	76.19	41.06	61.98	78.07	79.03	79.17
L.S.D. (5%) Conc Vs Time	**0.71**					**0.69**				

Values are mean of three replicates
Temperature - 30°C
pH - 4.0

TABLE 28

PERCENTAGE ADSORPTION OF Cr(VI) ON MODIFIED SUGARCANE BAGASSE AND GROUNDNUT SHELL CARBONS AT DIFFERENT CONTACT TIME WITH VARYING INITIAL SORBATE CONCENTRATIONS

Initial concentration of Cr(VI) (mg/l)	Modified sugarcane bagasse					Groundnut shell carbon				
	Contact time (minutes)									
	30	60	90	120	150	30	60	90	120	150
20	36.17	62.02	84.53	85.67	85.82	28.50	52.22	79.50	80.03	80.60
40	33.11	60.22	79.50	80.07	80.17	26.76	50.37	76.03	77.21	77.20
60	31.00	58.45	76.38	76.90	76.99	25.60	49.10	73.99	74.90	75.10
80	28.54	55.16	73.10	73.14	73.31	23.05	47.27	69.75	69.84	70.21
100	26.08	50.13	69.10	70.11	70.18	21.18	42.21	65.10	66.05	66.15
L.S.D. (5%) Conc Vs Time	**0.92**					**1.11**				

Values are mean of three replicates
Temperature - 30°C
pH - 4.0

It could be suggested from the tables that the percentage removal of Cr(VI) was dependent on contact time and maximum removal was obtained at 120 minutes and thereafter no significant increase in Cr(VI) was obtained with all the selected agrowaste adsorbents. The results further revealed that the equilibrium time was independent of initial sorbate concentration. The percentage removal of Cr(VI) was found to be maximum at the end of 150 minutes contact time and the lowest initial concentration (20 mg/l) of the sorbate with all the selected agrowaste adsorbents. The efficiency of Cr(VI) removal was in the following decreasing order : nitrated coconut shell carbon (93.36%), sulphonated coconut shell carbon (91.36%), sawdust carbon (90.50%), coirpith carbon (88.33%), modified sugarcane bagasse (85.82%) and groundnut shell carbon (80.60%).

Manju and Anirudhan (1997) demonstrated that the percentage adsorption of Cr(VI) on coconut fibre pith based pseudo activated carbon increased rapidly initially and maximum adsorption took place within 2 hours and the equilibrium was attained in 3 hours.

Swamy *et al.* (1998) observed that the maximum removal of o-cresol was obtained at 120 minutes of contact time with laboratory grade activated carbon and bagasse flyash, whereas the maximum adsorption of o-cresol obtained with commercial grade activated carbon was found to be at 90 minutes of contact time.

4.5.5 Adsorption isotherm

The adsorption equilibrium data obtained for the removal of Cr(VI) using nitrated coconut shell carbon, sulphonated coconut shell carbon, coirpith carbon, saw dust carbon, modified sugarcane bagasse and groundnut shell carbon were analysed using Freundlich and Langmuir equations.

Various researchers have used isotherms to examine the importance of different factors on metal ion sorption. The distribution of metal ion between the

liquid phase and the sorbent is a measure of the position of equilibrium in the adsorption process and can generally be expressed by one or more of series of isotherms. The shape of an isotherm may be considered with a view to, predict if a sorption system is 'favourable' or 'unfavourable'. The two most popular isotherm theories are the Langmuir and Freundlich. The Langmuir isotherm is valid for monolayer sorption on a surface containing a finite number of binding sites. The treatment assumes uniform energies of sorbate in the plane of the surface. In contrast, the Freundlich isotherm theory says that the ratio of the amount of solute adsorbed onto a given mass of sorbent to the concentration of the solute in the solution is not a constant at different solution concentrations (Brown *et al.,* 2000).

The adsorption data (Tables 29, 30, 31, 32, 33 and 34) of all selected adsorbents at different initial concentrations of Cr(VI) with a constant dose of 20 g/l was fitted to linearised Freundlich adsorption isotherm, which is of the form.

$$\log\frac{x}{m} = \log K + \frac{1}{n}\log C_e$$

where, (x/m) is the amount of Cr(VI) adsorbed per unit weight of adsorbent (mg/g), C_e is the equilibrium concentration of sorbate, K is a constant which is a measure of adsorption capacity and 1/n is a measure of the adsorption intensity. The linear plots of log 1/n vs log C_e suggest the applicability of the Freundlich model for Cr(VI) sorption (Siddiqui *et al.,* 1999). Figures 12, 13, and 14 show the plot of log x/m versus log C_e for Cr(VI) removal using selected adsorbents.

Values of Freundlich isotherm constants are presented in Table 35. The two constants K and 1/n represent all the factors affecting the sorption process from solution (Chandrasekar and Chakravarthy, 2000).

The value of 1/n characterises the selectivity of adsorbent for the adsorbate. The values of 1/n less than 1 are indicative of favourable adsorption (Anirudhan and Sreedhar, 1999).

TABLE 29

ADSORPTION OF Cr(VI) ON NITRATED COCONUT SHELL CARBON WITH VARYING SORBATE CONCENTRATION

Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	1.33	0.335	0.1238	0.9701	0.7518	0.1071
40	2.8	18.6	0.4471	1.2695	0.3571	0.0537
60	5.74	27.13	0.7589	1.4334	0.1742	0.368
80	9.1	34.45	0.9590	1.5371	0.1098	0.0290
100	12.94	43.53	1.1119	1.6387	0.0772	0.0229
Correlation coefficient (r)	**0.991**				**0.997**	

Temperature - 30°C
Amount of sorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

TABLE 30

ADSORPTION OF Cr(VI) ON SULPHONATED COCONUT SHELL CARBON WITH VARYING SORBATE CONCENTRATION

Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	1.73	9.135	0.2380	0.9607	0.5780	0.1094
40	4.2	17.9	0.6232	1.2528	0.2380	0.0558
60	7.07	26.465	0.8494	1.4226	0.1414	0.0377
80	10.8	34.6	1.0334	1.5390	0.0925	0.0289
100	15.1	42.45	1.1789	1.6278	0.0662	0.0235
Correlation coefficient (r)	**0.998**				**0.999**	

Temperature - 30°C
Amount of adsorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

TABLE 31

ADSORPTION OF Cr(VI) ON COIRPITH CARBON WITH VARYING SORBATE CONCENTRATION

Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	2.34	8.83	0.3692	0.9459	0.4273	0.1132
40	5.49	17.255	0.7395	1.2369	0.1821	0.0579
60	10.19	24.905	1.0081	1.3962	0.0981	0.0401
80	15.47	32.265	1.1894	1.5087	0.0646	0.0309
100	23.82	38.09	1.3769	1.5808	0.0419	0.0262
Correlation coefficient (r)	**0.993**				**0.999**	

Temperature - 30°C
Amount of adsorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

TABLE 32

ADSORPTION OF Cr(VI) ON SAW DUST CARBON WITH VARYING SORBATE CONCENTRATION

Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	1.9	9.05	0.2787	0.9566	0.5263	0.1104
40	4.72	17.64	0.6739	1.2464	0.2118	0.0566
60	8.92	25.54	0.9503	1.4072	0.1121	0.0391
80	14.03	32.985	1.1470	1.5183	0.0712	0.0303
100	20.84	39.58	1.3188	1.5974	0.0479	0.0252
Correlation coefficient (r)	**0.996**				**0.999**	

Temperature - 30°C
Amount of adsorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

TABLE 33

ADSORPTION OF Cr(VI) ON MODIFIED SUGARCANE BAGASSE WITH VARYING SORBATE CONCENTRATION

Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	2.84	8.58	0.4533	0.9334	0.3531	0.1165
40	7.94	16.03	0.8998	1.2049	0.1259	0.0623
60	13.821.	23.1	1.1398	1.3636	0.0724	0.0432
80	35	29.325	1.3293	1.4672	0.0468	0.0341
100	29.82	35.09	1.4745	1.5451	0.0335	0.0299
Correlation coefficient (r)	**0.999**				**0.992**	

Temperature - 30°C
Amount of adsorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

TABLE 34

ADSORPTION OF Cr(VI) ON GROUNDNUT SHELL CARBON WITH VARYING SORBATE CONCENTRATION

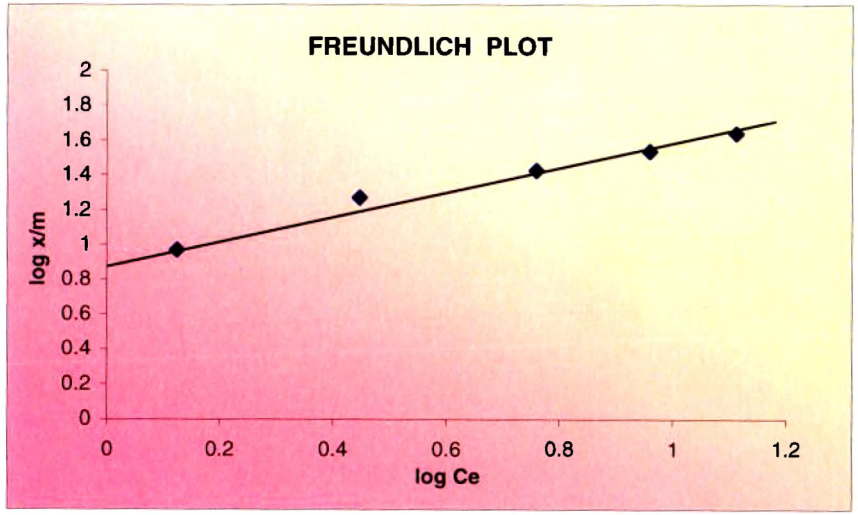
Initial concentration of Cr(VI) (mg/l)	C_e	$\frac{x}{m}$	$\log C_e$	$\log \frac{x}{m}$	$1/C_e$	$1/\frac{x}{m}$
20	3.88	8.06	0.5888	0.9063	0.2577	0.1240
40	9.12	15.44	0.9599	1.1886	0.1096	0.0647
60	14.94	22.53	1.1743	1.3527	0.0669	0.0443
80	23.83	28.085	1.3771	1.4484	0.0419	0.0356
100	33.86	33.07	1.5296	1.5194	0.0295	0.0302
Correlation coefficient (r)	**0.993**				**0.999**	

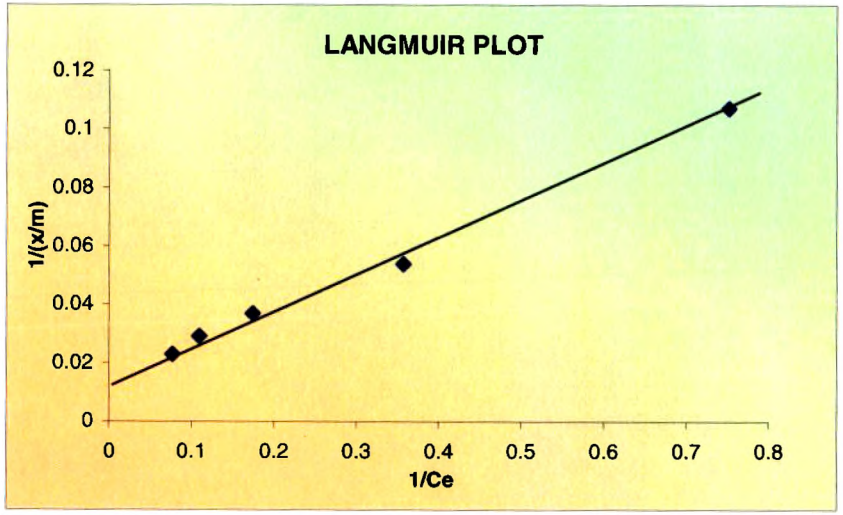
Temperature - 30°C
Amount of adsorbent - 2g
Contact time - 150 minutes
C_e - Equilibrium concentration of residual Cr(VI) in solution (mg/l)
x/m - The amount of Cr(VI) sorbed per unit weight of sorbent (mg/g)

FIGURE 12

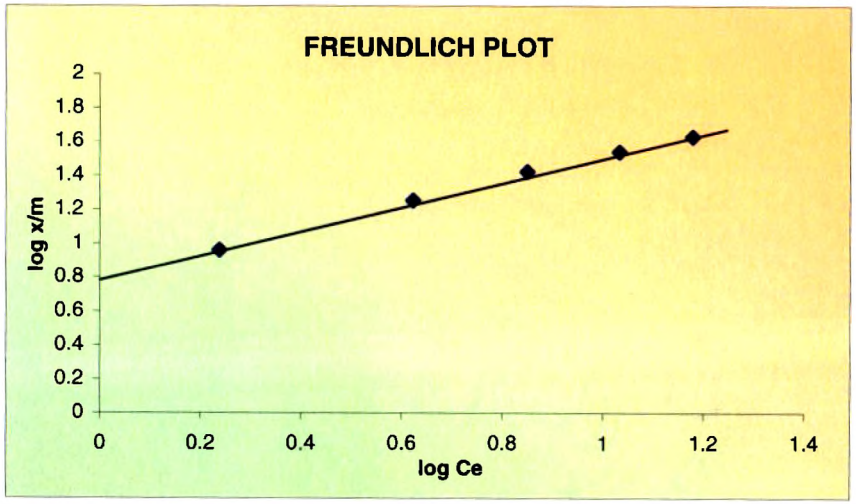
FREUNDLICH AND LANGMUIR ADSORPTION PLOTS FOR NITRATED AND SULPHONATED COCONUT SHELL CARBON AT DIFFERENT INITIAL Cr(VI) CONCENTRATIONS

NITRATED COCONUT SHELL CARBON





SULPHONATED COCONUT SHELL CARBON



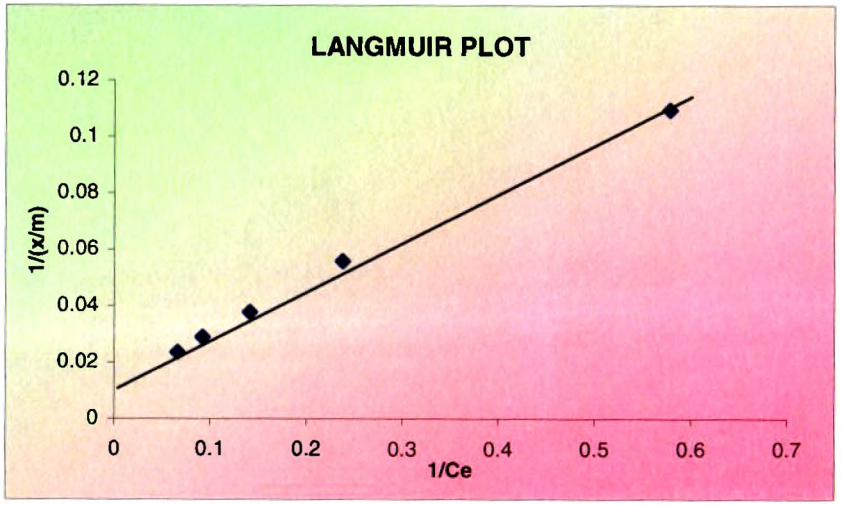
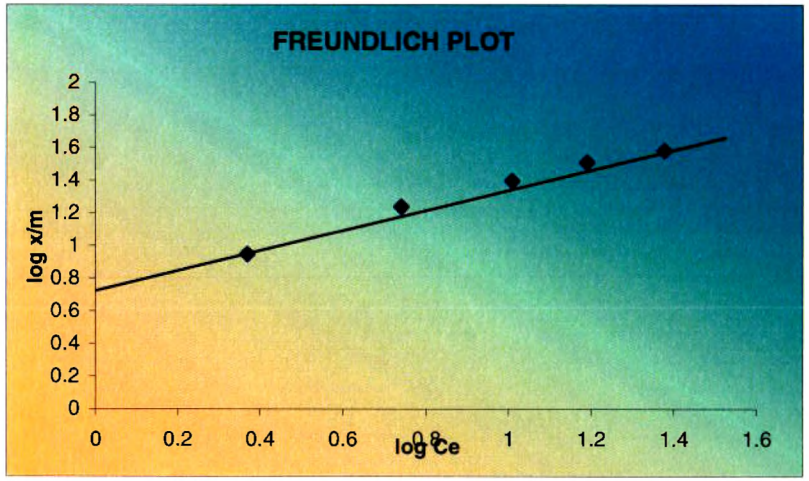
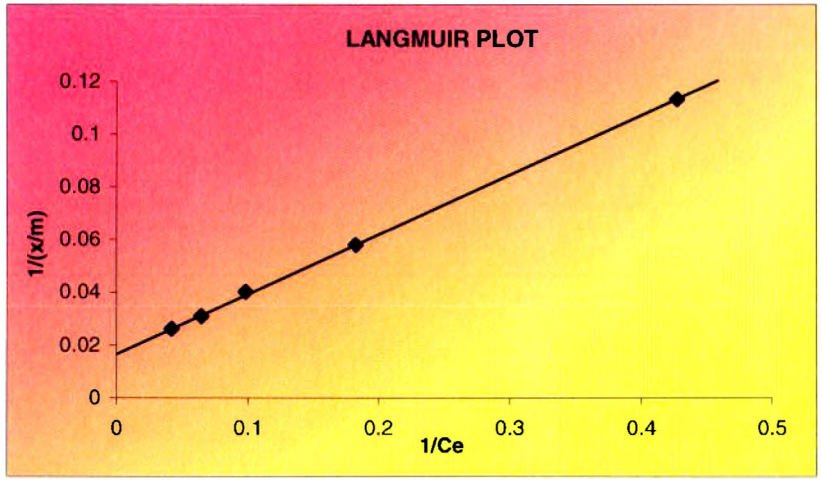


FIGURE 13

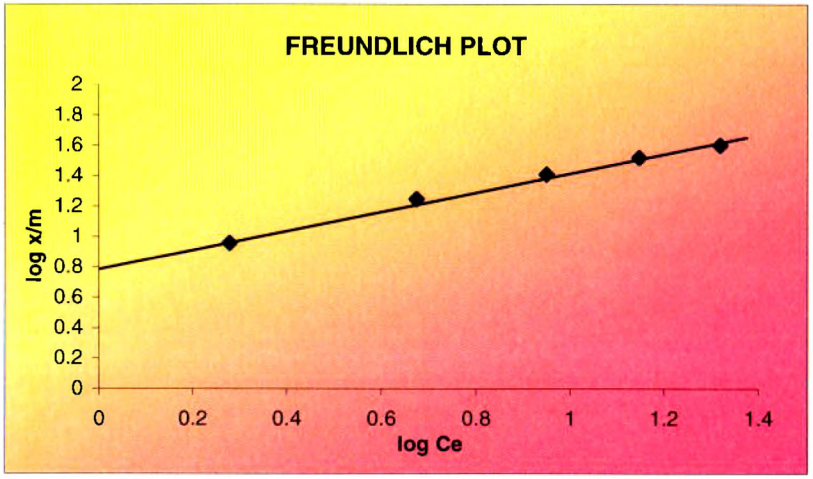
FREUNDLICH AND LANGMUIR ADSORPTION PLOTS FOR COIRPITH CARBON AND SAW DUST CARBON AT DIFFERENT INITIAL Cr(VI) CONCENTRATIONS

