COD FRACTIONATION OF TANNERY WASTE-WATER

A PROJECT REPORT

Submitted by

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The Report of the project work submitted by the above students in partial fulfilment for the reward of Bachelor of Technology degree in Biotechnology of Anna University, Chennai was confirmed to be the report of the work done by the above students and then evaluated.

(INTERNAL EXAMINER) **(EXTERNAL EXAMINER)**

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ABSTRACT

This study aims to establish the scientific link between particle size distribution (PSD) and bio-degradability of different COD fractions of tannery waste water by means of physio-chemical and biological techniques. Previous studies have reported that tannery waste- waters include total biodegradable COD portions comprising different fractions. In this study, we elaborate Low cost adsorbents like activated carbon (A1) rice husk (A2), cocoa grass(A3), sand(A4), E.cornia(A5) are used for adsorption. Standard amount (5gms) is measured for higher efficiency. Using different proportions of adsorbents, the degrading efficiency is calculated. By the application of adsorption, More than 95% of the COD get reduced and are degraded by these processes. Biodegradability related COD fractionation was determined at each size interval by optimizing effluent using different parameters. This study also enables to conclude the efficiency and the % fractionation of COD by both the processes. It also enabled the assessment of size distribution for each major COD fraction, as an original tool for better interpretation of specific biodegradation characteristic of the selected tannery waste water. Results also revealed a very slowly biodegradable / reducable COD component with significant inhibitory effect and also can make a comparative study for both the processes.

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LIST OF ABBERVIATIONS:

COD = Chemical Oxygen Demand (mg /l)

COD_r = COD Removal (%)

COD_s = Soluble COD (mg/l)

COD_t = Total COD (mg /l)

D = Days

DW = Dry Weight

TS = Total solid

TSS = Total suspended solids

RH & MRH= Rice husk and modified rice husk

AAS = Atomic adsorption spectroscopy

PSD = Particle size diameter

A1 = Activated charcoal

A2 = Rice husk

A3 = Sand

A4 = Ecornia leaves

A5 = Cocoa grass

CHAPTER 1

INTRODUCTION

Tannery effluent is a strong waste water with complex characteristics. It is associated with a high level of organic pollutants commonly characterized with chemical oxygen demand (COD) concentrations above 3000mgL^{-1} –6000 mgL^{-1} together with significant amount of inorganic compounds such as trivalent chromium and sulphide, capable of exerting toxic or inhibitory effects on biological treatments (Namasivayam et al, 1995). Stringent effluent limitations inevitably prescribe biological treatments for this level of organic content. Consequently achievement of effective removal requires elaborate evaluation of biodegradation characteristics imposed on the degradation process due to the nature of tannery wastewaters. COD is a useful collective design parameter reflecting the total organic content of waste water. Yet it has inherent deficiencies when used alone as it covers not only biodegradable organics, but also biologically resistant and refractory compounds and provides no information on significant organic fractions with different biodegradation kinetics. This missing information is apparently vital for the tannery waste water which embodies a wide spectrum of various organics. Previous studies have reported that the tannery waste waters include total biodegradable COD portions (Cs) comprising different fractions with markedly distinct biodegradability rates and inert COD fractions play an important role on the extent of treatment performance. The reorganization of different soluble and particulate COD fractions not only provided a major breakthrough in the conceptual understanding but also introduced respirometry as the main instrument of assessing COD fraction as an integral part of waste water characterization and modelling. While interpretation of our adsorbents enabled distinction of readily and slowly biodegradable COD fractions and assessment of major model coefficients COD fraction has been primarily based on a different size, different low cost adsorbents used for differentiation of particulate and reducing soluble COD components. Introduction of COD fractions with rapid and slow hydrolysis rates, although giving a clear picture of biodegradation profile within a soluble range did not include any additional information about particle size involved. The complex form of these effluents can also be fractionated with the treatment of microbial organisms. Integration of these two approaches also provide useful information for optimization of available treatment techniques indicating which size fraction is more important within the overall organic content and how the biodegradation characteristic of COD fraction differ in terms of treatment requirements.(Abele et al, 1974)

Research efforts has recently been directed towards particle size information for a better understanding COD fractionation and related biodegradation patterns combining physical particle size distribution of COD by means of sequential filtration/ultra filtration with parallel OUR experiments and model evaluation is the next step for a better interpretation of COD fractions and biodegradability. In this frame work objective of the study was to determine the major COD fractions in tannery waste water in different dosage levels of each low cost adsorbents. This goal was accomplished by means of physio-chemical/ biological together for exploring the relationship between physical adsorption and biodegradability. In this study the complexity of the tannery waste water is evaluated using AAS (Chadwick P et al, 1974)

CHAPTER 2

REVIEW OF LITERATURE

2.1.ADSORPTION:

Adsorption is an effective technique for reduction of COD from waste water. It is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms. Because of their high surface area and high potential adsorbents. The use of commercial activated carbon is not suitable for developing countries because of its high cost. Therefore, there is a need to produce low cost adsorbent, removal from cheaper and readily available materials which can be used economically large scale. Recently, removal of metals from aqueous solution was studied by natural, low cost material such as saw dust, Ecornia leaves, rice husk, cocoa grass, sand and Fe (III)/Cr (III) hydroxide. In the present study a non convection adsorbent from the leaves of the Almond leaves, saw dust, Ecornia leaves, rice husk, coco grass waste were developed as adsorbents which was used for the removal of toxic pollutant in aqueous medium. Adsorption capacity of these adsorbents were compared and reported in the literature. The parameter investigated in the present study included pH; contact time, initial Cr (VI) concentration and adsorbent dosage. The kinetics of adsorption on these adsorbents was also evaluated. The main disadvantages of the adsorbents are that they are expensive and also they pose a treat to the environment. The uses of low cost adsorbents like rice husk, sand etc. are widely studied. After keeping it in different time intervals for adsorption (1, 2, 3, 4, and 5) hrs, it is filtered off through watt man filter paper and collected in a separate funnel. Then it is taken for COD test and measured (Argus et al, 2006).

2. 2 ADSORBENTS

Adsorbents are used usually in the form of spherical pellets, rods, mouldings, or monoliths with hydrodynamic diameters between 0.5 and 10 mm. They must have high abrasion resistance, high thermal stability and small pore diameters, which results in higher exposed surface area and hence high surface capacity for adsorption. The adsorbents must also have a distinct pore structure which enables fast transport of the gaseous vapours.

Most industrial adsorbents fall into one of three classes:

- Oxygen-containing compounds – Are typically hydrophilic and polar, including materials such as silica gel and zeolites.
- Carbon-based compounds – Are typically hydrophobic and non-polar, including materials such as activated carbon and graphite.
- Polymer-based compounds - Are polar or non-polar functional groups in a porous polymer matrix.

The main disadvantages of the above adsorbents are that they are expensive and also they pose a threat to the environment. The use of low cost adsorbents like sugarcane baggase, dried leaves, rice husks, sawdust, maize and biogas residual slurry, brewery biomass etc. are widely studied.

2.3 COD FRACTIONATION:

2.3.1 COD fractionation using Activated Carbon:

The most studied adsorbent in adsorption of chromium is found to be the activated carbon derived from various raw materials such as sawdust, nut shells, coconut shells etc. (Kabdsali I et al, 1993) (Garrison et al, 1963) studied the removal of Cr (VI) from aqueous solution using GAC type Filtrasorb Water Air Soil Pollute 400. It was found that reduction in particle size of adsorbents increases its surface area for metal adsorption, and it results in higher removal efficiency on Cr6+. It was also indicated that the adsorption of heavy metals was more favourable at higher temperature. (Kabdsali I et al, 1993) prepared several activated carbons from Terminalia Arjuna nuts, an agricultural waste, by chemical activation with zinc that showed maximum removal of chromium at pH 1.0. (Garrison et al, 1963) used activated carbon produced by Sutcliffe Carbon starting from a bituminous coal to adsorb metals. It was found that the adsorption for the activated carbon strongly depends on solution pH and salinity. The main demerit of the process lies in its frequent desorption and regeneration and is dependent on the life of the adsorbent. In this study, different dosage levels of (A1) are treated with the effluents to denote their adsorbing efficiency. The values are noted and plotted down for 12 dilutions with respect to the dosage values vs. %removal.

2.3.2 COD fractionation using Rice husk:

The adsorption behavior of Cu (II) and Cd (II) ions onto rice husk and modified rice husk by Guo. The adsorption of the heavy metal ions by adsorbed are analysed by determing the COD concentration in samples. It was observed that the adsorption capacity of rice husk increased after treated with nitric acid. The pseudo first-order, pseudo second-order, second order and intra-particle diffusion models were used to analyze the kinetic data and the rate constants were evaluated. data was fitted very well with Freundlich and Langmuir isotherm models. The preliminary studies show that the RH and MRH can be used effectively in the removal of Cu (II) and Cd (II) from aqueous solution through adsorption. The equilibrium of Langmuir and Freundlich constants indicate favorable conditions for adsorption and the adsorption capacity of RH improved after treated with nitric acid (Guo et al, 2008).

2.3.3 COD fractionation using sand:

The use of agricultural products and by-products has been widely investigated as a replacement for current costly methods of removing heavy metals from water and wastewater. Some of the agricultural materials can be effectively used as a low-cost sorbent. Modification of agricultural by-product could enhance their natural capacity and add value to the by-product. Sands were modified by combinations of treatments following a 3^2 factorial design. Treatments consisted of either no wash, water wash or base wash followed by no modification or modification with 0.6M citric acid or 0.6M phosphoric acid. The percent of metal ions adsorbed per gram of adsorbent was significantly increased by each of the acid treatments, average values ranged from 19 to 34% compared with non-acid treated samples at 5.7%. The percent of metal ions adsorbed for base-washed samples were higher than water-washed or unwashed shells. Interaction between wash and acid treatment was not significant for most of the experimental conditions used. In solutions containing multiple metal ions, citric acid samples were found to be most effective and selective for Cu (II) compared with Cd (II), Ni (II), and Zn (II). In general, phosphoric acid-modified shells removed the most metals from solution for the experimental samples and were more effective in removing Cd (II) and Zn (II) than two of the three commercial resins. Acid-modified sands are promising as metal ion adsorbents. The removal of four heavy metals i.e. Pb, Cr, Cu, and Zn from their aqueous solutions, using ordinary sand as an adsorbent, was studied at 20°C. The amount of metal adsorbed to form monolayer on sand (a_m), exhibited the preference of metals for sand in the order Pb>Cr>Cu>Zn. The heavy metal-sand adsorption phenomena can be illustrated on the basis of the interaction between surface functional group of silicates (sand) and the metal ions. It is deduced that sand can be used as a low cost adsorbent for the removal of heavy metal from wastewater (containing low conc. of metals)

2.3.4 COD fractionation using Ecornia leaves:

The batch removal of arsenic from aqueous solution using low-cost adsorbent (E cornia leaves) under the influences of arsenic ion concentrations (0.50 to 1.50 mg/l), pH (3.2 to 11.5) and particle size (63 to 150 μm) were investigated. E cornia leaves were collected, washed with distilled water, air dried, ground into powder and sieved into different sieve sizes using British standard sieve. The study revealed that there was a slight reduction in the rate of adsorption of arsenic ion onto the larger particle size, but adsorption capacity and parameters were unaffected. E cornia leaves with particle size of 63 μm removed up to 99.6% of the 1.5 mg/l of arsenic ion in synthetic water within the first 6 hours but decreased to 98.4% and 97.4% when the E cornia leaves particle sizes were increased to 75 and 150 μm respectively. The pH optimum for arsenic removal was 6-7.

Heavy metals have been used in many industries for coloration purpose. Tannery industry is one of the prominent polluters releasing high concentrated effluent into the surrounding environment. Dyes contain carcinogenic materials which can pose serious hazards to aquatic life and end users of the water. Therefore, it is important to remove these pollutants from wastewater before their final disposal. This study investigates the potential of E cornia leaves powder as a low cost adsorbent for heavy metal removal. The adsorption was carried out to study different initial methylene blue concentration using batch technique.

The research is carried out to investigate the removal of lead from battery manufacturing wastewater by leaves. The effect of operating parameters i.e., initial pH, contact time, types of leaves and dose of leaves were investigated. The characteristics and chemical compositions of leaves were also investigated and experimental samples were analyzed using AAS, then the data was statistically processed using least significant difference at a 95% confidence level ($p<0.05$). The results indicated that the optimum pH for lead removal using 4 types of leaves were at pH 6, but at this pH final concentration of lead was too low for study. Therefore, unadjusted pH wastewater was used with an initial lead concentration of about 2.365 mg/l, initial pH of 1.35-1.45.

2.3.5 COD fractionation by Cocoa grass:

Heavy metals are present in abundance naturally and enter the water cycle through a variety of geochemical processes. Many metals are added to water by industrial processes. Disposal of untreated wastes, surface run-off and highway run-off also cause metal pollution of surface water.