COIRPITH CARBON





SAW DUST CARBON



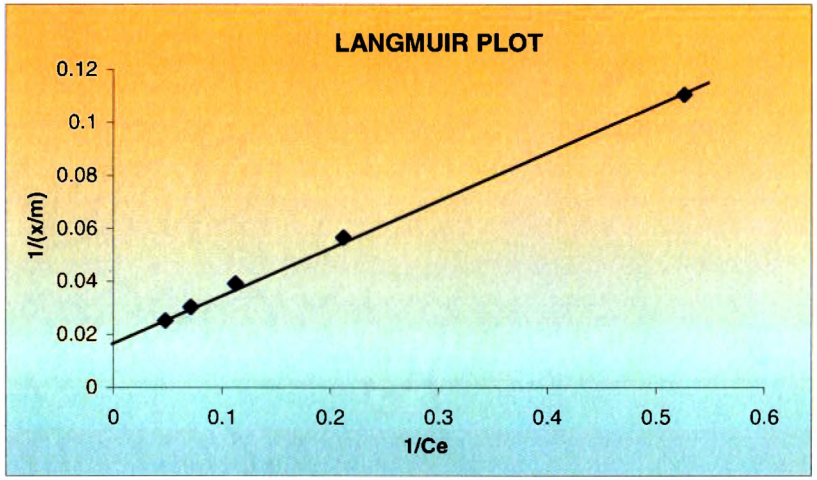
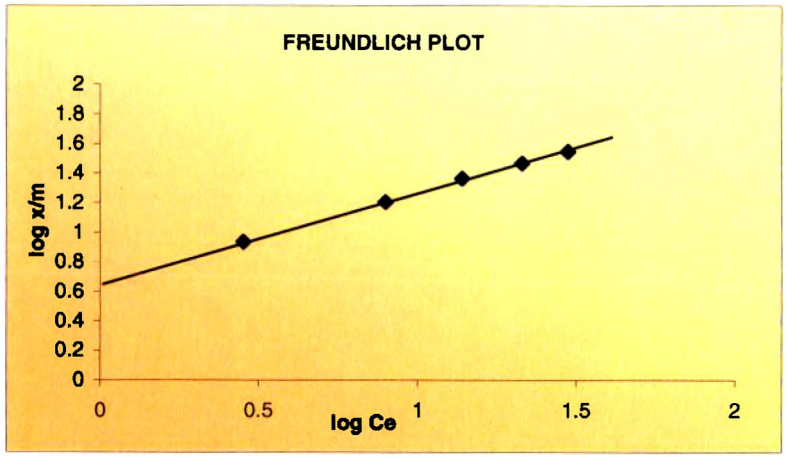
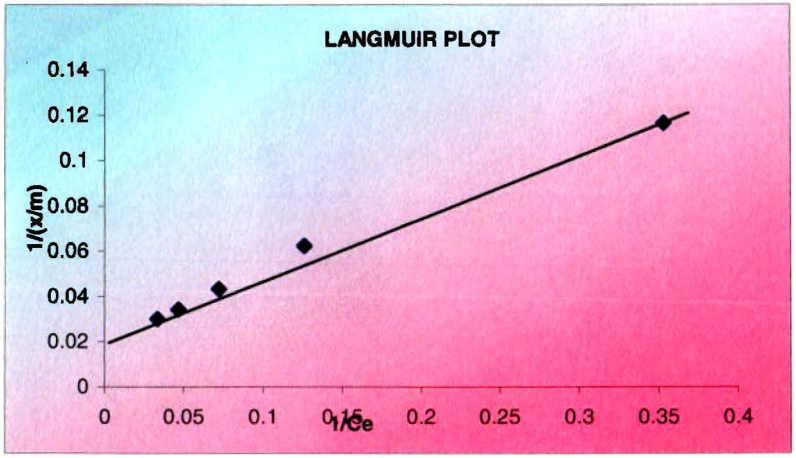


FIGURE 14

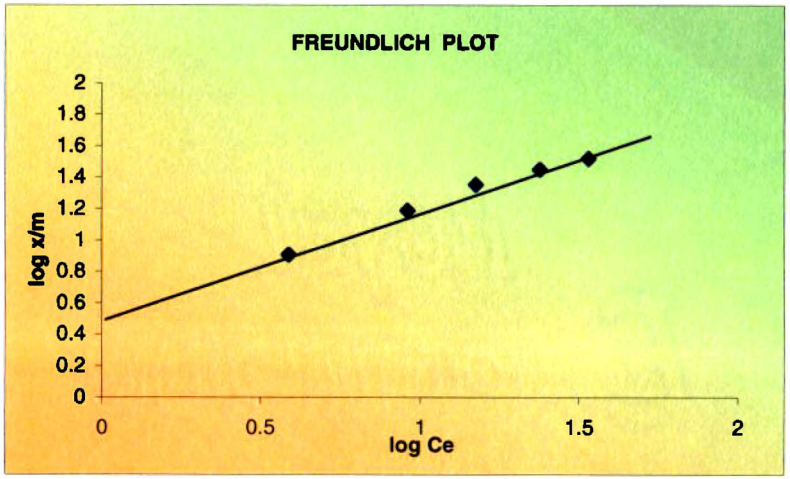
FREUNDLICH AND LANGMUIR ADSORPTION PLOTS FOR MODIFIED SUGARCANE BAGASSE AND GROUNDNUT SHELL CARBON AT DIFFERENT INITIAL Cr(VI) CONCENTRATIONS

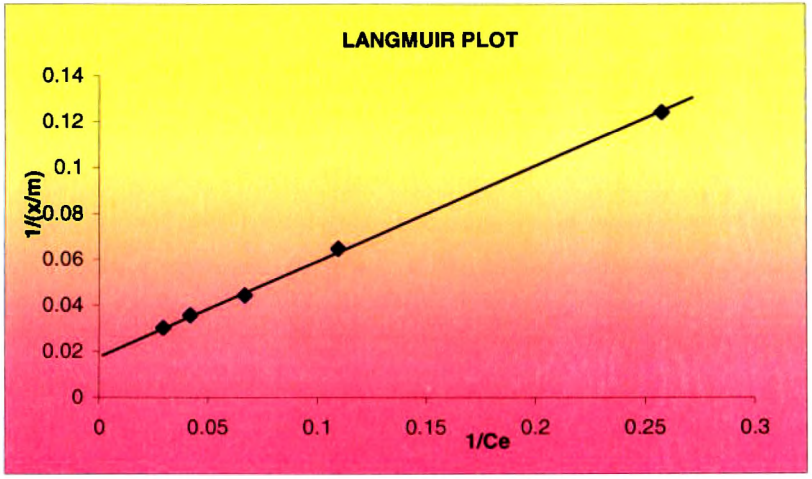
MODIFIED SUGARCANE BAGASSE





GROUNDNUT SHELL CARBON





In the present investigation, the values of 1/n all the cases were found to be less than 1, which suggests the favourable adsorption behaviour of Cr(VI) onto all the selected adsorbents. Jasuja *et al.* (1997) observed the value of 1/n less than 1 in their adsorption studies with *Ablesmoschus esculentus* for the removal of Cr(VI), which indicates a favourable adsorption. According to Chandrasekar and Chakravarthy (2000), the value of n greater than one represents favourable condition. In all the cases in the present study, the value of n obtained was found to be greater than 1 with all the selected adsorbents and this indicated favourable conditions for the sorption of Cr(VI) on these adsorbents.

The values of K was found to be maximum for nitrated coconut shell carbon which was followed by saw dust carbon, sulphonated coconut shell carbon, coripith carbon, modified sugarcane bagasse and groundnut shell carbon.

The regression analysis of the adsorption data revealed a correlation coefficient (r) for Freundlich adsorption isotherm to be between 0.991 – 0.999.

The sorption data for all the selected adsorbents analysed in the light of Langmuir's model are also presented in Tables 29, 30, 31, 32, 33 and 34.

The linear plots of 1/ x/m vs $1/C_e$ suggest the applicability of the Langmuir model which is in the form

$$\frac{1}{x/m} = \frac{1}{q_o} + \left(\frac{1}{q_o b}\right)\frac{1}{C_e}$$

where, C_e is the equilibrium concentration (mg/l) and x/m is the amount of Cr(VI) adsorbed at equilibrium (mg/g). q_o and b are Langmuir constants related to the adsorption capacity and equilibrium constant respectively (Swamy *et al.,* 1998). The linear plots of 1/x/m versus $1/C_e$ clearly indicate monolayer coverage of adsorbate at the outersurface of the sorbent (Raji and Anirudhan, 1999).

Figures 12, 13, and 14 show the plot of 1/ x/m versus $1/C_e$ for Cr(VI) adsorption on selected agrowaste adsorbents. The linear nature of all the plots showed the applicability of Langmuir isotherms.

The parameters of q_o and b have been calculated from the slope and intercept of the plots (Table 35). The Langmuir constant q_o was high for sulphonated coconut shell carbon when compared to the other adsorbents suggesting the higher adsorption capacity of sulphonated coconut shell carbon. The q_o value for the adsorption of o-cresol has been reported by Swamy *et al.* (1998) as 313.68, 179.04 and 69.08 mg/g for activated carbon (laboratory grade), bagasse flyash and activated carbon (commercial grade) respectively. The q_o values for the adsorption of As(III) have been reported by earlier workers as 240.73, 50.83, 3.3 and 0.03 mg/g for modified saw dust carbon (Raji and Anirudhan, 1999), activated carbon (Rajakovic and Mitrovic, 1992), coal fly ash (Sen and Arnab, 1987) and Ganga sand (Vaishya and Agarwal, 1993) respectively.

TABLE 35

FREUNDLICH AND LANGMUIR SORPTION ISOTHERM CONSTANTS FOR THE SELECTED AGROWASTE ADSORBENTS

Absorbent	Freundlich isotherm constants			Langmuir isotherm constants		
	K	$\frac{1}{n}$	r	q_o mg g^{-1}	b mg^{-1}	r
Nitrated coconut shell carbon	8.433	0.652	0.991	71.428	1.147×10^{-1}	0.997
Sulphonated coconut shell carbon	6.309	0.713	0.998	76.923	7.83×10^{-2}	0.999
Coir pith carbon	5.470	0.635	0.993	62.5	7.11×10^{-2}	0.999
Saw dust carbon	6.382	0.617	0.996	55.55	1.022×10^{-1}	0.999
Modified sugarcane bagasse	4.591	0.604	0.999	47.619	7.63×10^{-2}	0.992
Groundnut shell carbon	3.475	0.658	0.993	55.55	4.36×10^{-2}	0.999

The correlation coefficient (r) obtained by the regression analysis of Langmuir isotherm was between 0.992 and 0.999.

In the present study, it was revealed that the selected agrowaste adsorbents obey both Langmuir and Freundlich adsorption isotherm models at varying initial concentrations with fixed dose of adsorbent. Siddiqui *et al.* (1999) showed that the adsorption data for Zn^{2+} and Ni^{2+} sorption on phosphate treated saw dust were fitted to the linear form of both Freundlich and Langmuir models whereas Cu^{2+} did not follow Langmuir and Freundlich models. Bhalke *et al.* (1999) observed that the heavy metals Pb and Sr sorption on sunflower plant dry powder followed both Langmuir and Freundlich adsorption models.

4.5.6 Adsorption of Cr(VI) from tannery effluent using the selected agrowaste adsorbents

The suitability of the selected agrowaste adsorbents for the removal of Cr(VI) from tannery effluent was studied under the optimum pH, contact time and adsorbent dose (identified in the earlier studies as explained in section 4.5.2, 4.5.3 and 4.5.4) and the values are presented in Table 36.

TABLE 36

PERCENTAGE ADSORPTION OF CR(VI) FROM THE TANNERY EFFLUENT USING THE SELECTED AGROWASTE ADSORBENTS

Adsorbents	Percentage adsorption of Cr(VI)
Nitrated coconut shell carbon	82.21
Sulphonated coconut shell carbon	80.16
Coirpith carbon	70.82
Saw dust carbon	73.86
Modified sugarcane bagasse	65.28
Groundnut shell carbon	75.13

Values are mean of three replicates
Temperature - 30°C
Contact time - 3 hours
Adsorbent dose - 20g/l
pH - 4.0

Among the selected agrowaste adsorbents, nitrated coconut shell carbon recorded an adsorption of 82.21% of Cr(VI) which was followed by sulphonated coconut shell carbon (80.16%), groundnut shell carbon (75.13%), saw dust carbon (73.86%), coirpith carbon (70.82%) and modified sugarcane bagasse (65.28%). The removal of Cr(VI) from the tannery effluent with all the selected agrowaste adsorbents was less compared to the removal from the synthetic solution under optimum experimental conditions. This might be due to various interfering ions present in the tannery effluent. Vasanthy and Lakshmana perumalsamy (1993) reported that the leaf material prepared from *Thespia populnea*, adsorbed 90% of Cr(VI) from tannery waste water under optimum conditions.

Summary and Conclusions

5.0 SUMMARY AND CONCLUSION

Industrialization is linked with economic growth, national prosperity and high standard of living of the citizens. Increased industrialization leads to deterioration of environmental quality and subsequently to health hazards of man. However, industrialization cannot be sacrificed in view of the impending threat to environmental quality. Deployment of appropriate remedial measures for the treatment of effluents shall avoid environmental pollution.

Tannery is one of the important industrial ventures in several districts of Tamil Nadu. There is a phenomenal growth in Indian leather industry every year. A variety of chemicals and plant products are used in the tanneries. Chromium is one of the most widely used metals in the industry, resulting in large quantities of this element being discharged into the environment. Both trivalent chromium and hexavalent chromium exist in various bodies of water in a variety of forms. Cr(VI) is very toxic and is carcinogenic and mutagenic. Cr(III) on the otherhand, is much less toxic and significantly less soluble than Cr(VI). Also, the presence of relatively low concentration of Cr(VI) in effluent can significantly reduce the efficacy of biological sewage treatment.

Several physical and chemical methods are available for removing metal ions from industrial effluents. Some disadvantages of these methods include high operating costs, the requirement for preliminary treatment steps and the difficulty of treating the solid waste subsequently generated. Biological methods using microorganisms for the removal of metal ions from effluents has achieved growing attention. Lower cost and higher efficiency at low metal concentrations make biotechnological process very attractive in comparison to physicochemical methods for the heavy metal removal. Also, the application of activated carbon for the removal of heavy metals by adsorption process from the industrial wastes is a common practice. However, the high cost and the difficulty of procuring commercial activated carbon paved the way to produce indigenous low cost activated carbons from agricultural wastes. Hence, the present study was undertaken with a view to develop microbial processes for the removal of Cr(VI)

from tannery effluents. The study was also focussed to assess the possibility of using agro waste materials for Cr(VI) removal.

The study was carried out in five phases:

In the first phase, toxicological assessment of tannery effluent was attempted. The untreated effluent samples were collected from the discharge point from a tannery industry at Erode. These samples were analysed for the physicochemical parameters namely, colour, pH, electrical conductivity (EC), turbidity, total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorides, sulphates, ammoniacal nitrogen, oil and grease, sulphides, sodium, potassium, calcium, magnesium, phenolic compounds, total chromium, hexavalent chromium, copper, zinc and iron.

The second phase of the study included isolation and characterisation of bacteria and fungi from the tannery sludge and effluent samples. Several bacterial species were isolated from tannery effluent and sludge and were screened for Cr(VI) tolerance by inoculating into a medium amended with increasing concentrations of Cr(VI) ranging from 50 to 250 mg/.The bacterial species which were highly tolerant to Cr(VI) were selected and identified based on morphological and biochemical tests and their growth pattern was studied. The fungal species were isolated from the sludge sample after an enrichment for 15 days, screened for Cr(VI) tolerance by inoculating into the solid medium amended with various concentrations of Cr(VI) ranging from 10 to 30 mg/l. The fungal isolates which were highly tolerant to Cr(VI) were selected and identified based on their morphological characteristics.

The third phase of the study was performed in order to assess the efficacy of the selected bacterial isolates in the uptake of Cr(VI) from synthetic solution and the tannery effluent. The influence of various factors namely cell age, metal ion concentration, tannic acid, sodium chloride, metabolic state of selected bacterial isolates and incubation period on the uptake of Cr(VI) was studied.

The fourth phase of the study included Cr(VI) uptake processes using growing and dead cells of selected fungal isolates. The effect of various parameters namely chromium levels in the tannery effluent, temperature and pH on Cr(VI) uptake and growth were studied with live cells of selected fungal isolates. The fungal biosorption studies were carried out with dead cells at various experimental conditions namely pH, initial metal concentration and biomass concentration. The effect of Cr(VI) concentration on fungal metabolism was also studied in which mycelia were analysed for total carbohydrates, total protein, phosphorus, RNA and DNA. Cell walls of selected fungal isolates were analysed for total carbohydrates, total protein and phosphorus.

In phase five, chromium uptake studies were carried out using selected agrowaste adsorbents namely nitrated coconut shell carbon, sulphonated coconut shell carbon, coirpith carbon, saw dust carbon, modified sugarcane bagasse and groundnut shell carbon. Batch studies were carried out under varying experimental conditions namely pH, adsorbent dose, initial chromium concentration and contact time and the results were analysed using Freundlich and Langmuir adsorption isotherm.

Salient findings of the study are summarised as follows:

Phase I

The results of the study revealed that the tannery effluent was strongly coloured and was turbid. The pH of the effluent was found to be within the tolerance limit prescribed by the Bureau of Indian Standards for the discharge of waste water into inland surface waters. The physicochemical parameters studied in the effluent namely TSS, TDS, BOD, COD, chlorides, sulphates, sulphides, calcium, magnesium, sodium, potassium and ammoniacal nitrogen were very much above the permissible limits. However, phenolic compounds and oil and grease were found to be within the tolerance limit.

The heavy metals namely total chromium, hexavalent chromium, copper and iron in the tannery effluent were found to be very much above the tolerance limit whereas zinc content was found to be within the tolerance level.