The possibility of using cocoa grass produced in already working incineration plants, as adsorbents for waste water treatment, was studied. Showing very poor adsorption properties, they were improved by steam activation technique used in the conventional activated carbon manufacturing. It is concluded that various organic waste materials can be converted carbonaceous final products with a character similar to activated carbon. Their adsorption properties and pore size distribution are determined by the structure of the starting material. Adsorption tests with model waste waters confirmed that adsorption properties are strongly influenced by the character of the suface. The adsorption capacity of these samples can be utilized for the treatment of strongly polluted industrial waste waters. Considering that the raw material needed to manufacture these adsorbent is produced permanently and the adsorbents do not have to be regenerated, it might be worthwhile using these kinds of adsorbents in the primary treatment of industrial waste waters.

2.3.6 COD Fractionation using Micro- organisms

Many of the characteristics of Acinetobacter ecology, taxonomy, physiology and genetics point to the possibility of exploiting its unique features for future applications. Acinetobacter strains are often ubiquitous, exhibit metabolic versatility, are robust and some provide convenient systems for modern molecular genetic manipulation and subsequent product engineering. These characteristics are being exploited in various biotechnological applications including biodegradation and bioremediation, novel lipid and peptide production, enzyme engineering,biosurfactant and biopolymer production and engineering of novel derivatives of these products. It is anticipated that progress in these fields will broaden the range of applications of Acinetobacter for modern biotechnology. The method is effective, low cost but it is time defective.

2.4 ISOLATION AND CULTURE MEDIUM:

Blood Agar (BAP) Bacterial Growth Medium: Blood Agar is a bacterial growth medium that can distinguish normal from pathogenic bacteria based on the effect of bacterial hemolytic enzymes on red blood cells. A growth medium (plural: media) is a mixture of nutrients, moisture and other chemicals that bacteria require for growth. Media are used to grow bacterial colonies (millions of bacteria having arisen through the binary fission of a single progenitor).

Using Media to Identify Bacteria:

Some media can be used to do more than just grow bacteria; specialized agars can aid in bacterial identification. Differential and selective media are special types of agar that can exclude certain types of bacteria and even test for certain bacterial metabolic capabilities. MacConkey's (MAC), Blood agar (BAP) and Mannitol Salt (MSA) are three examples of these specialized types of media.

Blood Agar Is Not a Selective Medium:

If a bacterial growth medium is selective, that means that it grows only certain types of microbes while inhibiting the growth of other types of microbes. Blood agar is an enriched medium that provides an extra rich nutrient environment for microbes. Therefore, BAP is not a selective medium. Since it supports the growth of a wide range of organisms.

Blood Agar Is a Differential Medium:

A growth medium is considered differential if, when specific microbes are present, the medium or bacterial colonies themselves exhibit a color change that provides information about their identity. Blood agar (BAP) is a differential growth medium which microbiologists use to distinguish clinically significant bacteria from throat and sputum cultures. BAP contains 5% sheep blood. Certain bacteria produce enzymes (hemolysins) that act on the red blood cells to lyse or break them do.

CHAPTER 3

OBJECTIVES

- The main objective of this study is to analyse and characterise the sample and their COD and to reduce them effectively by means of low cost methods.

- To study the effectiveness of both the methods and degrading efficiency based of time and cost effectively.

- Other metals are targeted and they are removed with specific adsorbents to find out the adsorbing capacity of those adsorbents.

CHAPTER 4

MATERIALS AND METHODS:

4.1 STUDY AREA:

The tannery waste water (sample) is collected from Erode zone. It is highly stored in perfect conditions as per the requirements. The general characteristics of the sample are studied for convenience.

4.2 CHARACTERISATION:

Table 1. CONVENTIONAL WASTE WATER CHACTERISATION:

[illegible]	[illegible]	[illegible]
COD (mg/l)	**5536-6555**	**6250**
TSS (mg/l)	**8-10**	**9.68**
pH	**Acid**	**3.69**
color	**Dark bluish**	**Dark blue**

4.3 METHODS:

Conventional methods to reduce Heavy metals from the waste water stream includes

4.2.1 PHYSIO/CHEMICAL METHOD

4.2.2 BIOLOGICAL METHOD

4.3.1 PHYSIO-CHEMICAL METHOD

4.3.1.1 GENERAL:

The method must utilize maximum removal efficiency of heavy metal from contaminated waste water. Most commonly by adsorption (Ates E, orhon D, tunayO et al, 1997). It is the most common method for removing toxic heavy metals up to parts per million levels from water. Ates E in 1995 investigated the heavy metal removal from tannery wastewater using the process. Using this process metal concentration could be removed from 3,860 mg/l to less than 0.2 mg/l. Although the process is cost effective its efficiency is affected by low pH and the presence of other salts (ions). The process requires addition of other chemicals, which finally leads to the generation of a high water content sludge, the disposal of which is cost intensive. Also by Precipitation with lime, disulphide or ion exchange lacks the specificity and is ineffective in removal of the metal ions at low concentration.

4.3.1.2 CHEMICALS

All the chemicals used were of analytical reagent grade were used without any further purification. Potassium dichromate (salt) was purchased from Qualigens Fine Chemicals (Mumbai). 1,5-diphenylcarbazide, Acetone, 0.2N H_2SO_4, HCl, NaOH and distilled water were also used.

4.3.1.3 BATCH ADSORPTION STUDY

Batch adsorption experiments were conducted by agitating the flasks with each of the adsorbent and varying concentration of chromium solution (simulated effluent) from 20-100mg/l for a period of 8 hours at 30 ± 10°C in a mechanical shaker. Experiments were also carried out by varying the adsorbent dosage from 1g to 5g of all the adsorbents mentioned (Gupta *et al.,* 2005). The concentration of free chromium (VI) ions in the effluent after treatment was determined spectrophotometrically from the purple colour developed with 1,5-DPC in acidic solution.

4.3.1.3 ADSORBENT MATERIALS:

The adsorbent used here for the purpose of COD fractionation are as follows

- Activated carbon
- Sand
- Rice husk
- E.cornia leaves
- Cocoa grass

4.3.1.4 ADSORBENT PREPARATION:

Activated carbon:

Activated carbon were collected from the laboratory readily and measured. They were ground into fine powder and sieved using a mesh size of 150µm for fine results.

Sand:

Sand is collected from different sides of the college. It was dried continuously for reducing the moisture content present in it. It was sieved using 150µm sieve and the powder was stored.

Rice husk:

The rice husks were collected from rice mills near the college premises. It was then ground to fine powder and sieved using a 150µm sieve.

Ecornia leaves:

Leaves were collected from various conditions and dried heavily to activate it. Then it is sieved using 150 µm sieve.

Cocoa grass:

Cocoa grass were collected and soaked in 1% sodium hydroxide for 20 minutes and rinsed with distilled water. The moisture content was removed by keeping in a hot air oven at 50°C for an hour, powdered and sieved using 150μm sieve.

4.3.2 BIOLOGICAL METHOD

4.3.2.1 GENERAL:

This chapter describes materials used and outlines the experimental design for biodegradation through batch process. It also gives an overview of the isolation and characterization of bacterial strains. Optimization of culture and process parameters for the biomass growth and degradation kinetics is also studied. Treatment of mineral processing effluent at optimum conditions is studied to observe the efficiency of the indigenous strain.

4.3.2.2 CHEMICALS:

Pure and analytical grade chemicals were used in all experiments including media preparation for growth.

4.3.2.3 GLASSWARES AND APPARATUS:

All glass wares (Conical flasks, Measuring cylinders, Beakers, Petric plates and Test tubes etc.) are purchased from under the name Borosil. The instruments and apparatus used throughout the experiment.

4.3.2.4 ANALYSIS OF ENRICHMENT CULTURE:

Acinetobacter is a gram negative genus of Bacteria belonging to the Gammaproteobacteria. Non motile, Acinetobacter species are oxidase negative, and occur in pairs under magnification. They are important soil organisms where they contribute to the mineralisation, for example, aromatic compounds. Acineto bacter are a key source of infection in debilitated patients in the hospital. For isolation of chromium resistant bacteria, 1 ml of the wastewater sample was spread on blood agar medium (agar plates containing 100 ml of Cr6+/ml supplemented as K_2Cr2O7 to the medium).

4.3.2.5 ISOLATION AND CULTURE CONDITIONS:

For isolation of chromium resistant bacteria, 1 ml of the wastewater sample was spread on Mac Conkey and blood agar medium agar plates containing culture to the medium. Agar plates were prepared. The medium was autoclaved at 121° C and 15 lb pressure for 15 min. The growth of the bacterial colonies was observed after 24 h of incubation at 37°C. Isolated colonies were picked up with sterilized wire loop and streaked on blood agar medium plate containing 100 mg/ ml. Cr6+. It was again incubated at 37°C for 24 h. This process was repeated with successively higher concentrations (12.5, 25, 50, 75, 100μl/ml) until the minimum inhibitory concentration (MIC) of bacterial isolate was obtained. Significant growth and rapid kinetics of the specific bacterial species in the presence of 25, 50 and 100 μl/l Cr during twenty four hours of incubation at 30°C were considered as metal resistant. A single strain capable of growing at this condition was selected for further experiments

CHAPTER 5

RESULTS AND DISCUSSION

5.1 STANDARD GRAPH CALIBRATION

Table 2. Estimation of Cr (VI)-COLORIMETRIC METHOD

REAGENTS	B	S1	S2	S3	S4	S5
Volume of working standard(ml)	-	20	40	60	80	100
Concentration of working standard (100mg/ml)	-	20	40	60	80	100
Volume of working sample(ml)	-	2	2	2	2	2
Volume of 0.2N H_2SO_4(ml)	5	5	5	5	5	5
Make up to 100ml with distilled water						
Kept at room temperature for 15 minutes						
Volume of 1,5- DPC reagent added(ml)	5	5	5	5	5	5
Kept at room temperature for an hour						
Absorbance at 540nm	0.00	.086	.196	.275	.369	.421

2ml of known standard Chromium solution sample was taken and 5ml of 0.2N Sulphuric acid was taken in a standard flask and made upto 100ml. After 15 minutes, 5 ml of 1,5- DPC was added and mixed well. The absorbance was measured at 540 nm using a Beckman BU 530 spectrophotometer after an incubation period of 1 hour. The standard graph was plotted by taking concentration along X axis and absorbance along Y axis. A straight line was obtained with a slope value of 0.0044 and R2 value of 0.9914 as shown.

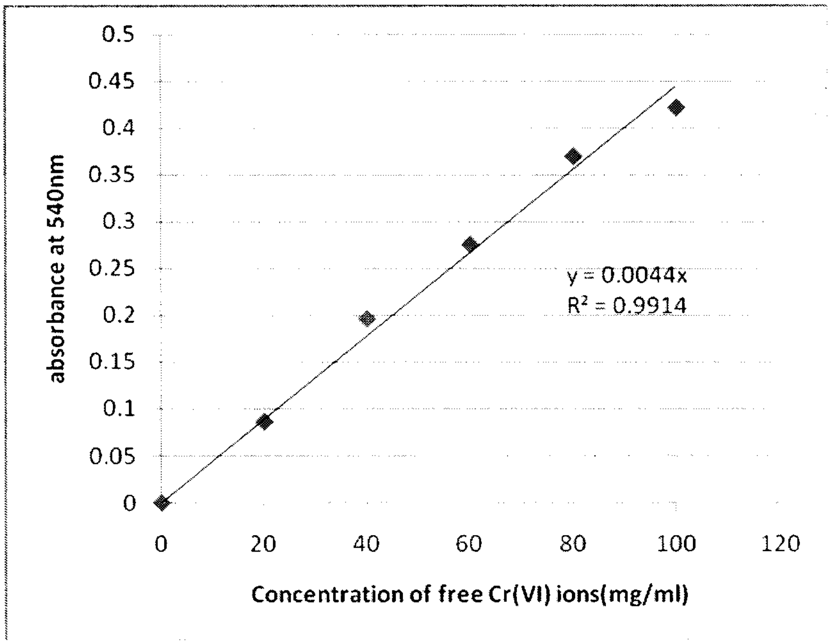


Fig1. Standard graph for Cr (VI) estimation-Colorimetric Method

4.7.2 STANDARD TIME DURATION ON INITIAL CONCENTRATION FOR 3 HOURS:

To determine the effect of adsorbent dosage on adsorption with respect to revolution, 100 ml of heavy metal solution of known concentration (50mg/ml) was added to, 1 to 5 g of adsorbent in a 150 ml conical flask and the mixture was stirred on a thermostat shaker bath for a contact time of 3 hours (Bulut, Y and Baysal, Z. *et al.,* 2006). Then, the solution was centrifuged at the speed of 100-500 RPM, filtered, and then the supernatant was analyzed for metals estimation by COD method.

5.2 BATCH ADSORPTION STUDY

5.2.1 Adsorption using activated charcoal

Activated charcoal is used as the adsorbent for COD fractionation. Activated carbon has the efficiency to degrade chromium ions. It was found that reduction in particle size of adsorbents increases its surface area for metal adsorption, and it results in higher removal efficiency on Cr6+. It was also indicated that the adsorption of Cr6+ was more favorable at higher temperature (Gupta et al, 2005) prepared several activated carbons from Terminalia arjuna nuts, an agricultural waste, by chemical activation with zinc that showed maximum removal of chromium at pH 1.0. (Gupta et al. 2005) used activated carbon produced by Sutcliffe Carbon starting from a bituminous coal to adsorb metals. It was found that the adsorption for the activated carbon strongly depends on solution pH and salinity.