The results of phase I study clearly suggested the hazardous impact of the tannery effluent on the environment and this demanded the discharge of the waste water from this industry after proper treatment.

Phase II

The selected isolates from the effluent were identified as *Pseudomonas sp., Proteus sp., Bacillus sp.* and *Staphylococcus sp.* The growth pattern of these selected bacterial isolates indicated that the duration of exponential growth phase was 8-10 hours for *Pseudomonas sp., Proteus sp.* and *Bacillus sp.* The exponential growth phase for Staphylococcus sp. was found to be between 10 and 12 hours. The screening study for Cr(VI) tolerance revealed that Cr(VI) up to 200 mg/l permitted the growth of *Pseudomonas sp.,* up to 160 mg/l permitted the growth of P*roteus sp.,* up to 300 mg/l permitted the growth of *Bacillus sp.* and up to 400 mg/l permitted the growth of *Staphylococcus sp.*

Based on the morphological characteristics, the selected fungal isolates were identified as *Aspergillus terreus, Cladosporium sp.* and *Colletotrichum sp.* In view of Cr(VI) tolerance, *Aspergillus terreus* and *Cladosporium sp.* showed growth up to 160 mg/l concentration of Cr(VI) whereas *Colletotrichum sp.* was found to be unaffected up to 300 mg/l and no growth was observed after this concentration.

Phase III

The influence of cell age on Cr(VI) uptake revealed that maximum uptake of Cr(VI) was obtained when the cells used as inoculum were harvested at the end of 10 hours of growth in the case of *Pseudomonas sp., Proteus sp.* and *Bacillus sp.* and 15 hours of growth in the case S*taphylococcus sp.* From the study, it became evident that the ability of the cells to accumulate Cr(VI) was strongly influenced by culture age. Cr(VI) uptake was influenced by initial metal ion concentration where all the selected bacterial isolates showed a decreasing trend in the uptake of Cr(VI) when the initial metal concentration was increased.

The environmental parameters namely tannic acid and sodium chloride were found to influence the uptake of Cr(VI) by all the selected isolates where they recorded a decrease in the Cr(VI) uptake with increasing tannic acid and sodium chloride concentrations. A comparative study of Cr(VI) uptake by resting, dead and metabolically inhibited cells at varying incubation periods revealed that the resting cells *Pseudomonas sp.* recorded an increased Cr(VI) uptake with increasing incubation period upto 48 hours and thereafter no significant increase was observed with further increase in the time of incubation. However, the dead cells and metabolically inhibited cells of *Pseudomonas sp.* showed an increased percentage uptake of Cr(VI) upto the incubation period of 24 hours.

Resting and metabolically inhibited cells of *Proteus sp.* recorded an increased percentage uptake of Cr(VI) upto 24 hours whereas dead cells showed the percentage increase of Cr(VI) upto 18 hours. A significant increase in the percentage uptake of Cr(VI) was observed among resting and dead cells of *Bacillus sp.* and metabolically inhibited cells of *Staphylococcus sp.* upto 24 hours of incubation period whereas metabolically inhibited cells of *Bacillus sp.* and resting and dead cells of *Staphylococcus sp.*upto 48 hours of incubation period.

Phase IV

Cr(VI) uptake by growing cells of selected fungal isolates at various dilutions of tannery effluent showed an increasing trend in the uptake with increasing dilution. *Colletotrichum sp.* recorded a maximum uptake of 91.20% of Cr(VI) at the highest dilution of 1:100 which was followed by *Aspergillus terreus* (89.30%) and *Cladosporium sp.* (75.17%). The growth interms of biomass was also significantly increased with increasing dilution with all the three fungal isolates. The maximum uptake of Cr(VI) and growth were recorded at 40°C with *Aspergillus terreus*, 30°C with *Cladosporium sp.* and 35°C with *Colletotrichum sp.* The pH also had an influence on the growth and Cr(VI) uptake with the selected fungal isolates. The maximum uptake of Cr(VI) was observed at pH 5.0 with *Aspergillus terreus* and *Cladosporium sp.* whereas *Colletotrichum sp.* showed a maximum uptake of Cr(VI) at pH 6.0.

The Cr(VI) uptake by dead cells of the selected fungal isolates was also influenced by metal ion concentration, pH, biomass concentration and temperature. The dead biomass of *Colletotrichum sp.* was found to remove greatest percentage of Cr(VI) (98.9%) at the lowest initial concentration of 10 mg/l which was followed by *Aspergillus sp.* (95%) and *Cladosporium sp.* (93.8%)The maximum uptake of Cr(VI) was found to occur between pH 4 and 5 with the biomass of all the selected fungal isolates. It was observed that the percentage removal of Cr(VI) increased with increase in the biomass dose of all the selected fungal isolates indicating increased availability of the active sites for biosorption. The influence of temperature on Cr(VI) uptake by the dead biomass showed that the uptake of Cr(VI) increased with increasing temperature up to 30°C with all the selected fungal isolates.

The growth in terms of biomass was decreased with increasing concentrations of Cr(VI) with all the three fungal isolates. The total carbohydrate content in the mycelia of all the selected fungal isolates was significantly increased when compared to their respective controls. This might be due to the accumulation of carbohydrates in the mycelial mats which seemed to be a mechanism for the detoxification of metals by the fungi. In contrast, the carbohydrate content of the cell wall of all the selected fungal isolates was found to be decreased significantly with increasing concentrations of Cr(VI). The protein content of the mycelia of *Aspergillus terreus* was significantly increased with increasing concentration of Cr(VI) whereas a significant decrease was noticed in the mycelia of *Colletotrichum sp.* There was no significant difference in the protein content of *Cladosporium sp.* up to a concentration of 50 mg/l and thereafter a significant decrease was noticed with increasing concentrations. The protein contents in the cell walls were found to be significantly increased in all the selected fungal isolates, when compared to their controls. There was no significant change in the phosphorus content in the mycelia of all selected fungal isolates at lower concentrations whereas significant decrease was obtained at higher concentrations. In contrast, the phosphorus content in the cell wall of all the selected fungal isolates was found to be increased with increasing metal ion concentration as compared to their controls. The nucleic acids (RNA and DNA) level in all the selected fungal isolates was found to be increased at lower initial

concentrations of Cr(VI) whereas it was decreased at higher initial concentrations. These results provide evidence for the resistance of selected fungal isolates at lower concentrations and the toxic effects at higher concentrations of Cr(VI).

Phase V

The characteristics of the selected agrowaste adsorbents were studied and it was found that the bulk density was high in nitrated and sulphonated coconut shell carbons when compared to other agrowaste adsorbents. The lowest moisture content was recorded in nitrated coconut shell carbon and highest moisture content was recorded in modified sugarcane bagasse. The ash content and volatile matter content were low in nitrated and suphonated coconut shell carbons when compared to other agrowaste adsorbents.

All the selected agrowaste adsorbents recorded an increased percentage uptake of Cr(VI) with decreasing pH from 7.0 to 4.0. The percentage removal of Cr(VI) on all the selected agrowaste adsorbents was found to be significantly increased with increase in the adsorbent dose. The percentage removal of Cr(VI) was found to be greater with lower initial concentrations and lesser with higher initial concentrations with all the selected adsorbents. The study also revealed that the percentage removal of Cr(VI) was dependent on contact time and maximum removal was obtained at 120 minutes and thereafter no significant increase in Cr(VI) was obtained with all the selected agrowaste adsorbents. The adsorption data of all selected adsorbents at different initial concentrations of Cr(VI) with a constant dose of 20g/l was fitted to linearlised Freundlich and Langmuir adsorption isotherms.

Among the selected agrowaste adsorbents, nitrated coconut shell carbon recorded an adsorption of 82.21% of Cr(VI) which was followed by sulphonated coconut shell carbon (80.16%), groundnut shell carbon (75.13%), saw dust carbon (73.86%), coirpith carbon (70.82%) and modified sugarcane bagasse (65.28%). The removal of Cr(VI) from the tannery effluent with all the selected agrowaste adsorbents was less compared to the removal from the synthetic solution under optimum experimental conditions.

The following conclusions may be drawn from the present investigation:

- Pollution load of the effluent from tannery industry was found to be very high.

- The chromium tolerant bacterial and fungal isolates were proved to be efficient in the removal of Cr(VI) from tannery effluent.

- All the four selected bacterial isolates were able to reduce hexavalent chromium to trivalent chromium which seemed to be one of the mechanisms to reduce hexavalent chromium toxicity.

- Both live and dead cells of selected bacterial isolates were efficient in the removal of hexavalent chromium from tannery effluent and more than 98% removal of hexavalent chromium was achieved.

- All the selected fungal isolates were found to be efficient in the removal of hexavalent chromium at wide range of pH, temperature and initial metal ion concentrations.

- The metabolic studies indicated that hexavalent chromium was found to be toxic to selected fungal isolates at only higher concentrations.

- All the selected agrowaste adsobents were proved to be efficient in the removal of hexavalent chromium.

- The adsorption data was fitted to both Freundlich and Langmuir isotherms.

- Nitrated coconut shell carbon recorded maximum uptake of Cr(VI) from the tannery effluent.

These studies clearly hold promise for the effective, economical and eco-friendly metal remediation technologies for industrial exploitation and pollution free environment.

Scope for further studies:

- Large scale treatment technology can be designed using the selected microorganisms and agrowaste adsorbents for the bioremediation of tannery industry pollution load.

- Role of mixed culture of selected microorganisms in the biosorption process can be studied.

- Studies can be carried out on cell wall modification of microbial cells in order to enhance the metal binding capacity of biomass.

- Processes can be developed for metal recovery using suitable desorbing agents without damaging the biosorbent so that it could be regenerated.

- The viable and nonviable cells of microorganisms can be immobilized into suitable matrices and studied for their efficiency in the removal of hexavalent chromium.

- Rate of bioremediation can be further enhanced by developing engineered noval strains of microorganisms with increased Cr(VI) reducing efficiency.

- Even though the sorption capacity of selected agrowaste absorbents was good, studies can be carried out to assess the possibility of increasing this potential by means of either chemical or physical treatment.

Bibliography

BIBLIOGRAPHY

Abel, P.D. (1989), Water Pollution Biology, Ellis Horwood, England, 231-233.

Agrawal, K.C. (1999), Environmental Biology, Agro Botanica, India, 342 - 346.

Ajmal, M., Mohammad, A and Anwar,S.(2001), Sorption studies of heavy metals on teak leaves *(Tectona grandis)* using thin-layer and column chromatographic techniques, Pollution Research, 20(3), 425 - 428.

Aksu, Z and Donmez, G. (2001), A comparative study on the biosorption characteristics of some yeasts for remazol blue reactive dye, Chemosphere, 50(8), 1075-1083.

Aneja, K.R. (1996), Identification of unknown microorganisms, In: Experiments in microbiology, plant pathology, tissue culture and mushroom cultivation, Second Edition, Wishwa Prakashan New Age International (P) Limited, New Delhi, 218 - 234.

Anirudhan, T.S and Sreedhar, M.K.(1999), Mercury(II) adsorption and desorption characteristics of coconut husk based carbon- kinetics of self diffusion, Indian Journal of Environmental Protection, 19(1), 6 -10.

APHA. (1989), Standard Methods for the examination of water and wastewater, Seventeenth Edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington DC, 2005 - 2605, 5.2 - 5.10.

APHA. (1998), Standard Methods for the examination of water and wastewater, Twentieth Edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, 2005-2605, 2.36 - 2.39, 2.56 - 2.58, 3.1 - 3.99, 4.105 - 4.178, 5.2 - 5.10.

Arakawa, H., Ahmad, R., Naout, M and Riahi, H.A.T. (2000). A comparative study of calf thymus DNA binding to Cr(III) and Cr(VI) ions, Journal of Biological Chemistry, 275, 10150-10153.

Arica, M.Y., Kacar, Y and Genc,O. (2001), Entrapment of white rot fungus *Trametes versicolor* in Ca-alginate beads: Preparation and biosorption kinetic

analysis for cadmium removal from an aqueous solution, Bioresource Technology, 80(2), 121-129.
Arnfalk, P., Wasay, S.A and Tokunaga, S. (1996), A comparative study of Cd, Cr(III), Cr(VI), Hg and Pb uptake by minerals and soil materials, Water, Air and Soil Pollution, 87,131-148.

Athar, M and Vohora, S.B. (1995), Heavy metals and environment, New Age International Publishers Limited, New Delhi, 49 - 51.

Atlas, R.M. (1995), Bioremediation, Chemical Engineering News,3,32- 42.

Babich, H and Stotzky,G.(1978), Toxicity of zinc to fungi, bacteria and coliphages: Influence of chloride ions, Applied and Environmental Microbiology, 36,906 - 914.

Backyavathi, D.M and Kumar, N.V.N. (1995), Occupational environmental health hazards of chromate industrial workers, Journal of Industrial Pollution Control, 11(1), 29-37.

Bader, J,L., Gonzalez, G., Goodell, P.C., Ali, A.S and Dillai S.D. (1999), Chromium-resistant bacterial populations from a site heavily contaminated with hexavalent chromium, Water, Air and Soil Pollution, 109,263-276.

Bai, S and Abraham, T.E. (2001), Biosorption of Cr(VI) from aqueous solution by *Rhizopus nigricans,* Bioresource Technology, 79(1), 73-81.

Baker, A.J.M., Reeves, R.D and Hajar, A.S.M. (1994), Heavy metal accumulation and tolerance in British populations of the metallophyte *Thiaspicaerulescens (Brassicaceae),* New Phytology, 127, 61-68.

Baldi, F., Vaughan, A.M and Olson, G.J. (1990), Chromium (VI). resistant yeast isolated from a sewage treatment plant receiving tannery wastes, Applied and Environmental Microbiology, 56(4), 913-918.

Balkose, D and Baltacioglu, H. (1992), Adsorption of heavy metal cations in aqueous solutions by wool fibers, Journal of Chemical Technology and Biotechnology, 54, 393 - 397.

Basketter, D., Horev, L., Slodovnik, S., Merimes, S., Trattner, A and Ingber ,A. (2001), Investigation of the threshold for allergic reactivity to chromium, Contact Dermatitis, 44,70-74.

Bergey's Manual of Determinative Bacteriology. (1994) (Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T and Williams, T (Eds)), Williams and Wilkins, Baltimore, Maryland , USA, 93-94, 151-184, 532-568.

Bhainsa, K.C and D'Souza, S.F, (2001), Uranium (VI) biosorption by dried roots of *Eichhornia crassipes* (water hyacinth), Journal of Environmental Science and Health. Part A Toxic /Hazardous Substances and Environmental Engineering. 36(9), 1621-1631.

Bhalke, S., Tripathi, R.M., Mahapatra, S., Sastry, V.N and Krishnamoorthy, T.M (1999), Uptake of heavy metals by sunflower plant-dry powder, Research Journal of Chemistry and Environment, 3(2), 9-14.

Bharani, A.(1999), Fungal biosorption - An alternative treatment for heavy metal bearing wastewaters, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22 & 23, Tamil Nadu Agricultural University, Coimbatore, India, 22 - 24.

Bhattacharya, R.N. (1986), First Edition, Experiments with Microorganisms, Emkay Publications, Delhi, 175-178.

Bolan, N.S., Adriano, D.C., Natesan, R and Koo, B.J. (2003), Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil, Journal of Environmental Quality, 32, 120-128.

Brawerman, G. (1974), Eukaryotic messenger RNA, Annual Review of Biochemistry, 43, 621-642.

Bridge, T.A.M., White, C and Gadd, G.M. (1999), Extracellular metal-binding activity of the sulphate-reducing bacterium *Desulfococcus multivorans*, Microbiology, 145, 2987-2995.

Brown, P.A., Gill,S.A and Allen, S.J. (2000), Metal removal from wastewater using peat, Water Research, 34(16), 3907-3916.

Cervantes, C and Corona, G.F. (1994) Copper resistance mechanisms in bacteria and fungi, FEMS Microbiology Reviews., 14,121-138.

Cervantes,C., Ohtake,H., Chu,L., Misra, T.K and Silver ,S.(1990) Cloning, nucleotide sequence and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid P^{UM} 505, Journal of Bacteriology, 172, 287-291.

Cervantes, C., Garcia, C.J., Devars,S., Corona, G.F., Tavera,L.H., Guzman, T.J.C and Sanchez, M.R. (2001), FEMS Microbiology Reviews., 25(3), 335 - 347.
Chandra,R. (2001), Microbial treatment of tannery effluent, Indian Journal of Microbiology, 41,103 -106.

Chandrasekar, M and Chakravarthy, T. (2000), Study of removal of zinc by coal and clay minerals, Journal of Indian Water Works Association, 32(4), 305 - 307.

Chhonkar, P.K., Datta, S.P., Joshi, H.C and Pathak, H (2000), Impact of Industrial effluents on soil health and agriculture- Indian experience: Part II - tannery and textile industrial effluents, Journal of Scientific and Industrial Research, 59, 446 - 454.

Codd, R., Dillon, C.T., Levina,A and Lay, P.A.(2001), Studies on the genotoxicity of chromium from the test tube to the cell, Coordination Chemistry Reviews., 216 - 217, 537 - 582.

Codina, J.C., Torrente, C.P., Garcia, A.P., Cazorla, F.M and Devicentc, A.(1995), Comparison of microbial tests for the detection of heavy metal genotoxicities, Archives in Environmental Contamination and Toxicology, 29,260 - 265.

Cole, M.A. (1977), Lead inhibition of enzyme synthesis in soil, Applied and Environmental Microbiology, 33, 262.

Dadhich, A.S., Maruthi, Y.A and Srinivas, P. (2002), The fate of chromium in water and soil: A case study of Andhra Pradesh tanneries, Journal of Ecotoxicology and Environmental Monitoring, 12(2), 139 - 145.

Dayan, A.D and Paine, A.J. (2001), Mechanisms of chromium toxicity, carcinogencity and allergenicity: Review of the literature from 1985 to 2000, Human and Experimental Toxicology, 20, 439 - 451.

De Flora, S. (2000), Threshold mechanisms and site specificity in chromium (VI) carcinogenesis, Carcinogenesis,21(4), 533 - 541.

De Leo, P.C and Ehrlich, H.L. (1994), Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB 300 in batch and continuous cultures, Applied Microbiology and Biotechnology, 40, 756 - 759.

Devi, P.S., Babu, G.V.A.K., Narasimha,G and Reddy, B.R.(1995), Effect of heavy metals on activities of amylase and invertase in soil, Proceedings of the National Symposium on Frontiers in Applied and Environmental Microbiology, December. 11-13, SES, CUSAT, Cochin, India, 217 - 219.

Dias, M,A., Rosa, C.A., Linardi, V.R., Conte R.A and De Castro H.F. (2001), Application of factorial design to the study of heavy metals biosorption by waste biomass from beverage distillery, Applied Biochemistry and Biotechnology, 91-93, 413 - 422.

Dias, M.A., Lacerda, I.C., Pimentel, P.F., De Castro, H.F and Rosa, C.A(2002), Removal of heavy metals by an *Aspergillus t*erreus strain immobilized in a polyurethane matrix, Letters in Applied Microbiology, 34(1), 46 - 50.

Elizabeth, K.M and Anuradha, T.V.R.(2000), Biosorption of hexavalent-chromium by non-pathogenic bacterial cell preparations, Indian Journal of Microbiology, 40, 263 - 265.

Fiske, C.H and Subbarow, Y. (1925), The colorimetric determination of phosphorus, Journal of Biological Chemistry, 66, 375.

Fontaine,T., Simenel,C., Duberucq, G., Adam,O., Delepierre,M., Lemoine,J., Vorgias,C.E., Diaquin,M and Latge,J.P. (2000), Molecular organisation of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall, Journal of Biological Chemistry, 275(36), 27594 - 27607.

Fourest, W., Canal, C and Roux J.C. (1994), Improvement of heavy metal biosorption by mycelial dead biomasses *(Rhizopus arrhizus, Mucor miehei* and *Penicillium chrysogenum):* pH control and cationic activation, FEMS Microbiology Reviews, 14(4): 325 - 332.

Freeman, S and Katan.T. (1997) Identification of *Collectotrichum* species, Phytopathology, 87, 516 - 521

Fude,L., Harris,B., Urrutia, M.M and Beveridge, T.J .(1994), Reduction of Cr(VI) by a consortium of sulfate-reducing bacteria (SRB III), Applied and Environmental Microbiology, 60, 1525 - 1531.

Fujie,K., Tsuchida,T., Urano, K and Ohtake, H. (1994), Development of bioreactor system for the treatment of chromate wastewater using *Enterobacter cloacae* HO -1, Water Science and Technology, 30, 235 - 243.

Gadd,G.M.(1990), Heavy metal accumulation by bacteria and other microorganisms, Experientia, 46, 834 - 840.
Gadd, G.M.(2000), Bioremedial potential of microbial mechanisms of metal mobilization and immobilization, Current Opinion in Biotechnology, 11, 271-279.

Gadd, G.M and Griffiths, A.J. (1980), Effect of copper on morphology of *Aureobasidium pullulans,* Transactions of the British Mycological Society, 74, 387.

Gadd, G.M and White.C.(1993), Microbial treatment of metal pollution-a working technology, Trends in Biotechnology, 2, 353 - 359.

Galli, A., Boccardo, P., Del Carratore, R., Cundani, E and Bronmetti, G. (1985), Conditions that influence the genetic activity of potassium dichromate and chromium chloride in *Saccharomyces cerevisiae*, Mutation Research, 144, 165-170.

Gao,S., Walker,W.J, Dahlgren, R.A and Bold, J. (1997), Simultaneous sorption of Cd, Cu, Ni, Zn, Pb and Cr on soils treated with sewage sludge supernatant, Water, Air and Soil Pollution, 93, 331 - 345.

Gendusa,T.C., Beitinger, T.L and Rodgers, J.H. (1993), Toxicity of hexavalent chromium from aqueous and sediment sources to *Pimephales promelas* and *Ictalurus punctatus*, Bulletin of Environmental Contamination and Toxicology,50,144 -151.

Giles, K.W and Meyer, A. (1965), An improved diphenylamine method for the estimation of DNA, Nature, 206, 93 - 94.

Gilman,J.C.(1967), A manual of soil fungi, Revised Second Edition, Oxford and IBH Publishing Co., Calcutta, Bombay, New Delhi, 215 - 234, 334.

Goel, P.K. (1997), Water Pollution: Causes, effects and control, New Age International (P) Limited Publishers, New Delhi, 269.

Golab, Z and Orlowska,B.(1991), Biosorption of lead and uranium by *Streptomyces sp.*, Water, Air and Soil Pollution, 60, 99-106.

Gunasekaran, G. (1999), Microbial decontamination of heavy metals, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22&23, Tamil Nadu Agricultural University, Coimbatore, India,1-3.

Gupta, R.D, (2000), Metal pollution-hazards, sources and management, Environment and People, 9,10 -13.
Gupta, R.D. (2002), Heavy metal pollution - threat to human life, Environment and People, 7, 32 - 35.

Gupta, B and Anjum, N. (2002), Radiation grafted membranes: Innovative materials for the separation of toxic metal ions from industrial effluent, Indian Journal of Environmental Health, 44(2), 154 -163.

Gupta, R and Mohapatra, H.(2003), Microbial biomass: An economical alternative for removal of heavy metals from waste water, Indian Journal of Experimental Biology, 41, 945 – 966.

Gupta,R., Ahuja,P., Khan,S., Saxena,R.K and Mohapatra,H.(2000), Microbial biosorbents:Meeting challenges of heavy metal pollution in aqueous solutions, Current Science, 78, 967-973.

Gvozdyak, P.I., Mogilevich, N.F., Rylskii, A.F and Grishchenko, N.I. (1986), Reduction of hexavalent chromium by collection strains of bacteria, Mikrobiologiya, 55, 770-773.

Hansen, M.B., Rydin, S.R., Menn, T and Johansen, J.D.(2002), Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather, Contact Dermatitis, 47(3), 127-134.

Hemalatha,S., Vasanthy,M and Lakshmanaperumalsamy,P. (1997), Biosortpion of chromium by *Bacillus sp.*HL-1 and *Pseudomonas sp.* HL-2.Journal of Ecotoxicology and Environmental Monitoring, 7(3), 181-184.

Hodge, J.E and Hofreiter, B.T. (1962), Determination of reducing sugars and carbohydrates, In: Methods in Carbohydrate Chemistry (Whistler, R.L and Wolfrom, M.L (Eds)), Academic Press, New York, 380-394.

Horitsu, H., Futo, S., Miyazawa, Y., Ogai, S and Kawai, K. (1987), Enzymatic reduction of hexavalent chromium by hexavalent tolerant *Pseudomonas ambigua* G-1, Agricultural and Biological Chemistry, 51, 2417-2420.

Ingole, N.W and Bhole, A.G. (2000), Bio-accumulation of chromium, nickel and zinc by water hyacinth, Pollution Research, 19(4), 575 - 583.

International Agency for Research on Cancer (IARC). (1990), Monographs on the evaluation of carcinogenic risk to humans: Chromium, nickel and welding, Geneva, Switzerland, 49, 49-256.

Iqbal, M and Saeed, A. (2002), Removal of heavy metals from contaminated water by petiolar felt-sheath of palm, Environmental Technology, 23(10), 1091 -1098.

Ishibashi, Y., Cervantes, C and Silver, S. (1990), Chromium reduction in *Pseudomonas putida,* Applied and Environmental Microbiology, 56(7), 2268-2270.

Ismail, I.M.K., Abdel-Rahman, T.M.A., Ali, M.I.A and Sharaf, E.F. (1991), Effect of zinc and copper on metabolic activities of some fungi, Egyptian Journal of Microbiology, 26(1), 1-13.

Jasuja, K., Parwana, H.K and Rao, A.L.J. (1997), Removal of Cr(VI) from wastewater by *Ablesmoschus esculentus*, Indian Journal of Environmental Health, 39(2), 103-108.

Kamaludeen, S.P.B., Arunkumar, K.R., Arudainayagam,S and Ramasamy, K. (2003), Bioremediation of chromium contaminated environments, Indian Journal of Experimental Biology, 41, 972-985.

Kandasamy, M and Santhaguru, K. (1994), Influence of sewage on physico chemical characteristics of the river Vaigai, Journal of Ecobiology, 6(4), 315-317.

Kannan,N.(1991), A study on removal of nickel by adsorption on flyash, Indian Journal Environmental Protection, 11(7), 514-518.

Kannan,N. (1996), Laboratory Manual in General Microbiology,Palani Paramount Publications, Palani, India, 121-150.

Kannan,N and Vanangamudi, A.(1991), A study on removal of chromium (VI) by adsorption on lignite coal, Indian Journal of Environmental Protection, 11(4), 241-245.

Kapoor, A and Viraraghavan,T. (1995), Fungal biosorption-an alternative treatment option for heavy metal bearing wastewaters: A review, Bioresource Technology, 53. 195-206.

Khangan,V.W., Bankar, D.B and Dara, S.S. (1996), Binding of copper ions with modified *Aegle Marmelos* bark substrate, Journal of Industrial Pollution Control, 12(2), 97-108.

Khovrychev, M.P., Semenov, A.M and Rabotnova, I.L. (1980), The effect of zinc ions on *Candida utilis*, Mikrobiologiya, 49,59 - 62.

Khurshid, S., Basheer,A., Zaheeruddin and Shabeer,M.U.(1998), Effect of waste disposal on water quality in parts of Cochin and Kerala, Indian Journal of Environmental Health, 40(1), 45-50.

Konopka,A., Zakharova,J and Bischoff,M.(1999), Microbial biomass and activity in lead contaminated soil, Applied and Environmental Microbiology, 65(5), 2256 - 2259.

Kotrba, P., Doleckova,L., de Loreuzo,V and Ruml,T. (1999a),Enhanced bioaccumulation of heavy metal ions by bacterial cells due to surface display of short metal binding peptides, Applied and Environmental Microbiology, 65, 1092-1098.

Kotrba,P., Pospisil, P., de Loreuzo, V and Ruml. T. (1999b), Enhanced metallosorption of *Escherichia coli* cells due to surface display of beta-and alpha-domains of mammalian metallothionein as a fusion to LamB protein, Journal of Receptors and Signal Transduction Research, 19, 703 - 715.

Kowshik, M and Nazareth,S. (1999), Biosorption of metals by *Fusarium solani,* Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 1-2, 57-61.

Krejpcio, Z. (2001), Essentiality of chromium for human nutrition and health, Polish Journal of Environmental Studies, 10(6), 399-405.

Krupadam, R.J and Rao, A.V.S.P. (1997), Removal of lead from industrial effluents using coconut shell carbon, Environmental Pollution Control Journal, 11, 52-53.

Lakshmi, S and Sundaramoorthy, P. (2003), Effect of chromium on germination and biochemical changes in blackgram, Journal of Ecobiology, 15(1), 07-11.

Lasat, M.M. (2002), Phytoextraction of toxic metals: A review of biological mechanisms, Journal of Environmental Quality, 31, 109-120.

Lin, R.I and Schjeide, O.A. (1969), Microestimation of RNA by the cupric ion catalysed orcinol reaction, Analytical Biochemistry, 27, 473.

Liu. K.J and Shi, X.(2001), *In vivo* reduction of chromium (VI) and its related free radical generation, Molecular and Cellular Biochemistry, 222(1-2), 41-47.

Liu, K.J., Jiang, J., Swartz, H. M and Shi, X. (1994), Low-frequency EPR detection of Cr(V) formation by Cr(VI) reduction in whole live mice, Archives of Biochemistry and Biophysics, 313, 248-252.

Liu, M., Zhang,H., Zhang, X., Deng,Y., Liu,W and Zhan,H.(2001), Removal and recovery of chromium (III) from aqueous solutions by a spheroidal cellulose adsorbent, Water Environmental Research, 73(3), 322-328.

Lokesha, S and Somashekar, R.K. (1991), Effect of heavy metals on the growth of some fungi-A preliminary assay, Indian Journal of Environmental Health, 33(1), 123-125.

Losi,M.E., Amrhein, C and Frankenberger, W.T.(1994), Biodegradation and bioremediation: Bioremedation of chromate-contaminated groundwater by reduction and precipitation in surface soils, Journal of Environmental Quality, 23, 1141-1150.

Lovely, D.R and Phillips, E.J.P. (1994), Reduction of chromate by *Desulfovibrio vulgaris* and its C_3 cytochrome, Applied and Environmental Microbiology, 60, 726 -728.

Lovely, D.R., Philips, E.J.P., Gorby, Y.A and Landa, E.R. (1991), Microbial reduction of uranium, Nature, 350 (413-416).

Lowry, O.H., Rosebrough, N.J., Farr, A.L and Randall, R.J. (1951), Protein measurement with folin-phenol reagent, Journal of Biological Chemistry, 193, 265 - 275.

Lukaski, H.C. (1999), Chromium as a supplement, Annual Review of Nutrition, 19, 279-302.

Madamba, L.S.P and Pamulaklakin, M.A. (1994), Heavy metals in selected fish species collected from Laguna DeBay, Philippines Journal of Science, 123(2), 135 -146.

Manivasakam, N. (1997), Industrial effluents:Origin, characteristics effects, analysis and treatment, Sakthi Publications, Coimbatore, 85-98, 307-360, 438 - 458.

Manju, G.N and Anirudhan, T.S. (1997), Use of coconut fibre pith-based pseudo activated carbon for chromium(VI) removal, Indian Journal of Environmental Health, 39,4,289-298.

Manju, G.N., Raji, C and Anirudhan,T.S. (1998), Evaluation of coconut husk carbon for the removal of arsenic from water, Water Research, 32, 3062-3070.

Mariappan,V and Rajan,M.R.(2002), Effect of tannery effluent on seed germination and seedling growth of *Parkinsonia aculeate* and *Caesalpinia Coriaria,* Journal of Ecobiology,14(4), 241-246.

Marmur, J. (1961), A method for the isolation of deoxyribo nucleic acid from microorganisms, Journal of Molecular Biology, 3, 208-217.

Maruti, P.M and Akhtar, A. (1995), Bioremediation of toxic metal ions from polluted lake waters and industrial effluents by fungal biosorbent, Current Science, 69, 1023-1030.

Mazumder,S., Rahulkar and Chandhuri,N. (1998), Waste water management-practice for a chrome tanning industry-Part I, Indian Journal of Environmental Health, 40(1), 79-92.

McKay, G and Porter, J.F. (1997), Equilibrium parameters for the sorption of copper, cadmium and zinc ions onto peat, Journal of Chemical Technology and Biotechnology, 69, 309-320.

McLean,J and Beveridge.T.J.(2001), Chromate reduction by a Psendomonad isolated from a site contaminated with chromated copper arsenate, Applied and Environmental Microbiology,67(3),1076-1084.

Menzie,C., Burke, A.M., Grasso,D., Harnois,M., Magee,B., McDonald,D., Montgomery,C., Nichols,A., Pignatello,J., Price,B., Price,R., Rose,J., Shatkin, J.A., Smets,B., Smith,J and Svirsky,S. (2000), An approach for incorporating information on chemical availability in soils into risk assessment and risk-based decision making, Human and Ecological Risk Assessment, 6, 479 - 510.

Metwally, M and Abou-Zeid,A. (1996), Effects of toxic heavy metals on growth and metabolic activity of some fungi, Egyptian Journal of Microbiology, 31(1), 115-127.

Michael,P. (1984). Aquatic environment, In:Ecological Methods for Field and Laboratory Investigations, Tata Mc Graw Hill Publishing Company Limited, New Delhi,92,84-151.

Morales,G.C., Jimenez, M.M.D., Gonzalez, M.P.E and Cid, A.A.P. (2001), Removal of metal ions from aqueous solution by adsorption on the natural adsorbent CACMM2, Journal of Chromatography A, 938 (1-2), 237 - 242.

Muley,D.V., Kamble,G.B and Bhilave, M.P.(2000), Effect of heavy metals on nucleic acids in *Cyprinus carpio*, Journal of Environmental Biology, 21(4), 367-370.

Muller,A.K., Rasmussen,L.D and Sorensen,S.J.(2001), Adaption of the bacterial community to mercury contamination, FEMS Microbiology Letters, 10124, 1-5.

Muraleedharan, T.R., Iyengar, L and Venkobachar, C. (1991), Biosorption; An alternative for metal removal and recovery, Current Science, 61, 379-385.

Murugesan, M and Vasanthy, M. (2003), Biosorption of chromium using *Pseudomonas sp*-S5, Journal of Ecotoxicology and Environmental Monitoring, 13(1), 69-71.

Muzzarelli,R.A.A. (1985), Removal of uranium from solutions and brines by a derivative of chitosan and ascorbic acid, Carbohydrate Polymers,5,85-89.

Nair,S and Krishnamurthi,V.S. (1991), Effect of chromium on growth of *Pseudomonas aeruginosa*, Indian Journal of Experimental Biology, 29(2), 140-144.

Narin,I., Soylak. M., Kayakirilmaz, K., Eki,L and Dogan, M.(2002), Speciation of Cr(III) and Cr(VI) in tannery wastewater and sediment samples on Ambersorb 563 resin, Analytical Letters, 35(8), 1437-1452.

National Leather Industry Development Programme (NLIDP). (2000), SriLanka, 3 -13.

Neves, R.P., Santos, M.T., Pereira, M.L and Jesus, J.P. (2002), Comparative histological studies on liver of mice exposed to Cr(VI) and Cr(V) compounds, Human and Experimental Toxicology, 21, 365-369.

Nies, A., Nies, D.H and Silver, S. (1989), Cloning and expression of plasmid genes encoding resistance to chromate and cobalt in *Alcaligenes eutrophus,* Journal of Bacteriology, 171, 5065 - 5070.

Nygren,O and Wahlberg, J.E. (1998), Speciation of chromium in tanned leather gloves and relapse of chromium allergy from tanned leather samples, Analyst, 123(5), 935 - 937.

Osman, M and Elwy, A.E. (1989), Effect of aluminimum, cobalt and lead nitrate on growth, total protein and nucleic acid in *Verticilioun agaricinium* (link) corda, Bulletant of Faculty of Science, Cairo University, 57, 241-249.

Panda, A. (1995), Uptake studies of copper and zinc pollutants by bone mineral, Indian Journal of Environmental Health, 37(3), 188 -191.

Panswad,T., Chavalparit,O., Chandung,C and Anotai,J.(2001), Economic considerations of chromium recovery from tanning waste water, Waste Management Research, 19(5), 450-455.

Pazirandeh, M., Wells, B.M and Ryan,R.L. (1998), Development of bacterium based heavy metal biosorbents:Enhanced uptake of cadmium and mercury by *Escherichia coli* expressing a metal binding motif, Applied and Environmental Microbiology, 64, 4068 - 4072.

Pearson, D. (1970), The Chemical Analysis of Food, Sixth Edition, J & A, Churchill, London, 289, 462.

Prakash, N.B. (2001), Biokinetic studies of tannery effluent under aerobic oxidation process, Journal of Scientific and Industrial Research, 60,344-347.