Table 3. Adsorption using activated charcoal after 3 hours

DOSAGE In gms	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
1gm	8	0.183	4860	22%
	9	0.156	2180	65.12%
	10	0.144	2035	67%
	11	0.132	2240	64.16%
	12	0.116	2101	64.96%
2gm	8	0.092	1996	68.06%
	9	0.048	816	86.94%
	10	0.032	783	87.47%
	11	0.018	540	91.36%
	12	0.009	334	94,60%
3gm	8	0.076	1401	77.58%
	9	0.036	782	87.48%
	10	0.028	580	90.72%
	11	0.020	544	91.29%
	12	0.018	424	93.21%

4gm	8	0.065	1235	80.24%
	9	0.032	783	87.4%
	10	0.028	545	91.28%
	11	0.020	539	91.37%
	12	0.012	412	93.40%
5gm	8	0.042	1010	83.84%
	9	0.031	760	81.84%
	10	0.016	525	91.6%
	11	0.012	478	92.35%
	12	0.007	286	95.42%

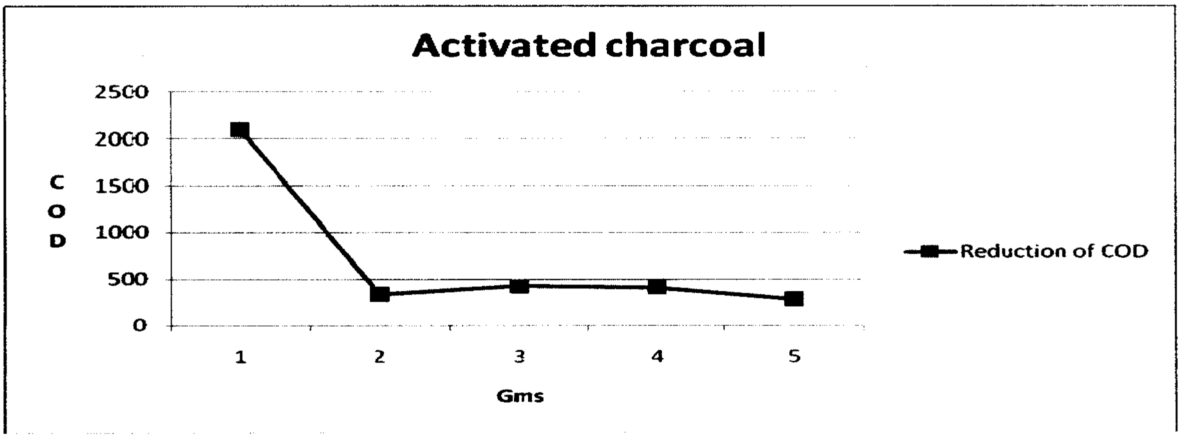


Fig2. COD reduced vs Amount of adsorbent (Activated charcoal) in gms

Fig2. COD reduced vs Amount of adsorbent (Activated charcoal) in gms

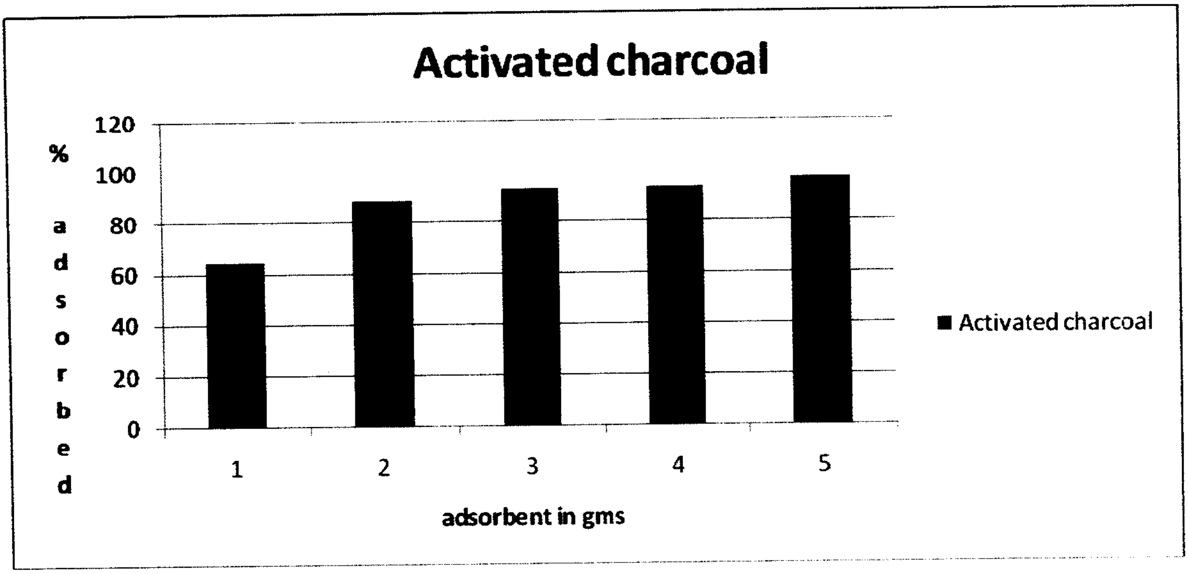


Fig3. % adsorbed vs Amount of adsorbent (Activated charcoal) in gms

The percentage removal of activated charcoal used as adsorbent is highly efficient in tannery effluent. The degrading efficiency and the COD fractionation is about 90% and it cleaves the problem for the lack of low cost adsorbents.

5.2.2 Adsorption using Rice husk.

The adsorption behavior of Cu (II) and Cd (II) ions onto rice husk and modified rice husk. The preliminary study shows that the RH and MRH can be used effectively in the removal of Copper and Cadmium from aqueous solution through adsorption. Adsorbents are taken for different dosage levels to identify their efficiency of fractionation. It unveils the dramatic degrading effects of heavy metals by adsorption and can be used as a low cost adsorbent. The preliminary studies show that the RH and MRH can be used effectively in the removal of Cu (II) and Cd (II) from aqueous solution through adsorption. The equilibrium of Langmuir and Freundlich constants indicate favorable conditions for adsorption and the adsorption capacity of RH improved after treated with nitric acid (Guo et al, 2008).

Table 4. Adsorption using Rice husk after 3 hours

DOSAGE In gms	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% REMOVAL
1gm	8	0.112	2300	63.20%
	9	0.108	2122	66.04%
	10	0.072	1823	70.83%
	11	0.058	1212	80.60%
	12	0.052	1001	63.98%
2gm	8	0.102	2101	66.36%
	9	0.092	2008	68.87%
	10	0.063	1545	75.28%
	11	0,052	1001	83.98%
	12	0.050	0910	85.54%
3gm	8	0.092	2008	68.87%
	9	0.072	1823	70.83%
	10	0.058	1212	80.60%
	11	0.048	858	80.27%
	12	0.042	841	86.54%

4gm	8	0.081	1842	70.52%
	9	0.068	1645	73.68%
	10	0.053	1001	83.90%
	11	0.042	841	80.54%
	12	0.031	636	89.84%
5gm	8	0.078	1643	73.71%
	9	0.063	1545	75.25%
	10	0.044	891	85.74%
	11	0.031	751	87.98%
	12	0.020	541	91.34%

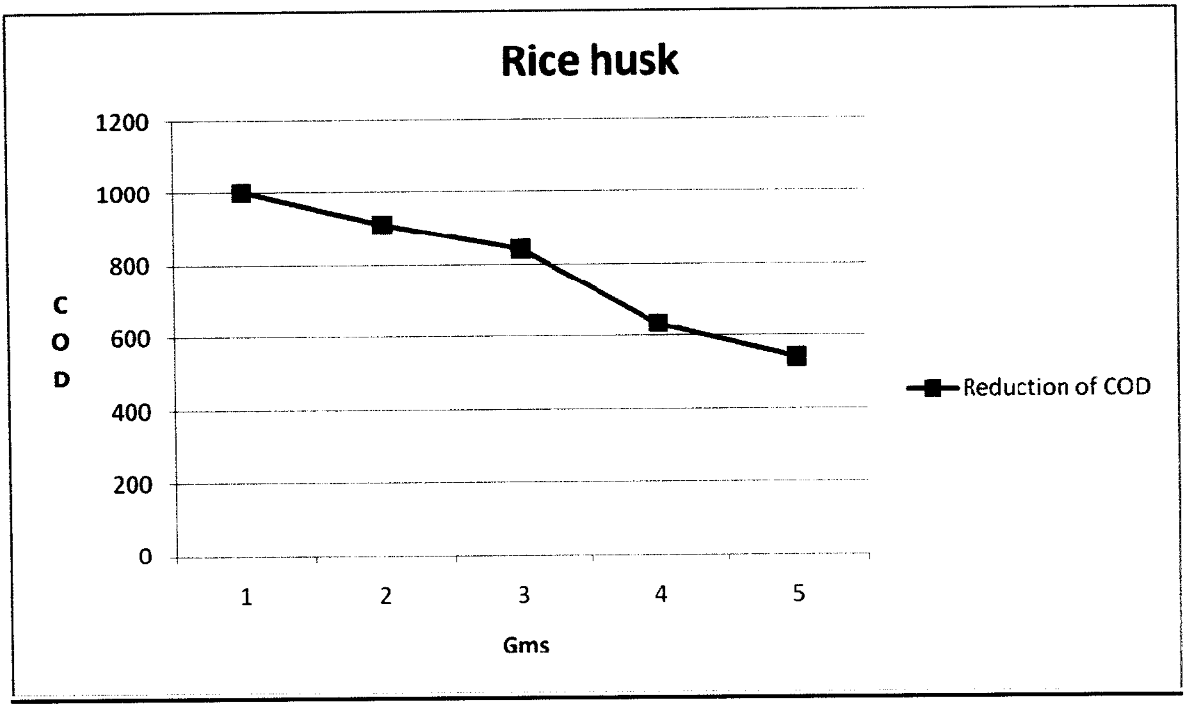


Fig4. COD reduced vs Amount of adsorbent (Rice husk) in gms

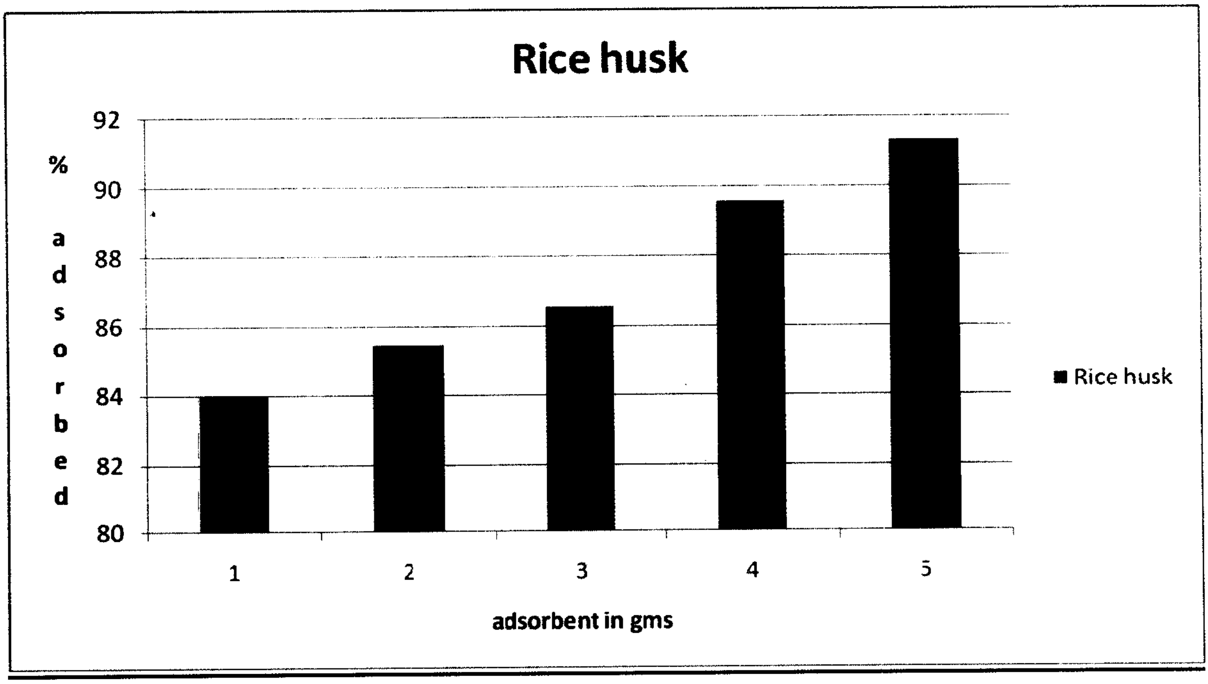


Fig5. % adsorbed vs Amount of adsorbent (Rice husk) in gms

The percentage removal of metals while, Rice husk used as adsorbent is highly efficient in tannery effluent. The degrading efficiency and the COD fractionation is about 92- 94% and it cleaves the problem for the lack of low cost adsorbents.