Radha, R., Rao, B.P., Gnanamani, A., Arichandran,R and Mariappan,M. (1995), Microbes in tannery wastewater, Proceedings of the Nation Symposium on Frontiers in Applied and Environmental Microbiology, December 11-13, SES, CUSAT, Cochin, India, 19-21.

Raghuramulu, N., Nair, K.M and Sundaram, S.K. (1983), A Manual of Laboratory Techniques, First Edition, National Institute of Nutrition, ICMR, Hyderabad, India, 31-33.

Rahman, K.S.M and Lakshmanaperumalsamy,P. (1999), Bioremediation of hydrocarbon contamination, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22 &23, Tamil Nadu Agricultural University, Coimbatore, India, 44-48.

Rajakovic, L.V and Mitrovic, M.H.(1992), Arsenic removal from water by chemisorption filters, Environmental Pollution, 75, 279-287.

Rajendran, P., Muthukrishnan, J and Gunasekaran, P. (2003), Microbes in heavy metal remediation, Indian Journal of Experimental Biology, 41, 935 - 944.

Raji,C and Anirudhan,T.S. (1999), Sorption characteristics of As(III) on surface modified saw dust carbon, Indian Journal of Environmental Health, 41(3), 184-193.

Raji,C., Shubha,K.P and Anirudhan,T.S. (1997), Use of chemically modified sawdust in the removal of Pb(II) ions from aqueous media, Indian Journal of Environmental Health, 39(31), 230-238.

Ramasamy, K and Thanga, V.S.G.(1999), Soil environmental pollution and remedial measures, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22&23, Tamil Nadu Agricultural University, Coimbatore , India, 34 -35.

Ramteke, P.W. (2000), Biosorption of nickel(II) by *Pseudomonas stutzeri*, Journal of Environmental Biology, 21(3) 219 -221.

Rao, M and Bhole, A.G. (2001), Chromium removal by adsorption using fly-ash and bagasse, Journal of Indian Water Works Association, 33(1), 97-100.

Rapoport, A.I and Muter, O.A.(1995), Biosorption of hexavalent chromium by yeasts, Process Biochemistry, 30(2), 145-149.

Rathi, A.K.A and Puranik, S.A.(2002), Mixed streams wastewater treatment using adsorption, Indian Journal of Environmental Health, 44(2), 138 -153.

Rengaraj, S., Sivabalan, R., Banumathi, A and Murugesan, V.(2000), Adsorption kinetics of o-cresol on activated carbon from palm seed coat, Indian Journal of Chemical Technology, 7(5) 127-131.

Romanenko. V.I and Korenkov, V.N. (1977), A pure culture of bacteria utilizing chrand bichromates as hydrogen acceptors in growth under anaerobic conditions, Mikrobiologiya, 46, 329 - 332.

Ross, I.S. (1982), Effect of copper, cadmium and zinc on germination and mycelial growth in *Candida albicans*, Transactions of the British Mycological Society, 78, 543-551.

Ruby, M.V., Schoof, R., Brattin,W., Goldade,M., Post, G., Harnois, M., Mosby,E.E Casteel,S.W., Berti, W., Carpenter, M., Edwards, D., Cragin, D and Chappell, W. (1999), Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment, Environmental Science and Technology,3, 3697 - 3705.

Sag, Y and Kutsal, T. (1996), The selective biosorption of chromium (VI) and copper (III) ions from binary metal mixtures by *Rhizopus arrhizus,* Process Biochemistry, 31, 561 - 572.

Samuelson, P., Wernerus, H., Svedberg, M and Stahl, S. (2000), Staphylococcal surface display of metal binding polyhistidyl peptides, Applied and Environmental Microbiology, 66(3) 1243-1248.

Saxena, D., Levin R and Firer M.A. (2000), Removal of chromate from industrial effluent by a new isolate of *Staphylococcus cohnii*, Water Science and Technology, 42(1-2), 93-98.

Saxena,D., Gowri, P.M., Mago, R and Srivastava, S. (2001), Removal of copper by *Pseudomonas putida* strain S4 isolated from copper mines, Indian Journal of Experimental Biology, 39, 590 - 593.

Selvi,K., Pattabi, S and Kadirvelu,K. (2001), Removal of Cr(VI) from aqueous solution by adsorption onto activated carbon, Bioresource Technology, 80(1), 87-89.

Sen, A.K and Arnab, K.De. (1987), Adsorption of arsenic on coal fly ash, Indian Journal of Technology, 25, 259 - 261.

Shah,S and Thakur, I.S. (2002), Treatment of tannery effluent by pentachlorophenol - degrading bacterial community from the chemostat, Journal of Scientific and Industrial Research, 61, 1051-1055.

Shakibaie, M.R., Kapadnis, B.P., Dhakephalker, P and Chopade, A.(1999), Removal of silver from photographic wastewater effluent using *Acinetobacter baumannii* BL54, Canadian Journal of Microbiology, 45, 995 -1000.

Sharma, P.D. (1997), Seventh Edition, Ecology and Environment, Rastogi Publications, Meerut, 468 - 470.

Sharma, D.C and Forster, C.F (1995), Continuous adsorption and desorption of chromium ions by sphagnum moss peat, Process Biochemistry, 30(4), 293 -298.

Sharma, B.K., Singhal, P.C and Chugh, K.S. (1978), Intravascular haemolysis and acute renal failure following potassium dichromate poisoning, Post Graduate Medical Journal, 54, 414 - 415.

Sharma, D.C., Srivastava, P.C., Johri, B.N and Chandra, P. (1996), Environmental impact of tannery effluents on heavy metal phytotoxicity and health hazards, Journal of Industrial Pollution Control, 12(2), 137-142.

Shen, H and Wang, Y.T. (1994), Modeling hexavalent chromium reduction in *Escherichia coli* 33456, Biotechnology and Bioengineering, 43, 293 - 300.

Shin, Y.C and Paik, N.W. (2000), Reduction of hexavalent chromium collected on PVC filteres, American Industrial Hygiene Association Journal, 61(4), 563 - 567.

Shrivastava, S and Thakur, I.S. (2003), Biosorption potential of *Acinetobacter sp.* strain IST 103 of bacterial consortium for removal of chromium from tannery effluent, Journal of Scientific and Industrial Research, 62, 616 - 622.

Shuttleworth, K.L and Unz, R.F. (1993), Sorption of heavy metals to the filamentous bacterium *Thiothrix* strain A1, Applied and Environmental Microbiology, 59(5), 1274 -1282.

Siddiqui, B.A., Sharma, P.P and Sultan, M. (1999), Adsorption studies on phosphate treated saw dust; separation of Cr(VI) from Zn^{2+}, Ni^{2+} and Cu^{2+} and their removal and recovery from electroplating waste, Indian Journal of Environmental Protection, 19(II), 846 - 852.

Singh, D.K and Lal, J. (1992), Removal of chromium (VI) from aqueous solutions using waste tea leaves carbon, Indian Journal of Environmental Health, 32(2), 108 -113.

Singh, V.K and Tiwari, P.N. (1997), Removal and recovery of chromium (VI) form industrial wasterwater, Journal of Chemical Technology and Biotechnology, 69(3), 376 - 382.

Singh, D.K and Srivastava, B.(2000), Removal of some phenols by activated carbon developed from used tea leaves, Journal of Industrial Pollution Control, 16(I), 19 - 30.

Singh, D.K., Saksena, D.N and Tiwari, D.P. (1994), Removal of chromium (VI) from aqueous solutions, Indian Journal of Environmental Health, 36(4), 272 -277.

Singleton, I and Simmons, P. (1996), Factors affecting silver biosorption by an industrial strain of *Saccharomyces cerevisiae,* Journal of Chemical Technology and Biotechnology, 65, 21-26.

Sinha, S., Saxena, R and Singh,S. (2002), Comparative studies on accumulation of chromium from metal solution and tannery effluent under repeated metal exposure by aquatic plants: Its toxic effects, Environmental Monitoring and Assessment, 80(1), 17 - 31.

Sivakumar,A.A., Lekeshmanaswamy, M., Vasuki,C.A and Aruchami, M.(1999), Environmental status of the river Bhavani with special reference to primary production, Proceedings of the Workshop on Environmental Status of Rivers in Tamil Nadu, March 26&27. Bharathiar University, Coimbatore, India, 60 - 66.

Skowronski,G.A., Seide, M., Abdel-Rahman, M.S. (2001), Oral bioaccessibility of trivalent and hexavalent chromium in soil by simulated gastric fluid, Journal of Toxicology and Environmental Health, Part-A, 63, 351 - 362.

Smith, W.L and Gadd, G.M (2000), Reduction and precipitation of chromate by mixed culture sulphate - reducing bacterial biofilms, Journal of Applied Microbiology, 88(6), 983 - 991.

Sonawane, S.K., Pawar, R., Patel,A., Mishtra, S and Tewari,A. (2002), Effect of biophysico-chemical factors on biosequestering of chromium from the tannery effluent by marine cyanobacteria, Research Journal of Chemistry and Environment, 6(2), 43 - 47.

Sousa, C., Cebolla, A and de Lorenzo,V. (1996), Enhanced metalloadsorption of bacterial cells displaying poly-His Peptides, Nature Biotechnology, 14, 1017 - 1020.

Srinath, T., Garg, S.K and Ramteke, P.W. (2002), Chromium (VI) accumulation by *Bacillus circulans:* Effect of growth conditions, Indian Journal of Microbiology, 42, 141 - 146.

Srinivasan K., Balasubramanian, N and Ramakrishna, T.V. (1998), Studies on chromium removal by rice husk carbon, Indian Journal of Environmental Health, 30 (4), 376 - 387.

Stearns, D.M., Courtney, K.D., Giangrande, P.H., Phieffer, L.S and Wetterhahn, K.E.(1994), Chromium (VI) reduction by ascorbate: role of reactive intermediates in DNA damage invitro, Environmental Health Perspectives, 102(3), 21 - 25.

Stohs, S.J., Bagchi, D., Hassoun, E and Bagchi, M. (2000), Oxidative mechanisms in the toxicity of chromium and cadmium ions, Journal of Environmental Pathology, Toxicology and Oncology, 19(3), 201-213.

Strandberg, G.W., Shumate, S.E and Parrott, J.R. (1981), Microbial cells as biosorbents for heavy metals: Accumulation of uranium by *Saccharomyces cerevisiae and Pseudomonas aeruginosa*, Applied and Environmental Microbiology, 41, 237-245.

Sudgen, K.D and Wetterhahn, K.E. (1996), EPR evidence for chromium (V) binding to phosphate and pyrophosphate: Implications for Cr(V)-DNA interactions, Inorganic Chemistry, 35, 3727 - 3728.

Suhasini, I.P., Sriram, G., Asolekar, S.R and Kumar, S.G.K.(1999), Biosorptive removal and recovery of cobalt from aqueous systems, Process Biochemistry, 34, 239 – 243.

Sujatha,P and Gupta, A. (1996), Tannery effluent characteristics and its effect on agriculture, Journal of Ecotoxicology and Environmental Monitoring,6(1), 45 - 48.

Sultan,S and Fatima, T. (1999), Phytotoxicity of heavy metals on *Spirulina platensis*, Phykos, 38(1&2), 87 - 92.

Sumathi,K.M.S., Papitha,P., Sivasubramanian,K., Saravanan,K and Mahimairaja, S.(1999), Heavy metal contamination in Coimbatore urban environment - preliminary study, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22 & 23, Tamil Nadu Agricultural University, Coimbatore, India, 91-94.

Swamy, M.M., Mall, I.D., Prasad, B and Mishra, I.M. (1998), Sorption characteristics of o-cresol on bagasse fly ash and activated carbon, Indian Journal of Environmental Health, 40(1), 67-78.

Thangavel, P., Rajannan, G and Balagurunathan, R. (2003), Effect of tannery effluent on soil properties and yield of maize (*Zea mays*), Journal of Ecobiology, 15(3), 161-168.

Thorat, S.R and Wagh, S.B.(2000), Physico-chemical analysis of tannery waste, Journal of Industrial Pollution Control, 16(1), 107-109.

Tseng, J.K and Bielefeldt, A.R. (2002), Low temperature chromium (VI) biotransformation in soil with varying electron acceptors, Journal of Environmental Quality, 31, 1831-1841.

Turick, C.E., Apel, W.A and Carmiol, N.S.(1996), Isolation of hexavalent chromium-reducing anaerobes from hexavalent-chromium contaminated and noncontaminated environments, Applied Microbiology and Biotechnology, 44, 683 - 688.

Ueno, S., Kashimoto, T and Susa, N. (2001), Detection of dichromate (VI) induced DNA strand breaks and formation of paramagnetic chromium in multiple mouse organs, Toxicology and Applied Pharmacology, 170, 56 - 62.

Vadivoo, A.S and Vijayalakshmi, A. (1998), Utilization of tannery affected soils with stress tolerant cultivars, Proceedings of State Level Seminar on Environmental Problems in Urban Area, Government Arts College, Coimbatore, India, 5.

Vaishya,R.C and Agarwal,I.C. (1993), Removal of arsenic (III) from contaminated ground waters by Ganga sand, Indian Water Works Association, 21, 249 -253.

Vankova,S., Kupec,J and Hoffman, J.(1999), Toxicity of chromium to activated sludge. Ecotoxicology and Environmental safety, 42, 16 -21.

Vasant, C., Balamurugan, K Rajaram,R and Ramasami, T. (2001), Apoptosis of lymphocytes in the presence of Cr(V) complexes: Role in Cr(VI)-induced toxicity, Biochemical and Biophysical Research Communications, 285, 1354 -1360.

Vasanthy, M and Lakshmanaperumalsamy, P. (1993), Removal of chromium using various adsorbents, Journal of Ecotoxicology and Environmental Monitoring, 3(1) 47 - 50.

Vasudevan, N. (1999), Microbial detoxification and remediation of petroleum contamination, Proceedings of the Workshop on Bioremediation of Polluted Habitats, March 22&23, Tamil Nadu Agricultural University, Coimbatore, India, 36 -37.

Vasudevan, P., Padmavathy, V., Tewari, N and Dhingra, S.C. (2001), Biosorption of heavy metal ions, Journal of Scientific and Industrial Research, 60, 112 -120.

Venkateshwerlu, G and Stotzky. (1986), Copper and cobalt alter the cell wall composition of *Cunninghamella blakesleeana*, Canadian Journal of Microbiology, 32, 654-662.

Verma, A and Verma, A.P. (1995), Effect of tannery effluent on seed germination and chlorophyll content in *Phaseolus radiatus L.,* Journal of Industrial Pollution Control, II(I), 63 - 66.

Verma, N and Rehal, R. (1996), Removal of chromium by *Albizia lebbeck* pods from industrial wastewater, Journal of Industrial Pollution Control, 12(1), 55-59.

Vincent, J.B. (1999), Mechanisms of chromium action: Low-molecular weight chromium binding substance, Journal of the American College of Nutrition, 18(1), 6-12, 213.

Viti, C., Pace, A and Giovannetti, L. (2003), Characterisation of Cr(VI) resistant bacteria isolated from chromium contaminated soil by tannery activity, Current Microbiology, 46(1), 1-5.

Vogel, A.I. (1964), A textbook and quantitative inorganic analysis including elementary instrumental analysis. Third Edition, The English Language Book Society and Longmans Green and Company Limited, London, 56 - 60.

Wang, Y.T. (2001), Microorganisms and pollutant metals, Journal of Environmental Engineering, 3, 185 -186.

Wang, P., Mori,T., Komori,K., Sasatsu,M., Toda, M and Ohtake, H. (1989), Isolation and characterization of *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions, Applied and Environmental Microbiology, 55, 1665 -1669.

Wang, P.C., Mori, T., Toda, K and Ohtake, H. (1990), Membrane-associated chromate reductase activity from *Enterobacter cloacae*, Journal of Bacteriology, 172(3), 1670 -1672.

Wyszkowska, J., Kucharski, J., Jastrzebska, E and Hlasko, A. (2001), The biological properties of soil as influenced by chromium contamination, Polish Journal of Environmental Studies, 10(1), 37 - 42.

Yazdandoost, M.Y and Katdare, M.S. (1999), Study of the heavy metal toxicity accumulation in the tissues of fish from Pune-rivers- India, Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 1(1-2) 115 -118.

Ziochevskaya, I.V and Rukhadze, E.G. (1968), A study of the toxic action of some lead compounds, Journal of Microbiology, 37, 951.

Appendices

APPENDIX 1

DETERMINATION OF TOTAL SUSPENDED SOLIDS
Filtration method
(APHA, 1998)

Principle

A well mixed sample is filtered through a weighed standard glass fibre filter and the residue retained on the filter is dried to a constant weight at 103°C - 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume.

Procedure

Filtered 250ml of the effluent through a tared filter paper by applying suction. Washed the filter paper with distilled water to remove the soluble salts. Dried the filter paper for atleast one hour in an oven at 103°C - 105°C. The increase in weight was the total suspended solids.

Expressed the results as mg total suspended solids per litre of the sample.

APPENDIX 2

DETERMINATION OF TOTAL DISSOLVED SOLIDS
Filtration method
(APHA, 1998)

Principle

A well mixed sample is filtered through a standard glass fibre filter and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids.

Procedure

Filtered 250ml of the effluent through a glass microfibre filter paper. Evaporated the filtrate in a tared porcelain dish which was preheated at 105°C and then at 550°C for one hour in a muffle furnace and cooled and brought to constant weight. Kept the dish at 180°C for about 1 hour, cooled and weighed. The increase in weight denoted the total dissolved solids content.

APPENDIX 3

ESTIMATION OF BIOCHEMICAL OXYGEN DEMAND
Winkler's iodometric method
(APHA, 1989)

Principle

BOD determination involves the measurement of dissolved oxygen content of the sample, before and after 5 days incubation at 20°C. The reduction in oxygen content is due to the demand exerted by the microbiological population and it is a measure of oxidisable organic matter in the sample.

When manganous sulphate is added to the sample containing potassium iodide, manganous hydroxide is formed, which is oxidised by the dissolved oxygen of the sample to basic manganic oxide. On addition of sulphuric acid, the basic manganic oxide liberates iodine, equivalent to that of dissolved oxygen originally present in the sample. The liberated iodine is titrated with a standard solution of sodium thiosulphate using starch as an indicator.

Reagents for the preparation of dilution water

1. Calcium chloride solution : 27.5 g was dissolved in one litre of distilled water.
2. Magnesium sulphate solution: 25 g was dissolved in one litre of distilled water.
3. Ferric chloride solution: 0.25 g was dissolved in one litre of distilled water.

4. Phosphate buffer pH 7.2: 21.75 g dipotassium hydrogen phosphate, 33.4 g disodium hydrogen phosphate heptahydrate and 1.7 g ammonium chloride were taken in one litre standard flask, dissolved in distilled water and made up to the mark. The buffer was stored in a refrigerator to prevent mold growth.

Reagents for the estimation of dissolved oxygen

1. Manganous sulphate solution: Dissolved 91.0g manganous sulphate monohydrate and diluted to 250 ml with distilled water.

2. Alkali-iodide-azide reagent: Reagent A - 175 g potassium hydroxide and 37.5 potassium iodide were dissolved in 250 ml of water. Reagent B - 2.5 g sodium azide was dissolved in 10.0 ml of water. Mixed reagents A and B.

3. Concentrated sulphuric acid.

4. Phosphoric acid: 85 - 90%.

5. Sodium thiosulphate solution 0.1 N: 24.82 g was dissolved in distilled water and made up to one litre.

6. Sodium thiosulphate solution 0.025 N: Diluted 250ml of exactly 0.1 N sodium thiosulphate solution to 1000 ml with distilled water. 1.0 ml of 0.025 N sodium thiosulphate ≡ 0.2 mg dissolved oxygen.

7. Starch solution 1%.

Procedure

Preparation of dilution water: Added 1.0 ml each of calcium chloride, magnesium sulphate, ferric chloride and phosphate buffer solutions to one litre of aerated distilled water and mixed thoroughly. This is the standard dilution water prepared freshly every time.

Seeding of the dilution water: It is essential to seed the dilution water. The seeding material generally used is freshly settled raw sewage. 2.0ml of raw sewage was added to one litre of dilution water.

Dilution of the samples: The test samples were diluted with seeded dilution water sample (1%, 5% and 10%) in dilution mixture. Each dilution sample was taken in a set of two BOD bottles.

Determination of dissolved oxygen (DO) before and after 5 days incubation: In one set of flasks DO was determined immediately while the other set was kept for incubation at 20°C for 5 days. After 5 days, DO of the incubated sample was determined. Determination of DO is as follows:

To the contents of the BOD bottle, added 2.0 ml of manganous sulphate solution and 2.0 ml of alkali-iodide-azide solution. Stoppered the bottle and mixed thoroughly. A brown precipitate of basic manganic oxide was formed, which was allowed to settle completely leaving a clear supernatant liquid. Then added 2.0 ml of concentrated sulphuric acid along the sides of the bottle, stoppered and mixed for complete dissolution. Transferred the contents to a 500 ml conical flask and titrated immediately against 0.025 N sodium thiosulphate using starch as an indicator.

Calculation for DO:

Volume of 0.025N thiosulphate used up in the titration = DO in mg l^{-1}

DO at 0°C and 760 mm pressure = DO x 0.07 mg l^{-1}

Calculation for BOD:

$$\text{BOD (5 days at 20°C)} = \frac{(DO_0 - DO_5 - BC) \times 100}{\text{Percent sample}}$$

DO_0 = Initial DO

DO_5 = DO after 20°C incubation for 5 days

BC = Blank correction ie, difference in DO of blank on the initial day and after 5 days incubation

APPENDIX 4

ESTIMATION OF CHEMICAL OXYGEN DEMAND
Open-reflux titrimetric method
(APHA , 1998)

Principle

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion ($Cr_2O_7^{2-}$), the specified oxidant is reduced to the chromic ion (Cr^{3+}).

COD often is used as a measurement of pollutants in wastewater and natural waters. Most types of organic matter are oxidised by boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate. After digestion, the remaining unreduced potassium dichromate is titrated with ferrous ammonium sulphate to determine the amount of potassium dichromate consumed and the oxidisable matter is calculated in terms of oxygen equivalent.

Reagents

1. Mercuric sulphate crystals.

2. Sulphuric acid - silver sulphate reagent: Dissolved 10.1 g of silver sulphate in 1 litre of concentrated sulphuric acid. Allowed the solution to stand for two days for complete dissolution.

3. Potassium dichromate solution 0.125N: Dissolved 0.129g in distilled water and made up to 1 litre. 1.0 ml of 0.125N potassium dichromate ≡ 1.0 mg of oxygen.

4. Ferroin indicator solution: Dissolved 95 mg of ferrous sulphate in 500 ml of distilled water. Added 1.485g of 1,10 phenanthroline monohydrate and mixed thoroughly.

5. Ferrous ammonium sulphate solution 0.125N: 40g of ferrous ammonium sulphate was dissolved in distilled water. Added 20 ml of concentrated sulphuric acid. Made up to one litre with water. Standardised it with 0.125 N potassium dichromate.

Procedure

A refluxing flask of 250ml capacity was used with a ground glass 24/40 neck fitted with a 300mm double surface condenser to which a glass cap was fitted. Placed 50ml of the sample in the flask. Added mercuric sulphate of suitable quantity such that the ratio of chloride content of the sample to mercuric sulphate was 1:10 (For this, chloride content of the sample was estimated as given in Appendix 5). Then added 5.0 ml of sulphuric acid - silver sulphate reagent and dissolved the mercuric sulphate. Cooled in cold water while mixing. Pipetted 25ml of 0.125N potassium dichromate into the flask and mixed well. Added 70ml sulphuric acid - silver sulphate reagent very carefully and mixed. Added a few porcelain bits and attached the condenser. Started water circulation and refluxed for two hours. Removed the flame, allowed the flask to cool. Transferred the contents of the flask and diluted to about 350ml with distilled water. Added 2 to 3 drops of ferroin indicator and titrated against 0.125N ferrous ammonium sulphate solution. The end point was the sharp colour change from blue green to reddish brown. A blank was conducted using 50ml of distilled water instead of the sample.

Calculation

$$\text{COD in mg/l} = \frac{(\text{Blank titre value} - \text{sample titre value}) \times 0.125 \times 1000 \times 8}{\text{Volume of sample taken}}$$

APPENDIX 5

ESTIMATION OF CHLORIDE
Titrimetric method
(Vogel, 1964)

Principle

Silver nitrate reacts with chloride ions to form silver chloride. The completion of reaction is indicated by the red colour produced by the reaction of silver nitrate with potassium chromate solution which is added as an indicator.

Reagents

Chloride free double distilled water was used for all reagents.

1. Standard silver nitrate titrant 0.0282N: 4.791 g of silver nitrate was dissolved in 1 litre of distilled water. Standardised it against 0.0282 N sodium chloride solution. 1.0 ml of exactly 0.0282 N silver nitrate ≡ 1.0 mg chloride.

2 Standard sodium chloride titrant 0.0282N : 1.648 g of sodium chloride was dissolved in 1 litre of distilled water. 1.0 ml ≡ 1.0 mg chloride.

3 Potassium chromate indicator solution: Dissolved 25 g in 100 ml of distilled water. Added silver nitrate solution dropwise until a slight red precipitate was formed. Allowed to stand for 12 hours. Filtered and made upto 500 ml with distilled water.

4 Aluminium hydroxide suspension: Dissolved 100 g aluminium sulphate in 1000 ml distilled water. Warmed to 60°C and added with stirring 55 ml concentrated ammonia solution. Allowed the precipitate to settle for about an hour. Washed by decantation with distilled water to make the precipitate free from chloride. Checked it by treating a portion of the decanted solution every time with silver nitrate solution. After the precipitate was free from chloride, diluted it to 1000 ml with distilled water.

Procedure

Added 3.0 ml of aluminium hydroxide to a measured volume of the sample in a beaker. Stirred well and allowed to settle. Filtered, washed the precipitate with chloride free distilled water and combined the filtrate and washings. Pipetted out 100 ml of the sample into a porcelain dish. Adjusted the pH to be in the range of 7-9.5. Added1.0 ml of potassium chromate indicator solution. Titrated it against standard silver nitrate solution with constant stirring until a slight precipitable reddish colouration persisted. Conducted a blank by placing 100 ml chloride free distilled water instead of sample.

Calculations

If the silver nitrate solution is exactly 0.0282 N,

$$\text{Chloride (mg per litre)} = \frac{(\text{ml } AgNO_3 \text{ for sample - ml } AgNO_3 \text{ for blank})}{\text{ml sample taken for estimation}} \text{ X1000}$$

If the silver nitrate solution is not exactly 0.0282 N,

$$\text{Chloride (mg per litre)} = \frac{(\text{ml } AgNO_3 \text{ for sample - ml } AgNO_3 \text{ for blank }) \text{ x Normality of } AgNO_3 \text{ x } 35.45 \text{ x } 1000}{\text{ml sample taken for estimation}}$$

APPENDIX 6

ESTIMATION OF SULPHATES
Turbidimetric method
(APHA , 1998)

Principle

Sulphate ion is precipitated in an acetic acid medium with barium chloride so as to form barium sulphate crystals of uniform size. Light absorbance of the barium sulphate suspension is measured by a photometer and the sulphate ion concentration is determined by comparison of the reading with a standard curve.

Reagents

1. Buffer solution : Dissolved 30g magnesium chloride, 5g sodium acetate, 1.0g potassium nitrate and 20ml acetic acid (99%) in 500 ml distilled water and made upto 1000ml.

2. Barium chloride crystals, 20 to 30 mesh.

3. Standard sulphate solution: Dissolved 0.1479g anhydrous sodium sulphate in distilled water and diluted to 1000ml.

Procedure

Formation of barium sulphate turbidity: Measured 100ml sample into a 250 ml Erlenmeyer flask. Added 20ml buffer solution, mixed and stirred. While stirring, added a spoonful of barium chloride crystals and stirred for 60 ± 2 seconds at constant speed.

Measurement of barium sulphate turbidity: After stirring period had ended, poured solution into absorption cell of photometer and measured turbidity at 5 ± 0.5 minutes.

Preparation of calibration curve: Estimated sulphate concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying sulphate standards through the entire procedure. Spaced standards at 5 mg/l increments in the 0 to 40 mg/l sulphate range.

Correction for sample colour and turbidity: Corrected for sample colour and turbidity by running blanks to which barium chloride was not added.

Calculation

$$\text{mg sulphate / l} = \frac{\text{mg } SO_4^{2-} \times 1000}{\text{ml sample}}$$

APPENDIX 7

ESTIMATION OF AMMONIACAL NITROGEN
Titrimetric method
(APHA, 1998)

Principle

The sample is distilled and the distillate containing ammonia is titrated against sulphuric acid till an end point of pale lavender colour appeared.

Reagents

1. Mixed indicator solution: Dissolved 200 mg methyl red indicator in 100 ml 95% ethyl alcohol. Dissolved 100 mg methylene blue in 50 ml 95% ethyl alcohol. Combined two solutions.

2. Indicating boric acid solution: Dissolved 20g boric acid in ammonia free distilled water, added 10 ml mixed indicator solution and diluted to 1 litre.

3. Standard sulphuric acid titrant 0.02 N.

4. Borate buffer solution: Added 88 ml of 0.1N sodium hydroxide solution to 500ml of approximately 0.025M sodium tetraborate solution (9.5g $Na_2B_4O_7.10H_2O$/l) and diluted to one litre.

Procedure

Placed 100 ml of the sample in a beaker. Dechlorinated sample (if it contains any chlorine) with sodium sulphite solution. (1.0 ml N/70 sodium sulphite removes 0.5 mg. chlorine). Neutralised the sample with dilute acid or base. Added 250 ml borate buffer solution and adjusted to pH 9.5 with 6N sodium hydroxide. Transferred the contents of the beaker to the distillation flask. Distilled at a rate of 6.0 to 10ml/minute with the tip of the delivery tube below the surface of acid receiving solution. Collected distillate in a 500 ml Erlenmeyer flask containing 50 ml indicating boric acid solution. Diluted to 500 ml with water.

Titrated ammonia in distillate with standard 0.02N sulphuric acid titrant until indicator turns a pale lavender. Carried a blank through all steps of the procedure and applied the necessary correction of the results.

Calculation

$$\text{mg Ammoniacal nitrogen / l} = \frac{(A-B) \times 280}{\text{ml sample}}$$

A = Volume of sulphuric acid titrated for sample, ml

B = Volume of sulphuric acid titrated for blank, ml.

APPENDIX 8

ESTIMATION OF OIL AND GREASE
Soxhlet extraction method
(APHA, 1998)

Principle

Oil, grease and other extractable matter are dissolved in a suitable solvent and extracted from the aqueous phase. The solvent layer is then evaporated and the residue is weighed as oil and grease.

Reagents

1. Hydrochloric acid, 1+1. Added 50 ml of concentrated hydrochloric acid to 50ml distilled water and mixed well.

2. Trichloro – trifluoroethane

3. Diatomaceous - silica filter aid suspension : 10g / l distilled water.

Procedure

Collected one litre of sample in a wide mouth glass bottle and marked sample level in bottle for later determination of sample volume. Acidified to pH 2

or lower using hydrochloric acid. Prepared a filter consisting of a muslin cloth disk overlaid with filter paper. Wetted paper and muslin are pressed down. Using a vacuum, passed 100 ml filter aid suspension through prepared filter and washed with 1 litre of distilled water. Applied vacuum until no more water passes through the filter. Filtered the acidified sample. Using forceps, transferred filter paper to a watch glass. Added the material adhering to the edges of muslin cloth disk. Wiped sides and bottom of the collecting vessel and Buchner funnel with pieces of filter paper soaked in solvent, taking care to remove all films caused by grease and to collect all solid material. Rolled all filter paper containing sample and fitted into a paper extraction thimble. Wiped watch glass with a filter paper soaked in solvent and placed in paper extraction thimble. Dried filled thimble in a hot air oven at 103°C for 30 minutes. Filled thimble with glass wool or small glass beads. Weighed extraction flask. Extracted oil and grease in soxhlet apparatus, using tri-chloro - trifluoroethane at a rate of 20 cycles / hour for 4 hours. Distilled solvent from extraction flask in a water bath at 70°C. Placed flask on a waterbath at 70°C for 15 minutes and drawn air through it using an applied vacuum for the final 1 minute. Cooled in a desiccator for 30 minutes and weighed.

Calculation

If the organic solvent is free of residue, the gain in weight of the tared distilling flask is mainly due to oil and grease. Total gain in weight A, of tared flask less calculated residue, B, from solvent blank is the amount of oil and grease in the sample.

$$\text{mg oil and grease / l} = \frac{(A - B) \times 1000}{\text{ml sample}}$$

APPENDIX 9

ESTIMATION OF SULPHIDES
Iodometric method
(APHA, 1998)

Principle

Sulphide is oxidised with potassium iodide solution under acidic conditions. The excess iodine liberated is titrated with a standard solution of sodium thiosulphate.

Reagents

1. Hydrochloric acid 6N.

2. Standard iodine solution 0.025 N: Dissolved 20 to 25g potassium iodide in a little water and added 3.2 g iodine. After iodine had dissolved, diluted to 1000 ml and standardised against 0.025 N sodium thiosulphate, using starch solution as indicator.

3. Standard sodium thiosulphate solution 0.25N: Dissolved 6.205g sodium thiosulphate in distilled water. Added 1.5 ml 6N sodium hydroxide and diluted to 1000 ml. Standardised with di - iodate solution.

4. Starch solution: Dissolved 2g laboratory grade soluble starch and 0.2g salicylic acid, as a preservative, in 100ml hot distilled water.

Procedure

Measured from a burette into 500 ml flask an amount of iodine solution estimated to be an excess over the amount of sulphide present. Added distilled water, if necessary, to bring volume to about 20 ml. Added 2.0 ml 6N hydrochloric acid. Pipetted 200 ml sample into flask, discharging sample under solution surface. If iodine color disappeared, added more iodine so that the colour remained. Back titrated with sodium thiosulphate solution, adding a few

drops of starch solution as end point was approached, and continuing until blue colour disappeared.

If sulphide was precipitated with zinc as zinc sulphide, filtered out, returned filter with precipitate to original bottle and added about 100 ml water. Added iodine solution and hydrochloric acid and titrated as above.

Calculation

One ml 0.025 N iodine solution reacts with 6.4 mg sulphide.

$$\text{mg sulphide / l} = \frac{(AxB) - (CxD) \times 16{,}000}{\text{ml sample}}$$

where,

A = ml iodine solution

B = Normality of iodine solution

C = ml sodium thiosulphate solution

D = Normality of sodium thiosulphate solution.

APPENDIX 10

ESTIMATION OF SODIUM AND POTASSIUM

Flame photometric method

(APHA, 1998)

Principle

In flame photometry, the solution under test is passed under carefully controlled conditions as a very fine spray in the air supply to a burner. In the flame, the solution evaporates and the salt dissociates to give neutral atoms. A very small proportion of these move into a higher energy state. When these excited atoms fall back to the ground state, the light emitted of characteristic wave length is measured. Sodium and potassium are estimated at 590 nm and 770 nm respectively.

Reagents

1. Sodium stock solution: 2.524 g of sodium chloride was dissolved in deionised distilled water and made upto one litre. 1.0 ml = 1.0 mg sodium.

2. Potassium stock solution: 1.907 g of potassium chloride was dissolved in deionised distilled water and made up to one litre. 1.0 ml = 1.0 mg potassium.

Procedure

Estimation of Sodium and Potassium

Standardised the flame photometer before feeding the sample. Set the reading to zero using deionised water. Using the stock solutions of sodium and potassium, adjusted the reading to 100 at their specific wavelengths. Then fed the sample in the flame photometer and noted the readings to get the amounts of sodium and potassium directly.

APPENDIX 11

ESTIMATION OF CALCIUM
EDTA titrimetric method
(APHA, 1998)

Principle

The pH of the sample is made sufficiently high (12-13) to precipitate magnesium as hydroxide, and calcium only is allowed to react with EDTA in the presence of a selective indicator.

Reagents

1. Sodium hydroxide 1N: 40 g of sodium hydroxide was dissolved in 1 litre of distilled water.

2. Murexide indicator: 200 mg of the dye was ground with 100 g of sodium chloride.

3. Standard EDTA titrant 0.02 N: 3.723 g disodium ethylene diamine tetra acetate dihydrate was dissolved in 1 litre of water. It was standardised against standard calcium solution. 1.0 ml of 0.02 N EDTA ≡ 1.0 mg of calcium carbonate.

Procedure

Pipetted out 50 ml of the sample. Added 2.0 ml of sodium hydroxide to it to produce a pH of 12-13 and mixed well. Added 0.1 - 0.2 g of the indicator, titrated immediately with EDTA. The end point is from pink to purple.