5.2.3 Adsorption using Sand

Adsorption using Sand is a very effective method, as well as cost effective. Mainly it absorbs iron and magnesium more than 83%. The removal of four heavy metals i.e. Pb, Cr, Cu, and Zn from their aqueous solutions, using ordinary sand as an adsorbent, was studied at 20°C. Adsorbents are taken for different dosage levels to identify their efficiency of fractionation. It unveils the dramatic degrading effects of heavy metals by adsorption and can be used as a low cost adsorbent.

DECOLOURISATION:

It degrades the color of the effluent completely as normal colourless from dark bluish color.

Table 5. Adsorption using Sand after 3 hours

DOSAGE In gms	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
1gm	8	0.174	2242	64.12
	9	0.156	2034	67.45
	10	0.134	1844	70.49
	11	0.112	2300	63.32
	12	0.108	2122	66.04
2gm	8	0.163	2838	54.59
	9	0.142	2030	67.45
	10	0.124	2403	61.55
	11	0.092	1851	70.38
	12	0.089	1853	70.35
3gm	8	0.144	2035	67.44
	9	0.124	2403	61.55
	10	0.110	2300	63.20
	11	0.082	1842	70.52

	12	0.042	841	86.54
4gm	8	0.135	2454	66.76
	9	0.112	2300	63.20
	10	0.108	1835	70.64
	11	0.078	1503	75.95
	12	0.038	801	86.96
5gm	8	0.064	1244	80.09
	9	0.076	1344	78.90
	10	0.089	1631	73.40
	11	0.074	1441	86.90
	12	0.034	781	91.09

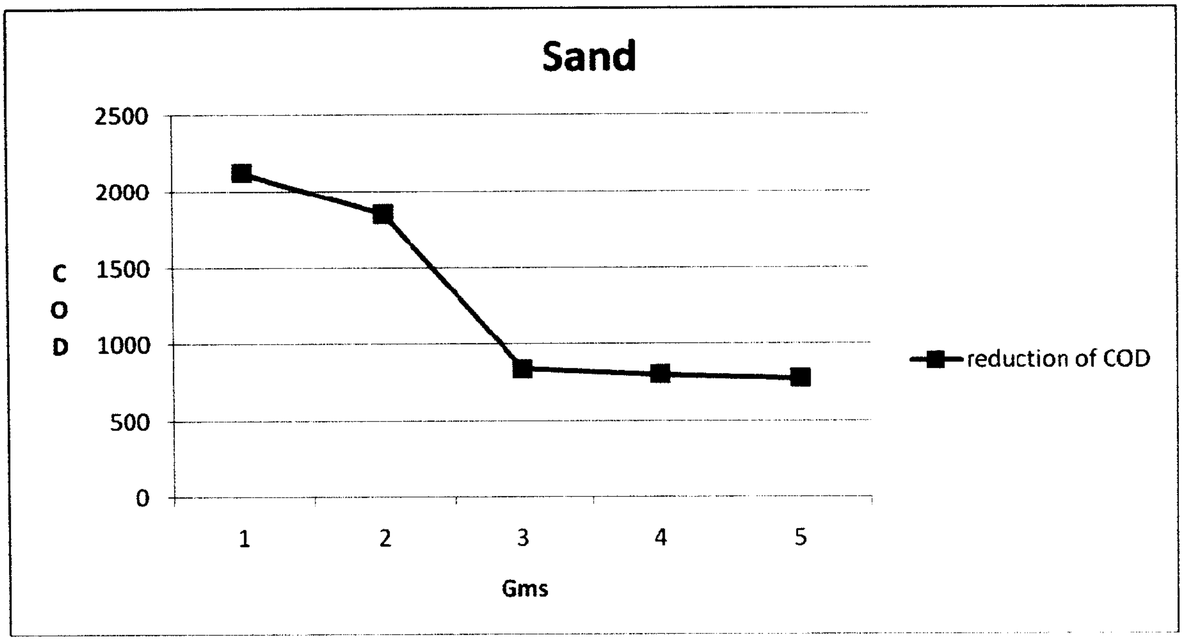


Fig6. COD reduced vs Amount of adsorbent (Sand) in gms

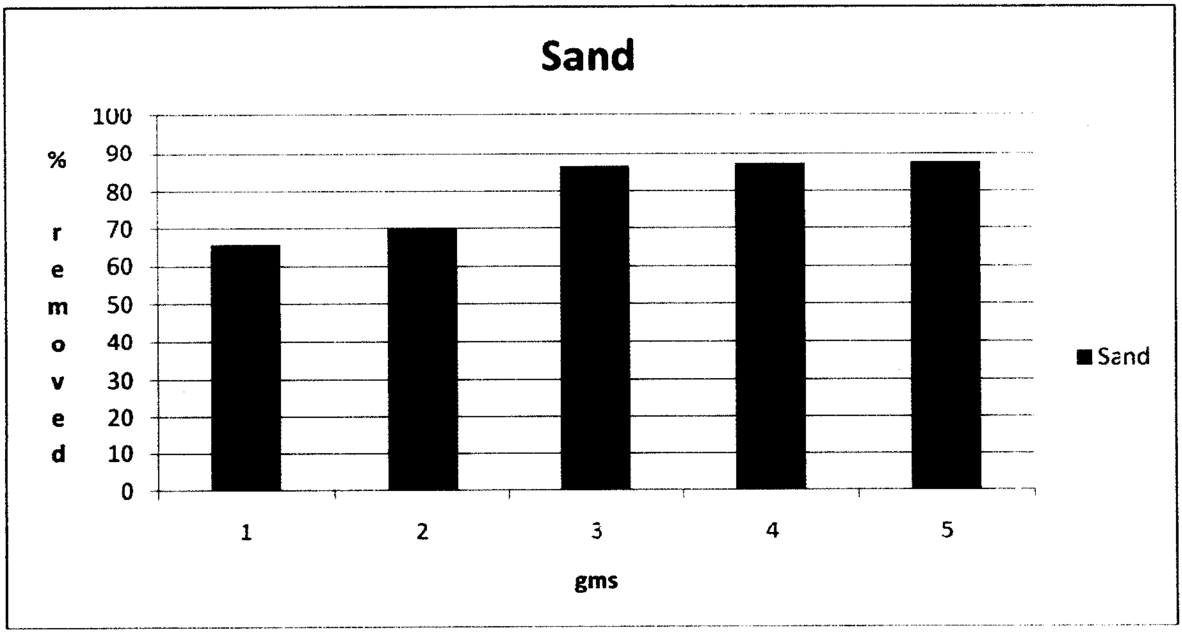


Fig7. % adsorbed vs Amount of adsorbent (Sand) in gms

The percentage removal of metals while, Sand used as adsorbent is highly efficient in tannery effluent. The degrading efficiency and the COD fractionation is more than 85% and it cleaves the problem for the lack of low cost adsorbents.It degrades the color of the effluent completely as normal colorless from dark bluish color.

5.2.4 Adsorption using Ecornia leaves

Ecornia leaves are collected from wet conditions and dried for removing the moisture conditions. Different dosage levels are measured and used for adsorption. The batch removal of arsenic from aqueous solution using low-cost adsorbent (Ecornia leaves) under the influences of arsenic ion concentrations (0.50 to 1.50 mg/l), pH (3.2 to 11.5) and particle size of Ecornia leaves (63 to 150 μm) were investigated. Ecornia leaves were collected, washed with distilled water, air dried, ground into powder and sieved into different sieve sizes using British standard sieve.The degrading efficiency and the amount of COD reduced are noted down.

Table 6. Adsorption using Ecornia leaves after 3 hours:

DOSAGE In gms	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
1 gm	8	0.210	5845	6.48
	9	0.232	6002	3.96
	10	0.189	4620	26.08
	11	0.144	2035	67.44
	12	0.142	2020	67.68
2gm	8	0.089	5010	19.84
	9	0.179	4235	32.24
	10	0.169	3232	48.28
	11	0.149	2045	67.44
	12	0.133	2021	67.66
3gm	8	0.078	3800	39.20
	9	0.177	3797	39.24
	10	0.163	2210	67.47
	11	0.144	2035	67.44
	12	0.142	2024	67.61

4gm	8	0.172	3725	40.40
	9	0.173	3800	39.20
	10	0.156	2180	67.47
	11	0.133	2070	66.88
	12	0.121	2011	67.74
5gm	8	0.169	3849	38.41
	9	0.142	3843	38.49
	10	0.144	2035	67.44
	11	0.121	2112	66.20
	12	0.062	1002	67.96

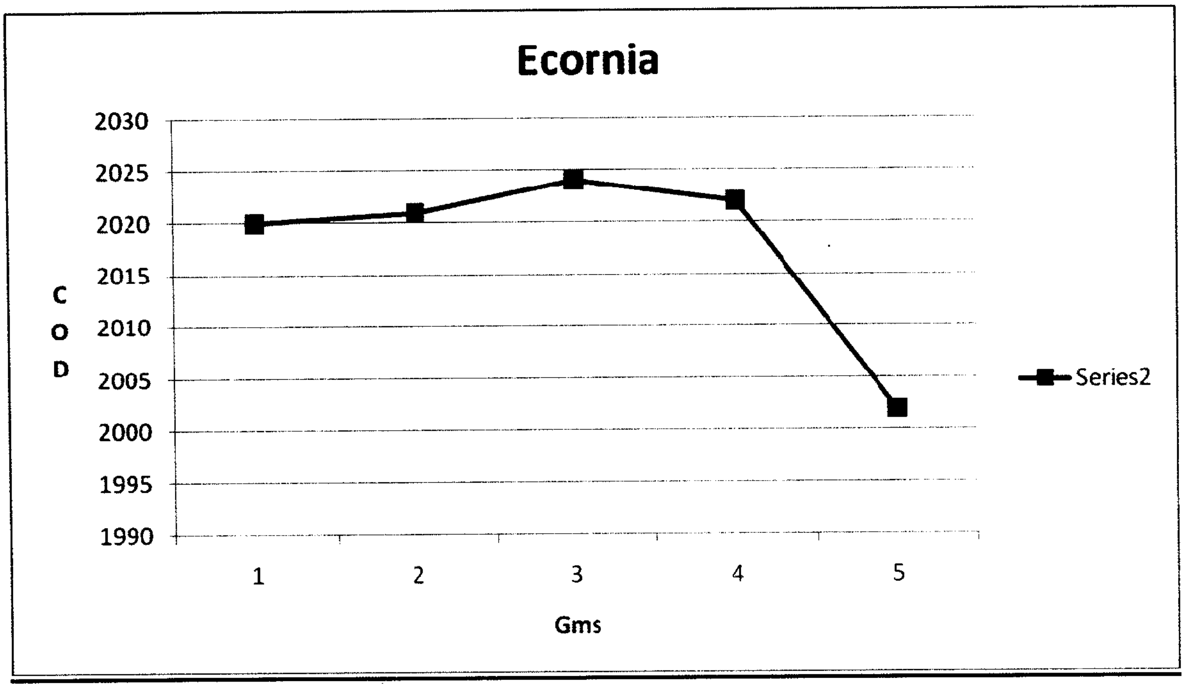


Fig8. COD reduced vs Amount of adsorbent (E cornia leaves) in gms

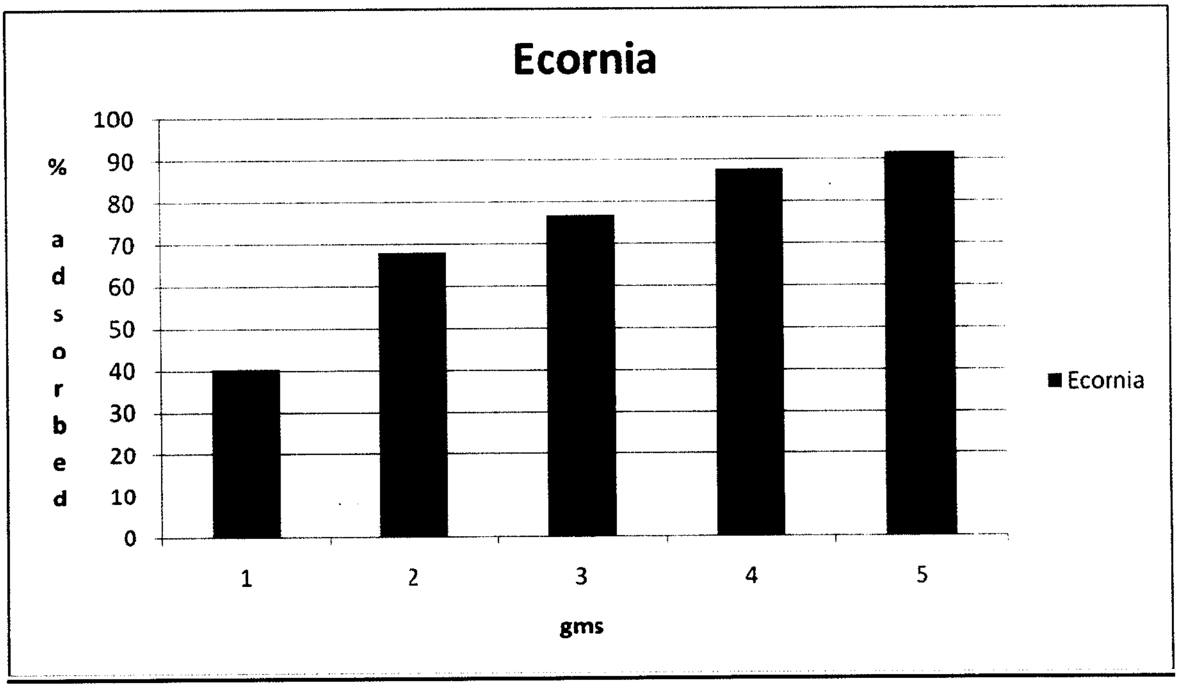


Fig9. % adsorbed vs Amount of adsorbent (Ecornia leaves) in gms

The percentage removal of metals while, Ecornia leaves used as adsorbent is highly efficient in tannery effluent. The degrading efficiency and the COD fractionation is about 92- 94% and it cleaves the problem for the lack of low cost adsorbents.

5.2.5 Adsorption using Cocoa grass

Heavy metals are present in abundance naturally and enter the water cycle through a variety of geochemical processes. Many metals are added to water by industrial processes. Disposal of untreated wastes, surface run-off and highway run-off also cause metal pollution of surface water. The possibility of using cocoa grass produced in already working incineration plants, as adsorbents for waste water treatment, was studied. Showing very poor adsorption properties, they were improved by steam activation technique used in the conventional activated carbon.

Table 7. Adsorption using Cocoa grass after 3 hours:

DOSAGE In gms	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
1gm	8	0.188	4980	20.32
	9	0.172	3725	40.40
	10	0.135	2241	64.14
	11	0.108	2002	67.96
	12	0.086	1744	72.09
2gm	8	0.153	2101	66.38
	9	0.144	2035	67.44
	10	0.132	2240	64.16
	11	0.125	2112	66.20
	12	0.108	2002	67.96
3gm	8	0.132	2240	64.14
	9	0.107	2011	67.82
	10	0.092	2008	67.87

	11	0.065	1545	75.28
	12	0.053	1443	76.91
4gm	8	0.098	2002	67.76
	9	0.096	2000	68.00
	10	0.083	1834	70.65
	11	0.032	761	87.82
	12	0.033	760	87.84
5gm	8	0.092	1841	70.54
	9	0.068	1545	75.28
	10	0.074	1343	78.51
	11	0.032	761	87.82
	12	0.016	525	91.60

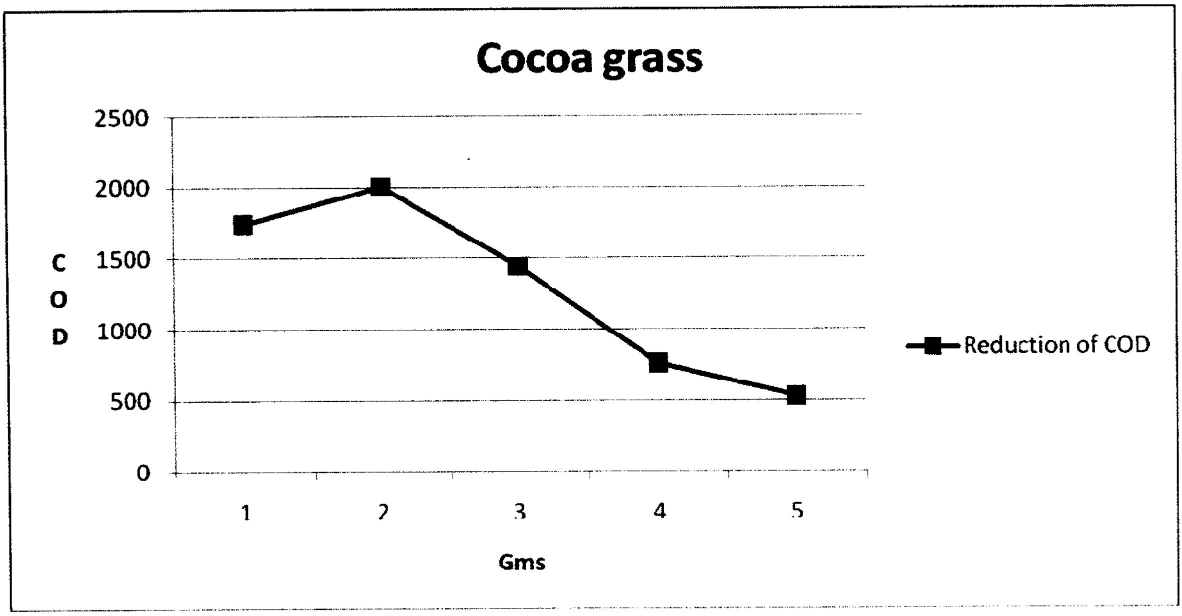


Fig10. COD vs reduced Amount of adsorbent (Cocoa grass) in gms

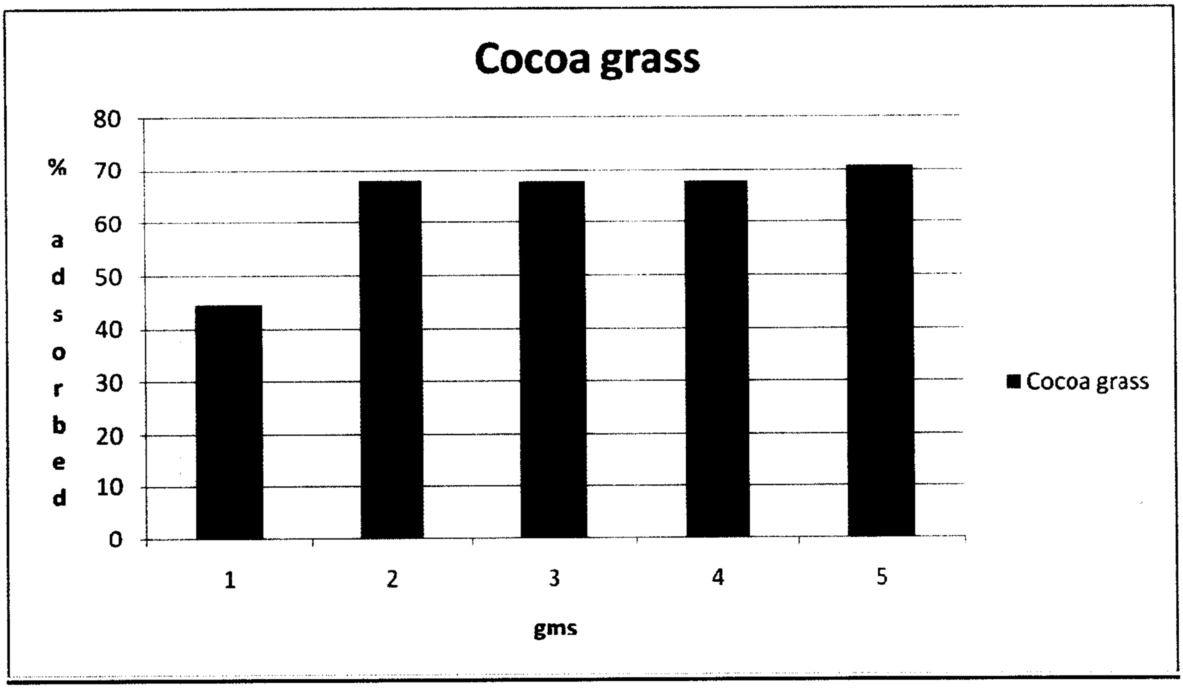


Fig11. % adsorbed vs Amount of adsorbent (Cocoa grass) in gms

The percentage removal of metals while, Cocoa grass used as adsorbent is highly efficient in tannery effluent. The degrading efficiency and the COD fractionation is about 92- 94% and it cleaves the problem for the lack of low cost adsorbents.

PROCESS OPTIMIZATION:

4.7.1 EFFECT OF pH

The adsorption of Cr (VI) from aqueous solution is dependent on the pH of the solution, which affects the surface charge of the adsorbent. It was therefore important to study the effect of pH on the adsorption of Cr (VI). The effect of the pH of the suspending medium on metal removal efficiency was studied by performing equilibrium sorption tests at different pH values ranging from pH 1-5 at an optimal simulated effluent concentration of 50mg/l with an adsorbent dosage of 2g for a time period of 8 hours. Adjustments to pH were made with HCl and NaOH (O.Karahan *et al.,* 2008).

Table 8. Effect of pH on effluent:

PH VALUE	TIME (In hrs)	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
5	0	0.274	6250	0
	1	0.232	5854	06.48
	2	0.216	4765	23.75
	3	0.144	2035	67.44
	4	0.132	1946	68.86
	5	0.062	1843	70.51
6	0	0.263	6180	01.12
	1	2.216	4765.5	23.75
	2	0.138	1998	68.03
	3	0.053	1643	73.71
	4	0.032	768	87.71
	5	0.018	530	91.52
7	0	o.254	6070	02.88
	1	0.208	5179.3	17.13

	2	0.155	2348.1	62.43
	3	0.043	994	84.09
	4	0.032	780	87.52
	5	0.016	525	91.60
8	0	0.272	6230	0.32
	1	0.243	5950.1	04.79
	2	0.212	4679	25.13
	3	0.164	2138	65.79
	4	0.139	1996	68.06
	5	0.040	780	75.04
9	0	0.296	6310	0.96
	1	0.263	6180	01.12
	2	0.216	4765.5	23.75
	3	0.166	2140	65.76
	4	0.144	1880	69.82
	5	0.121	1646	73.66

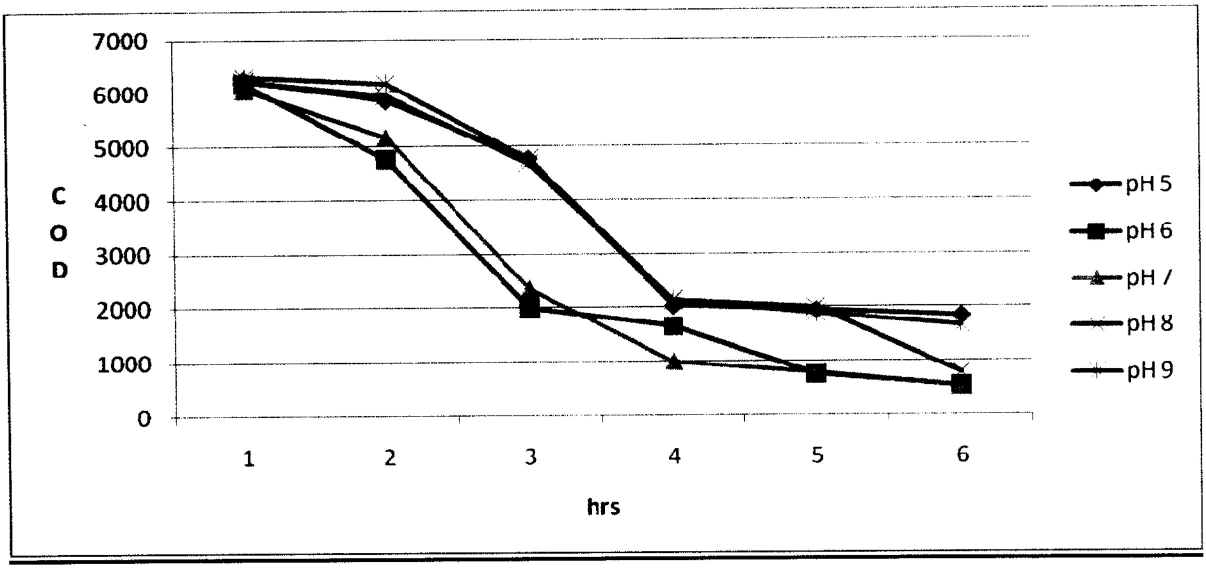


Fig 12: COD reduced vs pH (time in hrs)

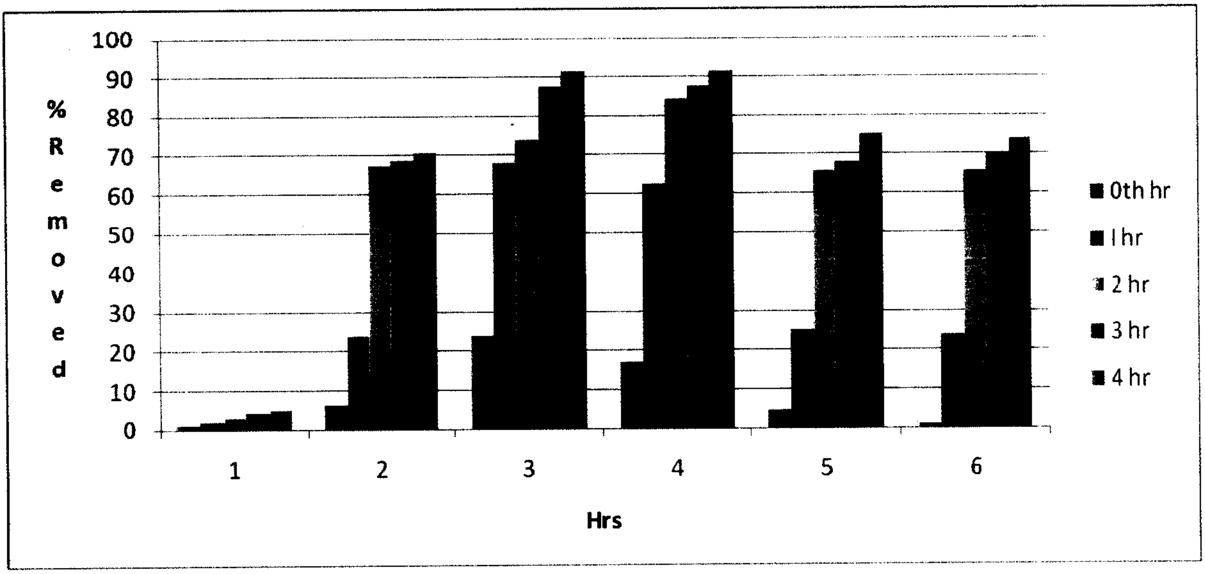


Fig 13: %Removed vs pH (time in hrs)

The optimal pH is concluded by the plot as 5-6. And also the degrading efficiency is merely to 90-95%. The removal efficiency is near to 10 in 0th hour. As the time increases, the degrading efficiency increases. Thus concluded the optimum pH as 5-6.