Calculation

If the EDTA titrant is exactly 0.02 N,

$$\text{mg/l Calcium (as } CaCO_3) = \frac{\text{ml EDTA titrant x 1 x 1000}}{\text{ml sample taken for titration}}$$

APPENDIX 12

ESTIMATION OF MAGNESIUM
By calculation
(APHA, 1998)

Calculation

mg/l Magnesium (as $CaCO_3$) = mg/l Total hardness as ($CaCO_3$) - mg/l Calcium (as $CaCO_3$).

APPENDIX 13

ESTIMATION OF PHENOLIC COMPOUNDS
Colorimetric method
(APHA, 1998)

Principle

Steam distillable phenolic compounds react with 4 - aminoantipyrine at pH, 7.9 ± 0.1 in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

Reagents

1. Stock phenol solution : Dissolved 1.0 g phenol in freshly boiled and cooled distilled water and diluted to 1000ml.

2. Intermediate phenol solution: Diluted 10.0 ml stock phenol solution in freshly boiled and cooled distilled water to 1000ml. 1 ml = 10.0 μg phenol. Prepared freshly.

3. Standard phenol solution: Diluted 50 ml intermediate phenol solution to 500 ml with freshly boiled and cooled distilled water. 1.0 ml = 1.0 μg phenol. Prepared within 2 hours of use.

4. Ammonium hydroxide 0.5N: Diluted 35ml fresh concentrated ammonium hydroxide to 1 litre with water.

5. Phosphate buffer solution: Dissolved 104.5 g dipotassium hydrogen phosphate (K_2HPO_4)and 72.3g monopotassium dihydrogen phosphate (KH_2PO_4)in water and diluted to 1 litre. The pH was 6.8.

6. 4-Amino antipyrine solution: Dissolved 2.0g 4-aminoantipyrine in water and diluted to 100ml. Prepared freshly.

7. Potassium ferricyanide solution: Dissolved 8.0g potassium ferricyanide in water and diluted to 100 ml. Filtered if necessary. Stored in a brown glass bottle.

Procedure

Placed 100ml distillate in a 250ml beaker. Prepared a 100ml distilled water blank and a series of 100ml phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol. Treated sample, blank and standards as follows : Added 2.5 ml 0.5 N ammonium hydroxide solution and adjusted to pH 7.9 ± 0.1 with phosphate buffer. Added 1.0ml 4-amino antipyrine solution, mixed well and then added 1.0 ml potassium ferricyanide solution and mixed well.

After 15 minutes, transferred to cuvettes and read absorbance of sample and standards against the blank at 500 nm.

Calculation

$$\text{mg phenol / l} = \frac{A \times 100}{B}$$

where

A = mg phenol in sample, from calibration curve and

B = ml original sample.

APPENDIX 14

ESTIMATION OF TOTAL CHROMIUM, COPPER , ZINC AND IRON
Atomic absorption spectrophotometric method
(APHA, 1998)

Principle

The technique involves determination of concentration of a substance by the measurement of absorption of the characteristic radiation by the atomic vapour of an element. When radiation characteristic to a particular element passes through the atomic vapour of the same element, absorption of radiation occurs in proportion to the concentration of the atoms in the light path. The source of characteristic radiation is a hollow cathode lamp, the cathode being made of the element desired to be estimated.

Reagents

1. Air: Cleaned and dried through a suitable filter to remove oil, water and other foreign substances. The source may be a compressor or commercially bottled gas.

2. Acetylene, standard commercial grade

3. Metal free water

4. Calcium solution: Dissolved 630mg calcium carbonate, in 50ml of 1+5 hydrochloric acid and diluted to 1000ml with water.

5. Hydrochloric acid, 1%, 10%, 20% (all v/v), 1+5, 1+1 and concentrated.

6. Lanthanum solution: Dissolved 58.65g lanthanum oxide (La_2O_3) in 250ml concentrated hydrochloric acid. Added acid slowly until the material was dissolved and diluted to 1000 ml with water.

7. Hydrogen peroxide, 30%.

8. Nitric acid, 2% (v/v), 1+1 and concentrated.

9. Aqua regia: Added 3 volumes concentrated hydrochloric acid to 1 volume concentrated nitric acid.

10. Standard metal solutions: Prepared a series of standard metal solutions in the optimum concentration range by appropriate dilution of the following stock metal solutions with water containing 1.5 ml concentrated nitric acid / l.

    (a) Chromium: Dissolved 0.1923g CrO_3 in water. Acidified with 10ml concentrated nitric acid and diluted to 1000ml with water. 1.0ml of this solution contains 100 μg of chromium.

    (b) Copper: Dissolved 0.1g copper metal in 2ml concentrated nitric acid, added 10.0 ml concentrated nitric acid and diluted to 1000ml with water. 1.0 ml = 100 μg copper.

    (c) Iron: Dissolved 0.1g iron wire in a mixture of 10ml 1+1 hydrochloric acid and 3.0 ml concentrated nitric acid. Added 5.0 ml concentrated nitric acid and diluted to 1000ml with water. 1.0 ml = 100 μg iron.

    (d) Zinc: Dissolved 0.1g zinc metal in 20ml 1+1 hydrochloric acid and diluted to 1000ml with water. 1.0 ml = 100 μg zinc.

Sample preparation

10ml of the effluent was taken in a 100ml Kjeldahl flask. Added 25ml of 3:2:1 triple acid mixture (concentrated nitric acid - concentrated perchloric acid - concentrated sulphuric acid) and left aside for 3 - 4 hours in a fume cupboard. Then heated for 30 minutes until the initial vigorous reaction had subsided. Heated more strongly for 4 hours until the nitrous fumes were removed and white fumes of perchloric acid were evolved. Allowed the contents to cool and transferred with 3 - 4 washings of deionised water to 10ml volumetric flask and made upto the mark with water.

Procedure

Selected atleast three concentrations of each standard metal solution to find out the expected metal concentration of a sample. Then aspirated each standard in turn into flame and recorded the absorbance.

Prepared a calibration curve by plotting the absorbance of standards versus their concentrations. Plotted calibration curve for chromium based on original concentration of standard before the addition of hydrogen peroxide. Rinsed nebulizer by aspirating water containing 1.5ml concentrated nitric acid / l. Aspirated the sample and determined its absorbance against blank. The estimations of chromium, copper, iron and zinc were done at the wavelengths of 357.9, 324.7, 248.3 and 213.9nm respectively.

Calculation

Calculated the concentration of each metal ion in milligrams per liter, by referring to the appropriate calibration curve.

APPENDIX 15

ESTIMATION OF HEXAVALENT CHROMIUM
Colorimetric method
(APHA, 1998)

Principle

The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red violet colour of unknown composition is produced which can be determined colorimetrically at 540 nm.

Reagents

1. Stock chromium solution: Dissolved 141.4 mg potassium dichromate in water and diluted to 1000ml. 1.0ml = 50 μg chromium.

2. Standard chromium solution: Diluted 10 ml stock chromium solution to 100 ml with distilled water. 1.0 ml = 5 μg chromium.

3. Diphenylcarbazide solution: Dissolved 250mg 1,5 - diphenylcarbazide in 50ml acetone.

4. Sulphuric acid 0.2 N.

Procedure

Pipetted measured volumes of standard chromium solution (5μg/ml) ranging from 2.0 to 20 ml corresponding to the concentration of 10 to 100 μg chromium into the conical flasks. Adjusted the pH of the solution to 1.0 ± 0.3 using 0.2N sulphuric acid. Transferred the solution to a 100ml volumetric flask, diluted to 100ml and mixed. Added 2.0 ml of diphenylcarbazide solution, mixed and let stand 5 to 10 minutes for full colour development. Transferred an appropriate portion to a 1 cm absorption cell and measured its absorbance at 540nm. Used distilled water as reference. Corrected absorbance reading of sample by subtracting absorbance of a blank. From the corrected absorbance, determined the amount of chromium present with reference to the calibration curve.

Calculation

$$\text{mg Chromium(VI) /l} = \frac{\mu\text{g chromium (in 102 ml final volume) x 100}}{\text{A}}$$

where,

A = ml original sample

APPENDIX 16

ESTIMATION OF TRIVALENT CHROMIUM
By calculation
(Saxena *et al.*, 2000)

Calculation

mg/l Trivalent chromium = mg/l total chromium - mg/l hexavalent chromium.

APPENDIX 17

BIOCHEMICAL TESTS FOR THE IDENTIFICATION OF BACTERIA
(Aneja, 1996; Kannan, 1996)

SIMPLE STAINING

In simple staining, the cells (smear) are stained by the application of a single staining reagent. A simple stain that stains the bacteria is a direct stain. The purpose of the simple staining technique is to determine cell shape, size and arrangement of bacterial cells. Simple staining is performed by using basic stains which have different exposure times (e.g. crystal violet, 2 - 60 seconds, carbol fuchsin, 15 - 30 seconds and methylene blue, 50 - 120 seconds).

Procedure

Prepared heat fixed bacterial smears of all the bacterial cultures. Kept a slide on the staining tray and applied about 5 drops of a stain (crystal violet) for

the designated period. Poured off the stain and washed the smear gently with slowly running tap water. Blot dried the slide using blotting paper and observed under the microscope.

GRAM STAINING

It is a very useful stain for identifying and classifying bacteria into two major groups : the gram positive and gram negative.

Procedure

Prepared heat fixed bacterial smears of all the bacterial cultures. Covered each smear with crystal violet for 30 seconds. Washed each slide with distilled water for a few seconds, using wash bottle. Covered each smear with iodine solution for 30 seconds. Washed off the iodine solution with 95% ethyl alcohol. Added ethyl alcohol drop by drop, until no more colour flows from the smear (the gram positive bacteria are not affected while all gram negative bacteria are completely decolourised). Washed the slides with distilled water and drained. Applied safranin to smears for 30 seconds (counter staining). Washed with distilled water and blot dried with absorbent paper. Air dried the slides and examined microscopically using oil immersion objective.

FERMENTATION OF CARBOHYDRATES

Fermentative degradation of various carbohydrates namely glucose, sucrose and lactose by microbes under anaerobic condition was carried out in a fermentation tube for the detection of gas production, as an end product of metabolism.

Procedure

Prepared a fermentation medium whose constituents are given below :

Peptose	-	10.0 g
* Carbohydrate	-	5.0 g

Sodium chloride	-	5.0 g
Phenol red	-	0.018 g
Distilled water	-	1000 ml
pH	-	7.3

(* a specific carbohydrate namely glucose, sucrose and lactose was added).

Broth taken into fermentation tubes was autoclaved at 12lb pressure for 15 minutes.

Labelled each of the specified fermentation tubes with the name of the organism to be inoculated. Inoculated the three types of sugar fermentation broths with each bacterium and kept one uninoculated tube of each fermentation broth as a comparative control. Incubated all the inoculated and uninoculated tubes at 35°C for 24 - 28 hours. Observed for change in colour (due to production of acid) or change in colour and appearance of bubbles (due to production of acid and gas) in the three fermentation media by comparing with uninoculated tubes (control).

INDOLE PRODUCTION TEST

Trytophan, an essential amino acid, is oxidized by some bacteria by the enzyme tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. The indole test is performed by inoculating a bacterium into tryptone broth. The indole produced during the reaction is detected by adding Kovac's reagent (dimethyl - aminobenzaldehyde) which produces a cherry - red reagent layer as illustrated.

$$\text{Tryptophan} \xrightarrow{\text{Tryptophananse}} \text{Indole + pyruvic acid + } NH_3$$

$$\text{Indole + Kovac's reagent} \xrightarrow[\text{Butanol}]{\text{HCl}} \text{Rosindole dye + } H_2O \text{ (Cherry red compound)}$$

Procedure

Prepared 1% tryptone broth by dissolving 10g of peptone in one litre of distilled water. Sterilized in the autoclave at 15 lb (121°C) for 15 minutes. Inoculated the tubes containing tryptone broth with each isolated bacteria and kept one tube as an uninoculated comparative control. Incubated inoculated and uninoculated tubes at 35°C for 48 hours. After 48 hours of incubation, added 1 ml of Kovac's reagent to each tube including control. Shook the tubes gently after intervals of 10 - 15 minutes. Allowed the tubes to stand to permit the reagent to come to the top. Examined the tubes as to the colour in the reagent "layer".

METHYL RED AND VOGES - PROSKAUER TESTS

The methyl red (MR) and the Voges - Proskauer (VP) tests are used to differentiate two major types of facultatively anaerobic enteric bacteria that produce large amounts of acid and those that produce the neutral product acetoin as end product. Both these are performed simultaneously because they are physiologically related and are performed on the same medium (MR - VP broth). In these tests, if an organism produces large amount of organic acids namely formic, acetic, lactic and succinic acid (end products) from glucose, the medium will remain red (a positive test) after the addition of methyl red, a pH indicator (ie pH remaining below 4.4). In other organisms, methyl red will turn yellow (a negative test) due to the elevation of pH above 6.0 because of the enzymatic conversion of the organic acids (produced during the glucose fermentation) to nonacidic end products such as ethanol and acetoin (acetylmethyl carbinol).

Procedure

Prepared MR-VP broth (pH 6.9) tubes of the following composition:

Peptone	-	7.0 g
Dextrose	-	5.0 g
Potassium phosphate	-	5.0 g
Distilled water	-	1000 ml

Poured the 5.0 ml broth in two sets of tubes sterilized by autoclaving at 15 lb pressure for 15 minutes.

Inoculated one set of MR-VP tubes with each isolated bacteria and kept one tube as uninoculated comparative control. Incubated the tubes at 35°C for 48 hours. Added 5 drops of methyl red indicator to all the tubes. Observed the change in colour of methyl red for MR test. Added 12 drops of VP reagent I (naphthol solution) and 2-3 drops of VP reagent II (40% potassium hydroxide) to the other set of tubes as well as to uninoculated control tube. Shook the tubes gently for 30 seconds with the caps off to expose the media to oxygen. Allowed the reaction to complete for 15 - 30 minutes. Observed the tubes for change in colour for the VP test. The development of a crimson to ruby pink colour is indicative of a positive test, while no change in colouration is a negative test.

CITRATE UTILISATION TEST

Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilise / ferment citrate as the sole carbon source. The utilisation of citrate depends on the presence of an enzyme citrase produced by the organism, that breaks down the citrate to oxaloacetic acid and acetic acid. These products are later converted to pyruvic acid and carbon dioxide enzymatically as shown below :

$$\text{Citric acid} \xrightarrow{\text{citrase}} \text{Oxaloacetic acid} + \text{Acetic acid} \xrightarrow{\text{enzymes}} \text{Pyruvic acid} + CO_2$$

The citrate test is performed by inoculating the microorganisms into an organic synthetic medium, Simmon's citrate agar, where sodium citrate is the only source of carbon and energy. Bromothymol blue is used as an indicator. When the citric acid is metabolised, the carbondioxide generated combines with sodium and water to form sodium carbonate, an alkaline product, which changes the colour of the indicator from green to blue and this constitutes a positive test.

$CO_2 + 2Na^+ + H_2O \longrightarrow Na_2CO_3$

(produced during citric acid metabolism) (alkaline pH) (blue colour)

Bromothymol blue is green when acidic (pH 6.9 and below) and blue when alkaline (pH 7.6 and higher).

Procedure

Prepared Simmon's citrate agar (pH 6.9) slants of the following composition:

Ammonium dihydrogen phosphate	-	1.0 g
Dipotassium phosphate	-	1.0 g
Sodium chloride	-	5.0 g
Sodium citrate	-	2.0 g
Magnesium sulphate	-	0.2 g
Agar	-	15.0 g
Bromothymol blue	-	0.8 g
Distilled water	-	1000 ml

Dissolved all the constituents, except phosphates which are to be dissolved separately in 100 ml of water and made the volume to 1 litre. Set the pH to 6.9. Poured the medium in the culture tubes and sterilised by autoclaving at 15 lb pressure for 15 minutes and prepared the slants.

Inoculated Simmon's citrate agar slants with each isolated bacteria. Kept one tube as an uninoculated comparative control. Incubated all the slants at 37°C for 48 hours. Observed the slant cultures for the growth and colouration of the medium.

NITRATE REDUCTION TEST

Among bacteria, there exists a variation in energy metabolism. A number of bacteria are capable of respiring under completely anaerobic conditions by utilising nitrate, sulphate or carbonate as a terminal electron acceptor.

$$NO_3^- + 2e + 2H^+ \longrightarrow NO_2^- + H_2O$$

The reaction is mediated by an enzyme nitrate reductase. The reduction of nitrate in the presence of a stable electron donor to nitrite can be tested by colorimetric reagent.

Reagents

1. Nitrite free medium

Potassium nitrate (nitrite free)	-	0.2 g
Peptone	-	5 g
Distilled water	-	1000 ml

2. Test reagent

Solution A : Dissolved 8.0 g of sulphanilic acid in 1 litre acetic acid.

Solution B : Dissolved 5.0 g of alpha naphtholamine in 1 litre of acetic acid.

Mixed equal volumes of solution A and B (immediately before use) to give the test reagent.

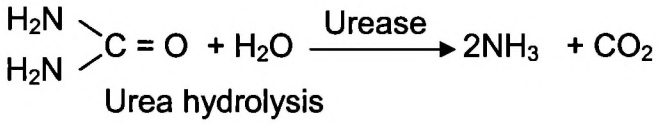
Procedure

Poured 5 ml of prepared medium in test tubes and sterilised at 120°C for 15 minutes. Inoculated the medium with the test culture and incubated at 35°C for 96 hours. Following incubation, added 0.1 ml of the test reagent to the test culture. Observed the development of colour in tubes. Colour development (red) within a few minutes indicates the presence of nitrate and hence the ability of the organism to reduce nitrate.

UREASE TEST

Urea is a major organic waste product of protein digestion in most vertebrates and is excreted in urine. Some microorganisms have the ability to produce the enzyme urease. Urease is a hydrolytic enzyme which attacks the

carbon and nitrogen bond in amide compounds (e.g. urea) with the liberation of ammonia as shown below :



Urea hydrolysis

It is a useful diagnostic test for identifying bacteria, especially to distinguish members of the genus *proteus* from the gram negative pathogens.

Urease test is performed by growing the test organisms on urea broth or agar medium containing the pH indicator phenol red (pH 6.8). During incubation, microorganisms possessing urease will produce ammonia that raises the pH of the medium. As the pH becomes higher, the phenol red changes from a yellow colour (pH 6.8) to a red or deep pink colour. Failure of the development of a deep pink colour due to no ammonia production is an evidence of a lack of urease production by the microorganisms.