4.7.2 EFFECT OF REVOLUTION ON INITIAL CONCENTRATION FOR 3 HOURS:

To determine the effect of adsorbent dosage on adsorption with respect to revolution, 100 ml of heavy metal solution of known concentration (50mg/ml) was added to, 1 to 5 g of adsorbent in a 150 ml conical flask and the mixture was stirred on a thermostat shaker bath for a contact time of 3 hours (Bulut, Y and Baysal, Z. *et al.,* 2006). Then, the solution was centrifuged at the speed of 100-500 RPM, filtered, and then the supernatant was analyzed for metals estimation by COD method.

Table 8: Effect of Revolution on effluent:

RPM in mins	DILUTION RATE	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
100	8	0.235	5945	48.80
	9	0.212	4780	23.52
	10	0.203	4650	25.60
	11	0.193	4323	30.83
	12	0.89	4100	34.40
200	8	0.212	4640	25.76
	9	0.211	4631	25.90
	10	0.201	4441	28.94
	11	0.180	4131	33.90
	12	0.177	3939	36.97
300	8	0.177	3939	36.97
	9	0.163	2254	63.93
	10	0.154	2112	66.20

	11	0.133	2019	67.69
	12	0.121	1987	68.20
400	8	0.154	2112	66.69
	9	0.143	1989	68.16
	10	0.133	2019	67.69
	11	0.121	1987	68.20
	12	0.110	1937	69.00
500	8	0.121	1987	68.69
	9	0.110	1937	69.00
	10	0.096	781	87.50
	11	0.063	621	90.06
	12	0.031	525	91.60

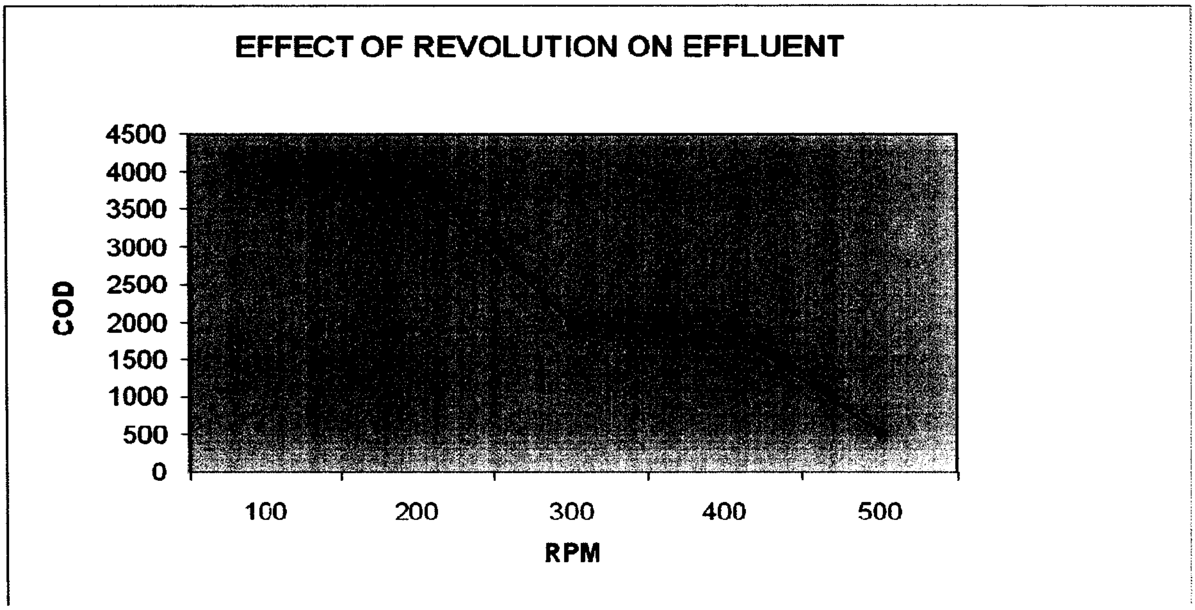


Fig 14: COD vs Rpm in mins

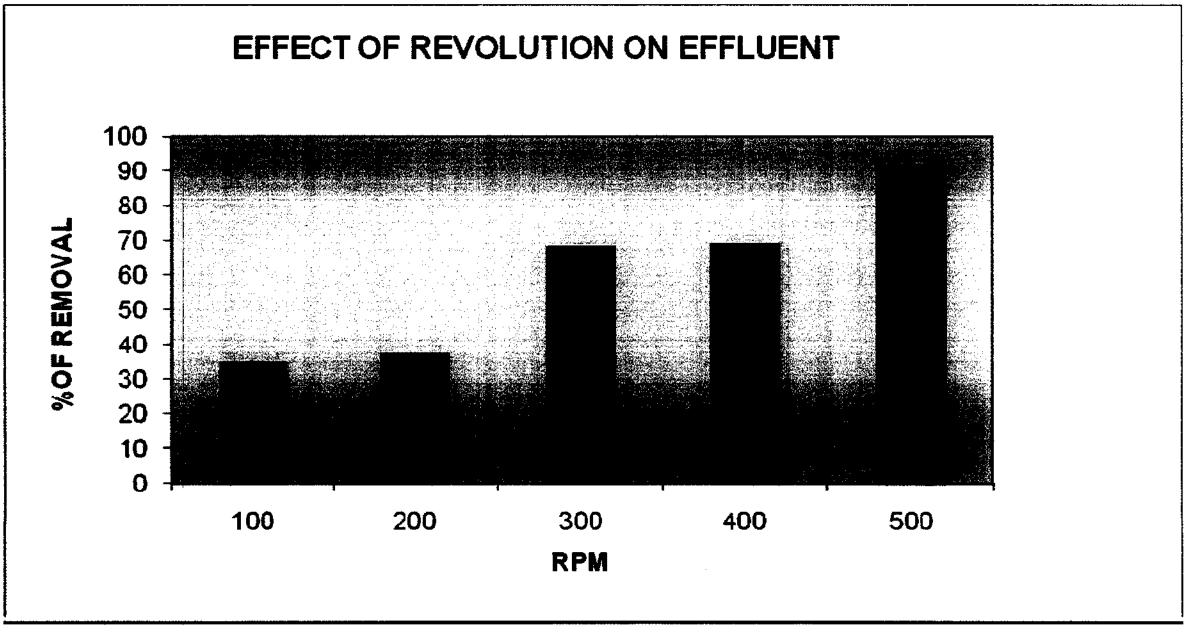


Fig 15: %Removal vs Rpm in mins

The revolutions are adjusted to 100-500 rpm for identifying the degrading efficiency and the level of COD fractionation. The growth of the culture is very effective at 500 rpm and the % removal is about 90 at the level.

Increase in revolution indirectly increases the growth rate of the organisms to degrade the metals.

High growth means the high removal efficiency. Here when the revolution was adjusted to 500, the % removal is about 90%.

EFFECT OF TIME ON EFFLUENT:

To determine the effect of adsorbent dosage on adsorption by means of time , 100 ml of the sample of high metal concentration was added to, 1 to 5 g of adsorbent in a 150 ml conical flask and the mixture was stirred on a thermostat shaker bath for a contact time of 3 hours (Gupta et al, 2005). Then, the solution was centrifuged at the speed of 6000 RPM, filtered, and then the supernatant was analyzed for Cr (VI) estimation by colorimetric method.

Table 9: Effect of time on effluent

TIME (In hrs)	DILUTION RATE (ml)	ABSORBANCE At 540 nm	COD (mg/l)	% OF REMOVAL
1h	8	0.267	5945	48.80
	9	0.254	5873	60.32
	10	0.244	5713	80.30
	11	0.185	4150	33.60
	12	0.118	2140	65.36
2 h	8	0.216	4635	25.84
	9	0.189	4180	33.12
	10	0.176	3100	48.00
	11	0.164	2138	65.74
	12	0.101	1980	68.22
3h	8	0.183	4179	33.13
	9	0.145	2434	66.05
	10	0.133	2019	67.69

	11	0.112	2010	67.89
	12	0.101	1986	68.22
4 h	8	O.104	2138	65.74
	9	0.135	2020	67.65
	10	O.141	1987	68.20
	11	O.121	1838	70.64
	12	0.101	1620	77.82
5h	8	0.133	2019	67.67
	9	0.121	1987	68.20
	10	0.103	1835	70.64
	11	0.096	1545	75.28
	12	0.034	761	89.84

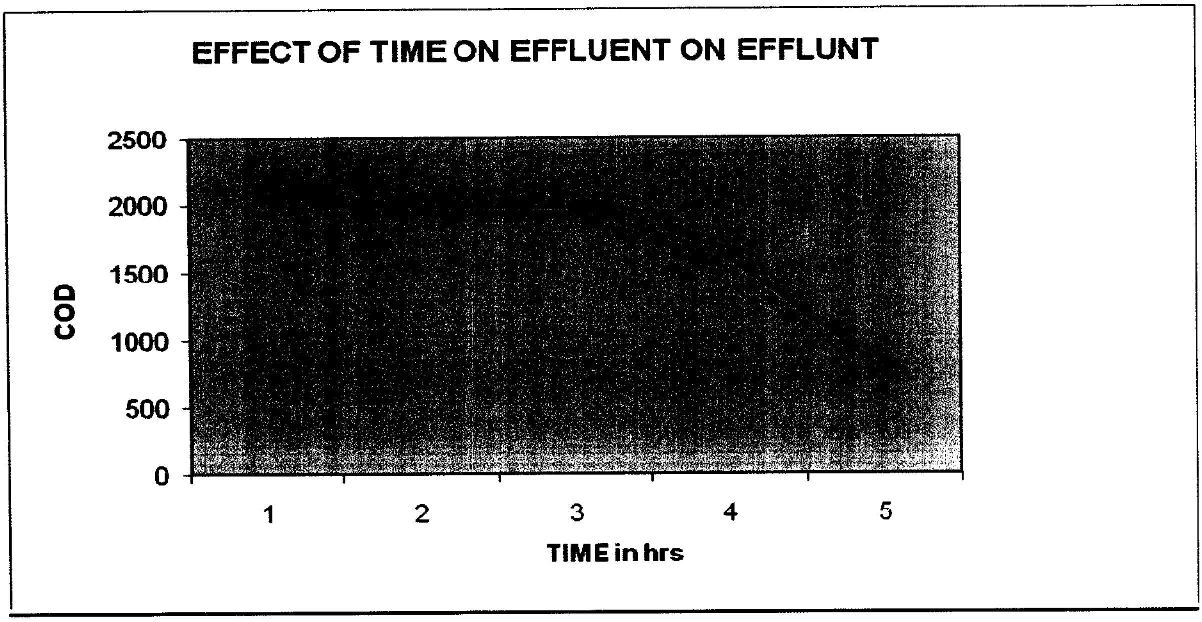


Fig 16: COD vs Time in hrs

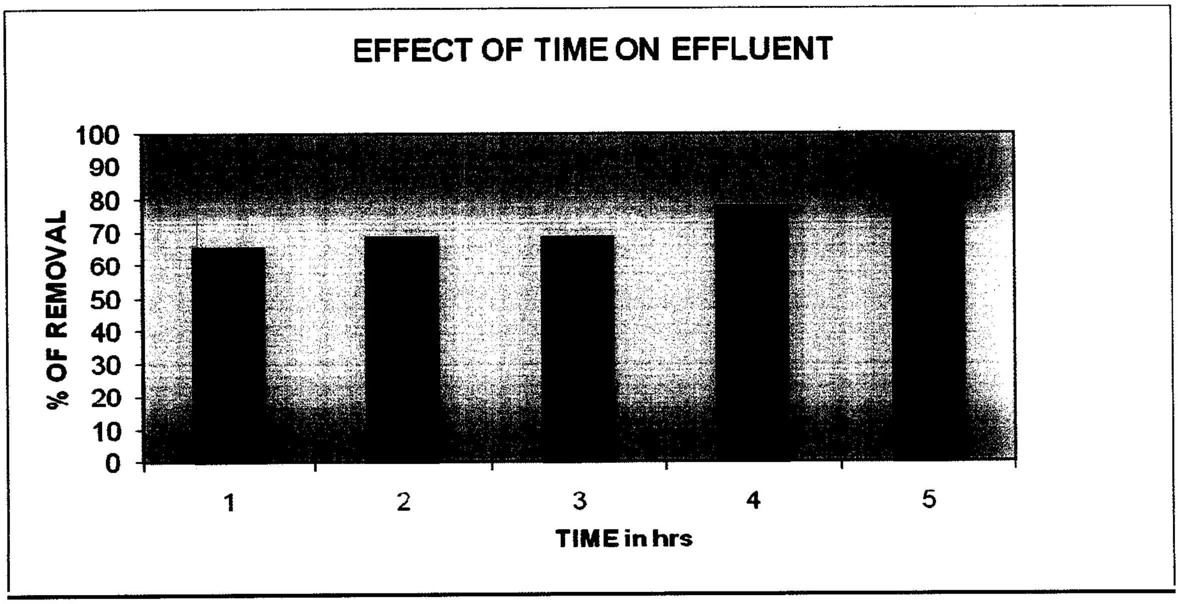


Fig 17: % Removal vs Time in hrs

The optimum time for the removal of heavy metal is about 5hrs. The degrading efficiency and the % removal is merely about 90%. The percentage removal of the

COD is high in 5hrs. COD level is reduced to 650mg/l and it is high degrading content with time.

EFFECT OF TEMPERATURE ON EFFLUENT:

The optimised temperature for the growth of microorganisms is well down by optimising the effluent with effect of temperature.

Table 10 : Effect of temp on effluent

TEMPERATURE (^{0}C)	DILUTION RATE	ABSORBANCE At 540nm	COD, (mg/l)	% OF REMOVAL
20	8	0.279	5536	11.43
	9	0.257	5845	06.48
	10	0.235	5945	04.88
	11	0.172	6055	01.52
	12	0.108	5540	11.36
25	8	0.238	5945	04.88
	9	0.212	4780	23.52
	10	0.198	4323	30.83

	11	0.186	4100	34.40
	12	0.113	2010	67.84
30	8	0.216	4635	25.84
	9	0.184	4180	33.12
	10	0.176	3200	48.00
	11	0.164	2138	65.57
	12	0.101	1986	68.55
35	8	0.183	4135	33.84
	9	0.164	2138	65.79
	10	0.154	2348	62.43
	11	0.133	2019	67.69
	12	0.096	1343	78.51
40	8	0.154	2348	62.43
	9	0.138	2016	67.74
	10	0.133	2019	67.69
	11	o.069	1854	70.33
	12	0.034	768	87.71

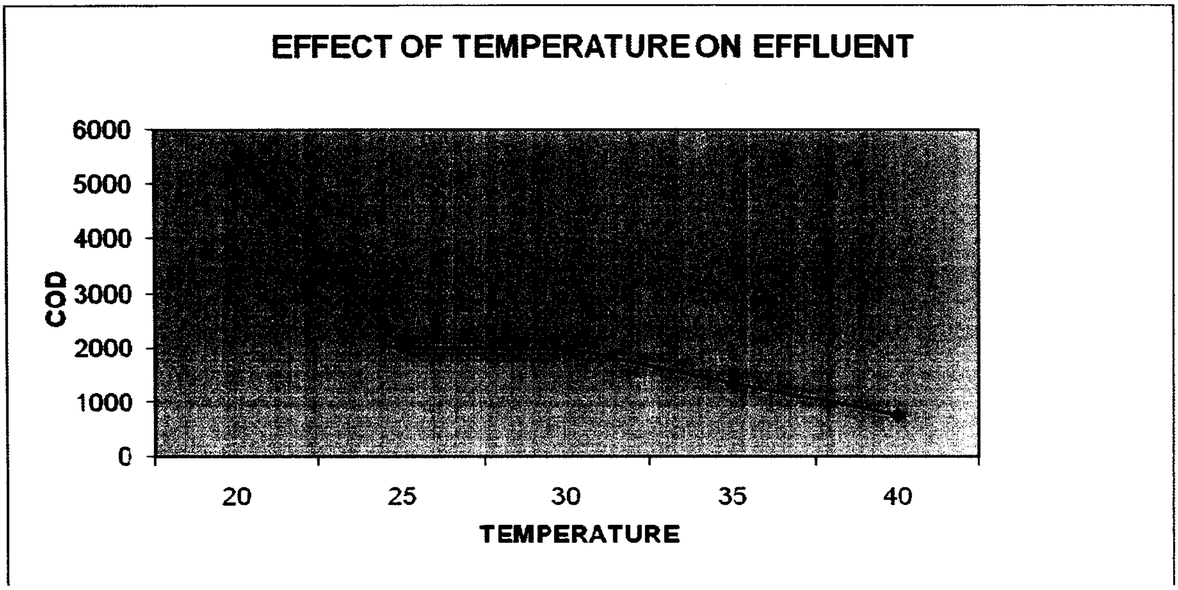


Fig 16: COD vs Temperature

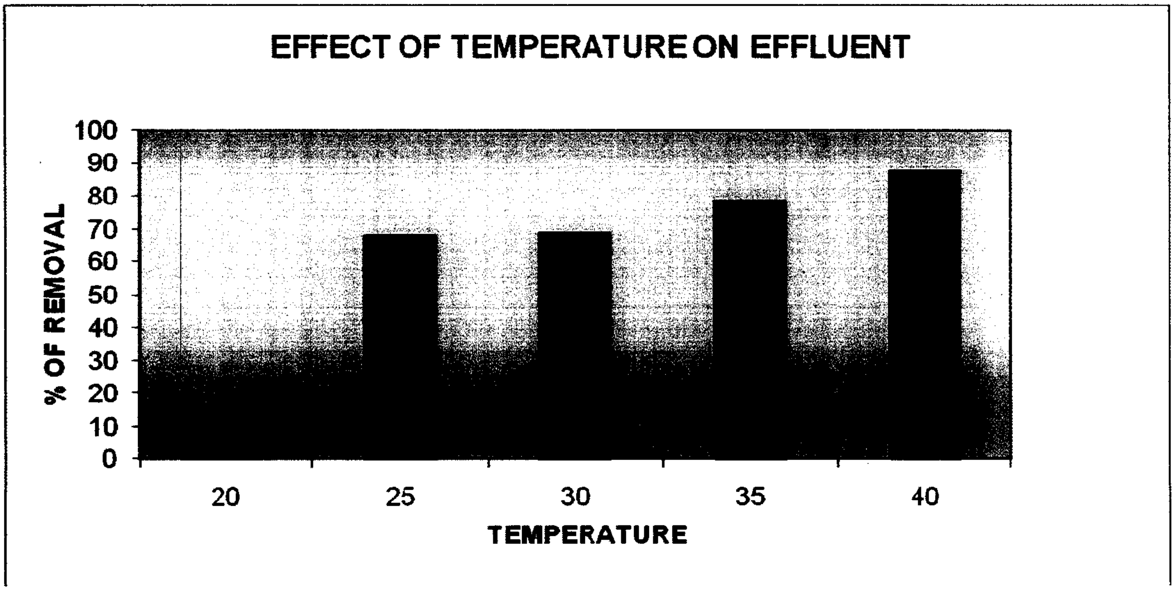


Fig 17: %Removal vs Temperature

The graph clearly describes the removal efficiency more than 80% and optimum temperature found is about 40° C. We can conclude that the growth of the organism is merely high at 40° C.

CHAPTER 6

CONCLUSION

PHYSIO-CHEMICAL METHOD:

The use of low-cost adsorbents may contribute to the sustainability of the surrounding environment. Undoubtedly low-cost adsorbents offer a lot of promising benefits for commercial purpose in the future. Few such low cost adsorbents generally available as wastes are the choice of materials in this study. Following conclusions are drawn from the above discussed results

- All the adsorbents used can be obtained without excessive cost.
- COD Fractionation increases with increase of adsorbent dosage.
- The degrading efficiency is nearly more than 80-95%. The COD got reduced from 6250 to 500mg/l.
- The removal efficiency of the selected adsorbents increases with the increasing dosage levels of the adsorbents

BIOLOGICAL METHOD:

- By the biological method, the degrading efficiency is high. Amount of organisms loaded into into the effluent is comparable to that of adsorbents.
- But, while when comparing both the methods, physio chemical method is more cost effective
- e than biological method.
- The maximum adsorption of heavy metals took place at pH 3 for all the adsorbent materials.
- The optimal time is found and the optimal Revolution is found as 500 rpm.

FUTURE PERSPECTIVES:

- Adsorbents can be subjected to various chemical treatments before performing batch and continuous studies.
- By using different methods to degrade heavy metals.
- Treatment using industrial effluent containing High COD.
- Analyze the results using Atomic Absorption Spectroscopy (AAS).
- To adsorb heavy metal effluent using the adsorbents and analyzing the data with various isotherms and kinetics.
- To find out the optimum adsorbent dosage, pH conditions and temperature.
- To apply the mentioned adsorbent materials for the removal of other toxic heavy metals like Pb, Cd, Zn, Fe etc. from effluents.
- To compare the effectiveness of the degrading methods.
- Also analyze the degrading efficiency of different low cost adsorbents.

APPENDICES

APPENDIX 1

COD FRACTIONATION:

- COD is a collective lump design parameter reflecting the total organic matter present in the waste water.
- Mostly, in dyes as well as effluents the COD will b high because of the presence of organic content and inorganic substances. The COD will be at 3000- 6000mg/l.
- Yet, it has inherent deficiencies when used alone as it covers not only biodegradable organics but also biologically resistant and refractory compounds.

PROCEDURE:

- Wash the tubes and dried it continuously for effective readings and to debug the errors.
- 2.5 ml of the sample are accurately measured in different dilutions
- Then add 1.5 ml of the pure concentrated acid solution and 3.5 ml of the potassium permanganate solution.
- Mix it gently for genuine readings.
- The tubes are incubated and kept in COD digestor for 2 hrs.
- Cool it at room temperature for not more than 15 mins and the absorbance readings are measured in UV spectrophotometry at 530 nm.

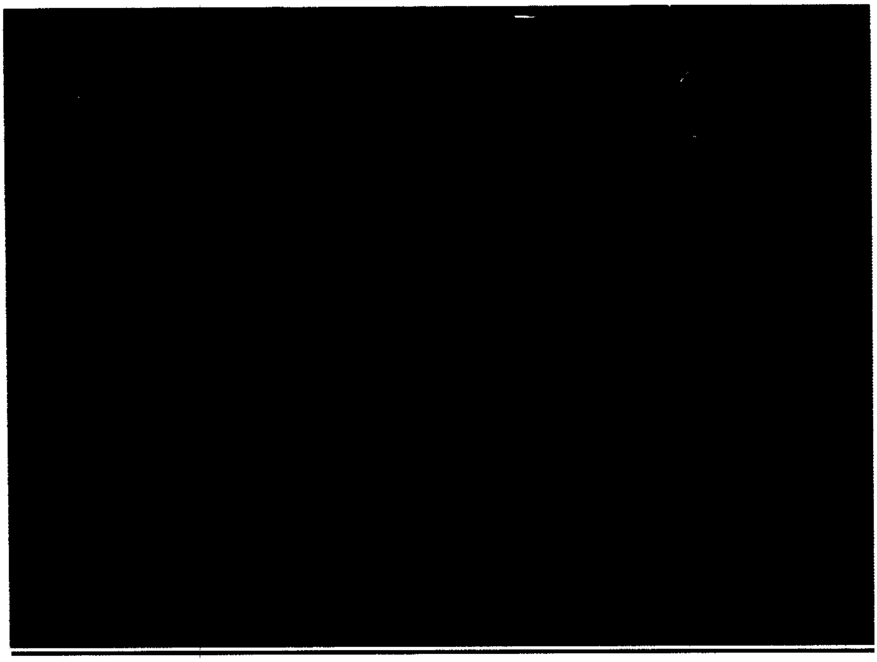


Fig 14: COD testing samples

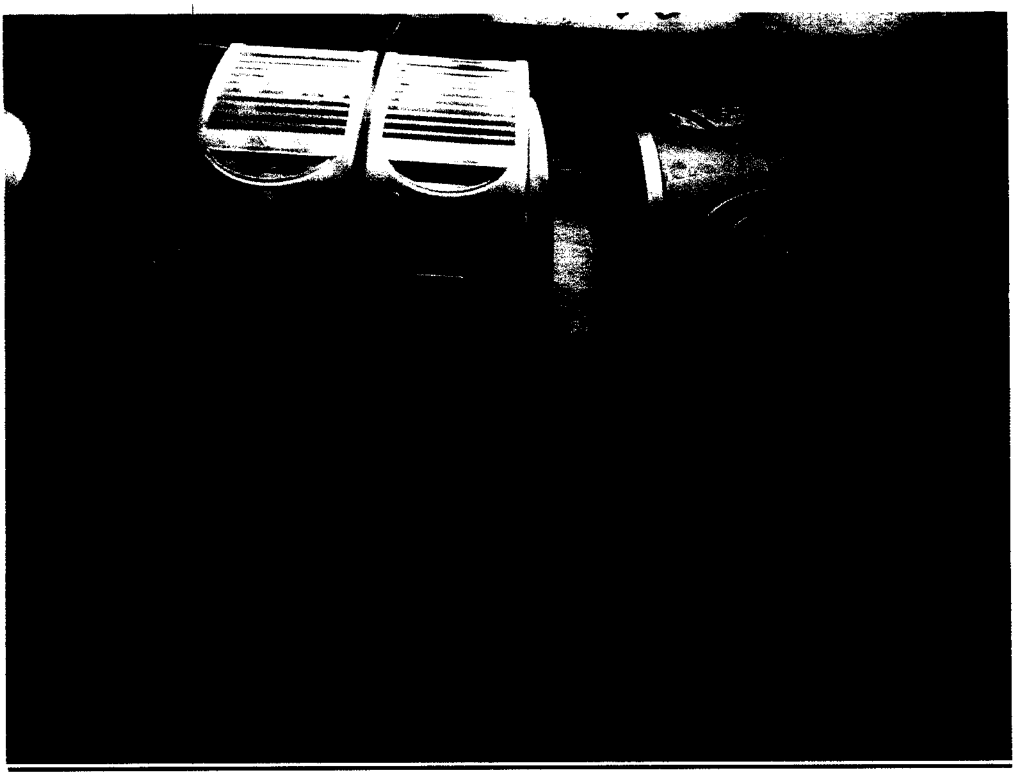


Fig 15:COD Digestor

TSS ANALYSIS:

- The TSS (Total Suspended Solids) analysis is done to identify the amount of suspended solids present in the sample.
- Crucible is taken and weighed in electronic balance.
- The sample of about 2.5 ml is centrifuged at 15000 rpm for 10 mins and poured into the crucible.
- The crucible is kept in hot air oven for a day and weighed next day.
- The difference shows the suspended solids in mg/l.