Procedure

Prepared urea agar medium with the following composition:

Peptone	- 1.0 g
Sodium chloride	- 5.0 g
Potassium monohydrogen phosphate	- 2.0 g
Agar	- 20 g
Distilled water	- 1000 ml

Dissolved the ingredients by heating, adjusted the pH to 6.8 and autoclaved at 121°C for 15 minutes and cooled to 50°C.

Glucose	- 1.0 g
Phenol red 0.2% solution	- 6.0 ml

Added to the molten base, steamed for 1 hour and cooled to 50°C.

Urea - 20% aqueous solution	- 100 ml

Mixed well and distributed into sterile containers, i.e. flasks, culture tubes and allowed the medium to solidify in a slanting position to form slopes.

Inoculated the tubes with each test organism. Incubated the inoculated slants for 24 - 48 hours at 37°C. Examined the slants as to their colour for the presence of urease (red colour) and for no urease (yellow colour).

CATALASE TEST

During aerobic respiration in the presence of oxygen, microorganisms produce hydrogen peroxide (H_2O_2) which is lethal to the cell. The enzyme catalase present in some microorganisms breaks down hydrogen peroxide to water and oxygen as shown below and helps them in their survival:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Catalase test is performed by adding H_2O_2 to trypticase soy agar slant culture. Release of free oxygen gas ($O_2\uparrow$) bubbles is a positive catalase test.

Procedure

Prepared trypticase soy agar (pH 7.3) slants of the following composition:

Trypticase	-	15.0 g
Phytone	-	5.0 g
Sodium chloride	-	5.0 g
Distilled water	-	1000 ml

Poured the medium in culture tubes and flasks were sterilised by autoclaving at 15 lb pressure for 15 minutes.

Inoculated the trypticase soy agar slants each with one test organism. Incubated the cultures at 35°C for 24 - 48 hours. While holding the inoculated tube at an angle, allowed 3 - 4 drops of hydrogen peroxide (3%) to flow over the

growth of each slant culture. Observed each culture for the appearance or absence of gas bubbles. A catalase positive culture will produce bubbles of oxygen within one minute after addition of hydrogen peroxide.

OXIDASE TEST

During aerobic respiration, oxidase enzymes play a vital role in the operation of electron transport system. Cytochrome oxidase catalyses the oxidation of a reduced cytochrome by molecular oxygen, resulting in the formation of water or hydrogen peroxide.

This test depends on the presence of certain oxidases in bacteria that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye - tetramethyl -p- phenylene - diamine dihydrochloride. The dye is reduced to a deep purple colour.

Procedure

The test cultures were grown on suitable solid medium. Following incubation period, poured on to the plate a freshly prepared 1% solution of tetramethyl -p- phenylene - diamine dihydrochloride solution so as to cover the surface and then decanted. Observed the colonies for the development of colour. The colonies of oxidase positive organisms rapidly develop a purple colour.

APPENDIX 18

ASSESSMENT OF GROWTH PATTERN OF BACTERIA

Turbidity method

(Bhattacharya, 1986)

Principle

Microorganisms do not settle out of suspension and they scatter light, creating turbidity. Turbidity is the effect of light scattering by colloid suspensions. Many growing microorganisms act like colloids. This character of microorganisms is used to measure microbial growth.

As cultures grow, the number of cells increase which is accompanied by a corresponding increase in turbidity. The amount of light scattered is directly proportional to the number of cells in a culture. Turbidity can be measured using spectrophotometer, which measures the amount of light that passes through a liquid medium.

Procedure

Made 5.0 ml suspension of bacteria in sterile distilled water and transferred it to a flask containing 200 ml of sterile nutrient broth. Mixed well and allowed to incubate at 37°C. Noted the time of inoculation. Determined the initial optical density (ie at T_o) at 600 nm wavelength. Aseptically transferred after 2 hours incubation, 5.0 ml of the culture to a cuvette and determined the optical density of the sample at 600 nm. Repeated this process at each two hours interval for a period of 24 hours. Plotted a graph showing optical density against time in hour. From the curve obtained, the growth pattern was assessed.

APPENDIX 19

ESTIMATION OF TOTAL CARBOHYDRATES
Colorimetric method
(Hodge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

Reagents

1. 2.5 N hydrochloric acid.
2. Anthrone reagent : Dissolved 200 mg anthrone in 100 ml of ice cold 95% sulphuric acid. Prepared freshly before use.

3. Stock standard glucose : Dissolved 100 mg in 100 ml water.

4. Working standard glucose : 10 ml of stock was diluted to 100 ml with distilled water.

Procedure

Weighed 100 mg of the sample into a boiling tube. Hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N hydrochloric acid and cooled to room temperature. Neutralised it with solid sodium carbonate until the effervescence was ceased. Made up the volume to 100 ml and centrifuged. Collected the supernatant and took 0.5 and 1.0 ml aliquots for analysis. Prepared the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. '0' was served as blank. Made up the volume to 1.0 ml in all the tubes including the sample tubes by adding distilled water. Then added 4.0 ml anthrone reagent. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X axis versus absorbance on the Y axis. From the graph, calculated the amount of carbohydrates present in the sample tube.

Calculation

Amount of carbohydrates present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

APPENDIX 20

ESTIMATION OF PROTEIN
Colorimetric method
(Lowry *et al.*, 1951)

Principle

The blue colour developed by the reduction of the phosphomolybdic - phosphotungstic components in the Folin - ciocalteau reagent by the amino acids

tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Reagents

1. 2% Sodium carbonate in 0.1N sodium hydroxide (Reagent A)

2. 5% Copper sulphate in 1% potassium sodium tartarate (Reagent B)

3. Alkaline copper solution : Mixed 50 ml of Reagent A and 1.0 ml of Reagent B prior to use (Reagent C)

4. Folin-ciocalteau reagent (Reagent D) - Refluxed gently for 10 hours, a mixture consisting of 100g sodium tungstate, 25 g sodium molybdate, 700 ml water, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid in a 1.5 litre flask. Boiled the mixture for 15 minutes without condenser to remove excess bromine. Cooled, diluted to 1 litre and filtered.

5. Protein solution (stock standard) : Weighed accurately 50 mg of bovine serum albumin and dissolved in distilled water and made upto 50 ml in a standard flask.

6. Working standard : 10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. 1.0 ml = 200μg protein.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. Weighed 500mg of the sample and ground well with a pestle and mortar in 5 - 10 ml of the buffer. Centrifuged and used the supernatant for protein estimation.

Estimation of protein

Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard into a series of test tubes. Pipetted out 0.1 ml and 0.2 ml of sample extract in two other test tubes. Made up the volume to 1.0 ml in all the test tubes. A tube with 1.0 ml of water served as the blank. Added 5.0 ml of Reagent C to each tube including the blank. Mixed well and allowed to stand for 10 minutes. Then added 0.5 ml of Reagent D, mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour was developed. The readings were taken at 660 nm. A standard graph was drawn and calculated the amount of protein in the sample.

APPENDIX 21

ESTIMATION OF PHOSPHORUS
Colorimetric method
(Fiske and Subbarow, 1925)

Principle

Inorganic phosphate reacts with ammonium molybdate in an acid solution to form phosphomolybdic acid. Addition of a reducing agent, 1,2,4 - amino naphthol sulphonic acid reduces the molybdenum in the phosphomolybdate to give a blue colour, but does not affect the uncombined molybdic acid. The blue colour produced is proportional to the amount of phosphorus present and measured at 640nm.

Reagents

1. Molydate reagent : 2.5% Solution of ammonium molybdate in 10N sulphuric acid

2. Amino naphtholsulphonic acid (ANSA) reagent : Dissolved 0.5 g 1,2,4 - aminonaphthol sulphonic acid in 195 ml of sodium bisulphite. To this added 5 ml of 20% sodium sulphite and mixed thoroughly to get a clear solution. (If the resultant solution is not clear, added 0.5 ml of 20% sodium sulphite each time followed by thorough mixing. This can be repeated thrice, beyond which it is not advisable)

3. Stock Standard: Dissolved 35.2 mg of potassium dihydrogen phosphate and made upto 100ml with double distilled water.
4. Working standard: 1.0 ml of the stock was diluted to 10ml with distilled water. 1.0 ml of this solution contains 8 μg of phosphorus.
5. Borate buffer (pH 8.0): Mixed 50ml of 0.2 M boric acid with 4.9 ml of 0.05 M solution of borax.
6. 20% (w/v) Trichloroacetic acid.

Sample extraction

100 mg sample material was kept in borate buffer at pH 8.0 and held it over night at 0°C. The mixture was centrifuged. Equal volumes of this extract were mixed with 20% (w/v) trichloro acetic acid and kept overnight at 0°C and then centrifuged.

Procedure

Pipetted out 1.0 to 5.0 ml of the working standard and made upto 7.6 ml with double distilled water. 1.0 ml of the sample was taken for the experiment and made upto the corresponding volume with water. 8.6 ml of water was taken as the blank. To all the tubes, added 1.0 ml of the ammonium molybdate and then added 0.4 ml of ANSA and kept the tubes as such for 5 minutes. Measured the blue colour formed at 640 nm. A standard graph was prepared with concentration of phosphorus on X-axis and O.D on Y-axis. From the standard graph, the amount of inorganic phosphorus present in the sample was calculated.

APPENDIX 22

ISOLATION OF DEOXYRIBONUCLEIC ACID
Precipitation method
(Marmur, 1961)

Principle

Extraction of DNA is accomplished by the rupturing of cell wall and nuclear membrane followed by deproteinization and precipitation of the nucleic acid using ethanol.

Reagents

1. Extraction medium, 0.15M sodium chloride, 0.1 M Na_2 EDTA
2. Lysozyme solution, 10mg / ml
3. 25% Sodium dodecyl sulphate solution
4. 5 M Sodium chlorate
5. Chloroform : Isoamyl alcohol (24 : 1)
6. 95% Ethanol
7. Saline citrate (1x), 0.15 M sodium chloride, 0.015 M trisodium citrate (also 10 – fold and 1/10 fold concentration)
8. 3M Sodium acetate
9. Isopropanol

Procedure

Ground 2g of the sample material in 25 ml of extraction medium in a pre – chilled pestle and mortar. Added 1.0 ml of lysozyme solution to the above suspension and incubated at 37°C for 30 minutes, shaking occasionally. After the incubation, completed the lysis by adding 2.0 ml of sodium dodecyl sulphate solution, heating this preparation for 10 minutes in a 60°C water bath and finally cooling the solution to room temperature in a bath of tap water. Added sufficient 5M perchlorate solution to the lysed preparation to a final concentration of 1M. Added an equal volume of chloroform - isoamyl alcohol (24:1) to the lysed preparation suspended in 1M perchlorate and slowly shaken (30 - 60 oscillations / minute) in a tightly stoppered flask for 30 minutes at room temperature.

Separated the resulting emulsion by centrifuging for 5 minutes at 10,000g at room temperature. After centrifugation, carefully pipetted off the top clear aqueous phase from the coagulated protein emulsion at the interface. Placed the aqueous phase containing the nucleic acids in a beaker. Gently stirred the

nucleic acid solution with a sterilised glass rod while slowly adding two volumes of 95% ethanol down the sides of the beaker so that ethanol is layered over the viscous aqueous phase. Continued stirring the preparation to mix ethanol throughout the entire aqueous phase and spooled all of the gelatinous, thread – like DNA rich precipitate on the glass rod. Drained off excess fluid from the spooled crude DNA by pressing the rod against the wall of beaker until no further fluid can be squeezed from the spooled preparation. Dissolved the crude DNA on stirring the glass rod with its spool of material in 9.0 ml diluted (1/10 fold) saline citrate in a test tube or small beaker. To the even suspension, added 1.0 ml of 3M sodium acetate, 1mM EDTA (pH 7.0) solution.

Transferred the preparation to a 100 ml beaker and gently swirled the sample while dripping in 5.4 ml of isopropanol. After fibrous DNA was readily apparent, collected the DNA threads by stirring and spooling with a sterilised glass rod as before. Finally, removed excess fluid from the spooled DNA by pressing the sample against the walls of the beaker. Washed the sample in test tubes containing, in turn, 10 ml of 70% ethanol and then 10 ml of 95% ethanol. Stored the DNA in a stoppered tube (2°C refrigerator) as a spool submerged on the rod in 95% ethanol. Removed alcohol from the spooled DNA by blotting with a clean piece of filter paper and then dissolved the DNA by stirring the glass rod in a test tube containing 9.0 ml of dilute (1/10 fold) saline citrate. When the DNA was dissolved, added 1.0 ml of concentrated (10x) saline citrate solution to achieve approximately a standard (1x) saline citrate concentration. This solution can be stored at 2°C with a few drops of chloroform.

APPENDIX 23

ESTIMATION OF DEOXYRIBONUCLEIC ACID
Colorimetric method
(Giles and Meyer, 1965)

Principle

Under extreme acid conditions, DNA is initially depurinated quantitatively followed by the dehydration of sugar to - hydroxylevulinylaldehyde. This

aldehyde condenses, in acidic medium, with diphenylamine to produce a deep - blue coloured condensation product with absorption maximum at 595 nm.

Reagents

1. DNA standard 0.5 mg/ml

2. Saline citrate 0.15 M sodium chloride, 0.015M trisodium citrate solution.

3. Diphenylamine Reagent : Mixed 5g fresh or recrystallised diphenylamine, 500 ml glacial acetic acid and 13.75 ml concentrated sulphuric acid.

Procedure

Prepared separate marked tubes containing 1.0 ml, 2.0 ml and 3.0 ml aliquots of the isolated DNA dissolved in standard saline citrate and similar aliquots of a 0.5 mg DNA / ml standard. Made all sample tubes and a separate blank, upto 3.0 ml with water. Added 6.0 ml of diphenylamine reagent to each tube, and after mixing, heated the tubes in a boiling water bath for 10 minutes. Cooled the tubes. Read the absorbance of blue solution at 600 nm against the blank. Constructed a standard graph A_{600} (ordinate) versus quantity of DNA (abscissa) and then calculated the concentration of DNA dissolved in the saline citrate solution.

APPENDIX 24

ISOLATION OF RIBONUCLEIC ACID
Precipitation method
(Brawerman, 1974)

Principle

The ribonucleoprotein complex is dissociated by sodium dodecyl sulphate (SDS) into RNA and protein, deproteinized by phenol and the free RNA left in aqueous solution is precipitated in the cold after adding alcohol.

the extraction and centrifugation steps at least five times or until the interphase showed no proteins. Finally, collected the upper aqueous phase containing RNA, dissolved in it about 250 mg NaCl, added two volumes of 96% cold ethanol and left the flask overnight at -20°C for RNA precipitation. Collected RNA by centrifugation at 2000 x g for 10 minutes. Washed the RNA pellet with 70% ethanol, ethanol : ether (1:v/v) and finally with ether. Dried the pellet gently 'in vacuo' for a few minutes. Dissolved the RNA completely in elution buffer for further analysis.

APPENDIX 25

ESTIMATION OF RIBONUCLEIC ACID
Colorimetric method
(Lin and Schjeide, 1969)

Principle

The method depends on the conversion of the pentose, ribose in presence of hot acid to furfural, which then reacts with orcinol to yield a green colour. The colour formed largely depends on the concentration of HCl, ferric chloride, orcinol and the time of heating.

Reagents

1. Standard RNA 50 μg RNA / ml.

2. Orcinol acid reagent : Added 2 ml of a 10% solution (w/v) of ferric chloride to 400 ml of concentrated hydrochloric acid.

3. 6% Alcoholic orcinol: Dissolved 6 g orcinol in 100 ml 95% ethanol.

Procedure

Prepared a standard RNA (50 μg RNA / ml) solution in ice - chilled 10mM Tris - acetate, 1 mM EDTA buffer (pH 7.2) or any other suitable buffer by dissolving RNA completely. Dissolved the isolated RNA in the above buffer

solution to an approximate concentration of 50 μg / ml. Prepared a series of tubes containing 0.5 ml, 1.0 ml, 1.5 ml and 3.0 ml of isolated RNA, 0.5 ml, 1.0 ml, 1.5 ml and 3.0 ml of 50 μg standard RNA / ml. Made up each tube to 3.0 ml with water. In addition, set a blank containing 3.0 ml of water. Added 6.0 ml of orcinol acid reagent to each tube. Added 0.4 ml of 6.0% alcoholic orcinol to each tube. Shook the tubes to mix contents and then heated all tubes in a boiling water bath for 20 minutes. Cooled the tubes and read the absorbance at 660 nm against the blank. A standard curve was drawn using A_{660} and the concentration of standard RNA. Calculated the amount of RNA in the isolated solution using the graph.

APPENDIX 26

ESTIMATION OF THE CHARACTERISTICS OF THE SELECTED ADSORBENTS

1. **Ash content** (Raghuramulu *et al.*,1983)

An empty crucible was cleaned well and heated to redness over a Bunsen burner, cooled in a desiccator and weighed. Heating, cooling and weighing were repeated to obtain constant weight.

2.0 g of the indigenous material was taken in the pre-weighed crucible and the total weight was noted. Then the crucible was heated over a Bunsen burner until the sooty flame disappeared. Then kept the crucible in the muffle furnace at 550 ° C for 6 hours. The crucible was then cooled in a desiccator and weighed. (Weight of the crucible + Ash) – (Weight of the empty crucible) gives the weight of the ash content of 2.0 g of the material. From this, the percentage of ash content was calculated as

$$\text{Ash content (\%)} = \frac{\text{Weight of the ash}}{\text{Weight of the sample taken}} \times 100$$

2. **Moisture content** (Raghuramulu *et al.*,1983)

An empty silica crucible with lid was cleaned well and heated to redness over a Bunsen burner. Cooled in a desiccator and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained.

2.0g of the indigenous material was taken in it and heated in the oven at 100°C for 6 hours. Cooled in a desiccator and weighed. This procedure was repeated until a constant weight was obtained. The difference in the weight of the crucible with the indigenous material before and after heating gives the moisture content of the material. From this value, the percentage of moisture content was calculated as

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

3. Bulk density (Pearson, 1970)

Placed 20 g of dried indigenous material in a 100 ml measuring cylinder. Leveled off the powder upto the mark and measured the bulk density in g/ml.

4. **Volatile solids** (APHA, 1998)

Transferred a sample of known weight to an evaporating dish and placed in a muffle furnace. Heated furnace to 550 ± 50°C and ignited for 1 hour. Cooled in a desiccator to balance temperature and weighed.

Calculation

$$\text{\% volatile solids} = \frac{A - C \times 100}{A - B}$$

A = Weight of dried residue + dish, mg

B = Weight of dish, mg

C = Weight of residue + dish after ignition, mg.