Crucible Wt (w1) = 34.469

Crucible Wt (w2) = 34.50

TSS= w2-w1/ volume of the sample taken in ml

TSS= 1240mg/l

Table 15: List of Instruments used during the whole experiment their make and function

Instruments:

Analytical Balance Weight Measurement
Laminar airflow Aseptic Environment
PH EuTech Instruments (Measurement of pH)
COD Incubator
Ultra Low Temperature freezer (U410) for Preservation of cultures
Ultra pure water system (Preparation of the stock solution) throughout the experiment etc.
UV/VIS Spectrophotometer (Estimation of Biomass and Degradation)
Incubator shaker Orbital Shaker Batch degradation
Ultra Centrifuge (Collection of pellet and Estimation)

% REMOVAL:

The removal efficiency is found using the calculation.

% R= (Initial concentration – Final concentration)/ Initial concentration.

APPENDIX 2:

ESTIMATION OF CHROMIUM (VI) CONCENTRATION

Preparation of Stimulated Effluent

A stock solution (100mg/ml) of Cr (VI) was prepared by dissolving 2.8287 g of 99.9% $K_2Cr_2O_7$ in 1000ml of distilled water.

Molecular Weight of Chromium = 294.19

100 mg/ml of Chromium = $\frac{294.19}{51.99 X 2}$ = 2.82g of 99.9% $K_2Cr_2O_7$ in 1000ml of distilled water.

Preparation of 1,5- DPC Complexing Agent

The classical method of Cr(VI) analysis for industrial hygiene use was colorimetry using 1,5-diphenylcarbazide (DPC) for colour development after acid extraction of the Cr (VI) from the sample. Dissolve 0.5 g of DPC in 100 ml of HPLC grade CH_3COCH_3 and add 28 ml of 98% H_2SO_4 to about 500 ml of distilled H_2O.Make additions very, very slowly, with mixing, and allow to cool. Mix the solutions carefully and dilute, with stirring, in a 1litre volumetric flask with distilled H_2O. Cool solution to room temperature. The reaction of the DPC with Cr (VI) will be incomplete if this solution is warm. Transfer the solution to the 1litre brown bottle and store in a cool dark place as the solution is sensitive to light. The solution is stable for up to 3 days but should only be prepared as it is used, 1litre at a time. The sensitivity of the method is dependent on the freshness of the DPC solution (NIOSH Manual of Analytical Methods).

Fig 12: Acid solution and Potassium permanganate solution for COD test

Estimation of Free Chromium (VI) Ions – Colorimetric Method

The working standard solutions with concentration ranging from 20-100 mg/l were prepared from the stock. The concentration of free Cr (VI) ions was measured by reacting the solution with DPC reagent and pink colored complex was formed. The absorbance was measured using a spectrophotometer at 540nm.

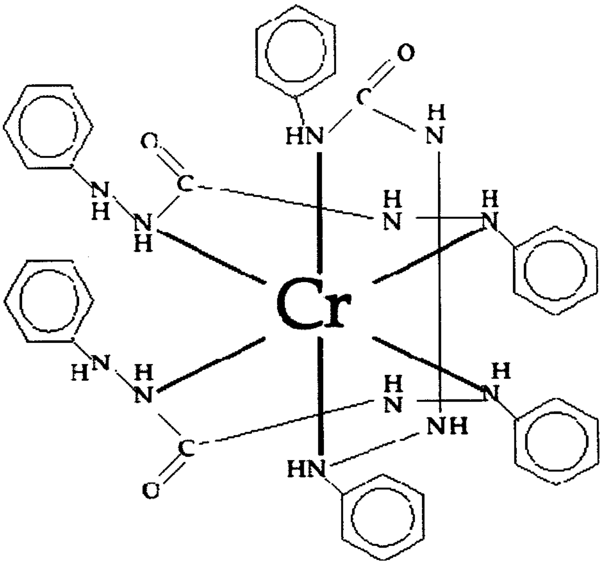


Fig 13: Interaction of free Cr (VI) with 1,5-DPC

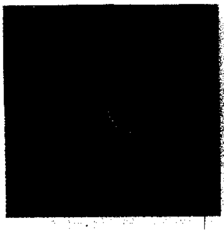
The working standard solutions with concentration ranging from 20-100 mg/l were prepared from the stock. Adsorbent feed was given and the experiment was carried out by agitating in a mechanical shaker for 8 hours. The concentration of free Cr (VI) ions was measured by reacting the solution with DPC reagent and a pink colored complex was formed. The absorbance was measured using a spectrophotometer at 540nm.

Table 11: Estimation of chromium (VI) free ions – Colorimetric Method

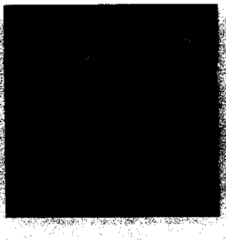
REAGENTS	**B**	**S1**	**S2**	**S3**	**S4**	**S5**
Volume of working standard(ml)	-	20	40	60	80	100
Concentration of working standard (mg/ml)	-	20	40	60	80	100
Volume of working sample(ml)	-	2	2	2	2	2
Volume of 0.2N H_2SO_4(ml)	5	5	5	5	5	5
Make up to 100ml with distilled water						
Kept at room temperature for 15 minutes						
Volume of 1,5-DPC added(ml)	5	5	5	5	5	5
Kept at room temperature for an hour						
Measure Absorbance at 540nm	0.00	.086	.196	.275	.369	.421

APPENDIX 3:

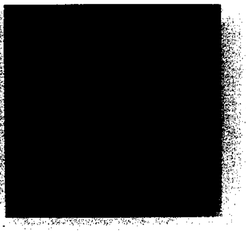
Fig 16: Medium preparation



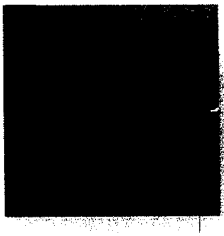
Sterile Plate of Blood Agar Purple (BAP)



Alpha Hemolysis Viewed From Underside of BAP Plate



Blood Agar (BAP) with Alpha-hemolytic Colonies



MacConkey's, Mannitol Salt & Blood Agar (bottom)

Beta Hemolysis on Blood Agar

REFERENCES

1. Abele, M.T. and J. R. Carlberg. (1974) 'A Simple Reliable Method for the Determination of Airborne Hexavalent Chromium' *Am. Ind. Hyg. Assoc. J.*,pp. 35, 229

2. Argus, M.E., Dursun, S., Ozdemir, C and Karatas, M. (2006); 'Heavy metal adsorption by modified oak sawdust': Thermodynamics and kinetics' *J. Hazard. Mater.* 141 (1), pp.77-85

3. APHA,WEF,AWWA,1998.in;clesceri,L.s.Greenberg,AE,eaton,A.D 'Standard methods for the examination water and wastewater' 20th Ed American public health association washington,DC.USA.

4. Ates, E., orhon, D., tunay, O., 1997 'Characterization of tannery wastewater for pre-treatment-selected case studies' water sci.technol.

5. Babu, B.V. and Gupta, S. (2005) 'Modelling and Simulation of Fixed Bed Adsorption Column: Effect of Velocity Variation' J. *Eng. & Technol.*, 1 (1),pp. 60-66.

6. Baral, S. S., Dasa, S. N., and Rath, P. (2006) 'Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust' *Biochem. Eng. J.,* 31 (3),pp. 216–222.

7. Bishnoi, N. R., Bajaj, M., Sharma, N and Gupta, A. (2004) Adsorption of Cr(VI) on activated rice husk carbon and activated alumina" *Bio resource Technol*., 91 (3), pp.305–307

8. Brown, P., Jefcoat, I. A., Parrish, D., Gill, S and Graham, E. (2000) Adv.Environ. *Res., 4*,pp. 19-29.

9. Bulut, Y and Baysal, Z. (2006) 'Removal of Pb (II) from wastewater using wheat bran' *J. Env. Mng*, 78 (2), pp.107-113.

10. Chadwick, P., G. J. Delisle, and M. Byer. 1974 'Biochemical Identification of hospital enterobacteria by replica agar plating Can. J. Microbiol. 20:pp.1653-1664.

11. Cunha, B. A., J. J. Klimek, J.Gracewski,C.McLaughlin,andR.Quintiliani. 1980 'A common source outbreak of AcinetobacterPulmonary infections traced to Wright respirometers' Postgrad.Med. J. 56:169-172Dikshoorn, L., H. M. Aucken, P. Gerner-Smidt, M. E. Kaufmann,VOL. 32, 1994.

12. Garrison, R. G. 1963 'The occurrence of Bacterium anitratum in Secretions of pulmonary origin Am. J. Clin. Pathol. 40:260-262.

13. Gennari, M., and P. Lombardi. 1993 'Comparative characterization

Of Acinetobacter strains isolated from different foods sources' Int. J. Med. Microbiol. Virol. Parasitol. Infect.Dis. 279:pp.553-564.

14. "International organization for standardization, 1986.In: Technical committee ISO/TC 147(Eds)", 'International standard ISO 6060': Water quality-determination of the chemical oxygen demand", Reference No.ISO 6060-1986(E).1st ed.ISO Switzerland.

15. Kabdsali, I., Tunay, O., Orhon, D., 1993 'The treatability of chromium tannery waste' Sci.technol.28 (2),pp. 97-105.

16. Gupta,.Karahan.,O.,Artan,N.,Orhon,D.,Hehze,M.,van loosdrecht,M.CM.,2005 'Experimental assessment of bacterial storage yield' ASCE J.Eng128(11),pp.1030-1035.

17. N.F.Fahim, B.N.Barsoum, A.E.Eid, M.S.Khail. 'Removal of chromium (III) from wastewater using activated carbon tannery industrial waste' B136 (2006) pp.303-309.

18. Namasivayam, C.; Yamuna, R. T., (1995) 'Adsorption of chromium (VI) by a low-cost adsorbent: biogas residual slurry' Chemosphere, 30 (3), pp.561-578.

19. NIOSH (1975) Criteria for a Recommended Standard: 'Occupational Exposure to Chromium (VI)' Cincinnati, OH: U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, DHEW (NIOSH) Publication, pp.76-129.

20. NIOSH/OSHA (1981) 'Occupational Health Guidelines for Chemical Hazards'. 'Occupational Health Guidelines for Chromic Acid and Chromates' U.S. Department of Health and Human Services (NIOSH) Publication,pp. 81-123.

21. Reichert,P.,Ruchti,J.,Simon., W.,1998.Aqusim 2.0 swiss federal 'Institute for Environment Science and Technology' (EAWAG), CH-8600 Duebendorf, Switzerland.

22. O.Karahan, S.dogruel, E.Dulekgurgen, D.Orhon. 2008 'COD fraction of tannery wastewater' (1) 1083-1092. Thomsen, E. and R.M. Stern (1979). "A Simple Analytical Technique for the Determination of Hexavalent Chromium in Welding Fumes and Other Complex Matrices" *Scand. J. of Work, Environ. And Health*, 5, pp.386-403.

23. Tunali, S., Cabuk, A. And Akar, T. (2006) 'Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil' *Chem. Eng. J.,* 115,pp.203–211.

24. Weston, A. and Murthy, M. (1996). Synthesis of Fullerenes: 'An Effort to Optimize Process Parameters' Carbon, 34,pp. 1267-1274.

25. Guo, X.Y., Zhang, A.Z and Shan, X.Q. (2008). 'Adsorption of metal ions on lignin', *J. Hazard. Mater.* 151,pp. 134-142.