Determination of Maximum Reduction of the Hexavalent Chromium Using Indigenous Bacterial Isolates

A. Senthil kumar, K. Nagarajan[1] and M. Moorthi[2]

PG & Research Department of Zoology, Chikkaiah Naicker College, Erode – 638 004.
[1]PG & Research Department of Zoology, Sri Vasavi College, Erode – 638 316.
[2]PG & Research Department of Zoology, AVC College (Autonomous), Mannampandal, Mayiladudhurai-609 305.

Abstract- Tanneries generate wastewater in the range of 30 to 35L kg^{-1} skin/hide processed with high salinity, high oxygen demand, high organic loading and a high salt content one of which is chromium which spoils the agricultural land and pollute the ground water. In the present investigation, the bacterial strains were isolated from the contaminated soil which was collected from in and around the tannery waste contaminated site. Previously grown indigenous bacterial culture was transferred into 100 ml nutrient broth containing different concentrations of hexavalent chromium (100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 600ppm and 700ppm). These were incubated for 72 hours in facultative conditions. The blanks were also maintained in same conditions without bacterial inoculation. Samples were collected from this experiment to evaluate hexavalent chromium at different time intervals (0hr, 12hrs, 24hrs, 36hrs, 48hrs, 60hrs and 72hrs). From the results it was inferred that the higher bacterial growth was observed at 48 hrs and the maximum chromium reduction was observed upto 500ppm, it was reduced at percentage of 90.40. The isolated microorganisms were identified as *Pseudomonas fluorescens* and *Bacillus subtilis.*

Index Terms- Tannery waste, Hexavalent chromium, Indigenous bacteria, Chromium reduction.

I. INTRODUCTION

Indian leather industry occupies an important position in foreign exchange earning. This phenomenal growth obviously calls for the processing of enormous amounts of hides and skins. The tanning process involves pre-tanning, tanning and post tanning operations. During the processing of leather, substantial amount of solid and liquid wastes are generated (Chakraborty, 2003), especially toxic metal chromium passing to wastewater and are not easily eliminated by ordinary treatment process (Franco *et al.,* 2005).

Chromium used by the leather industry to tan hides is not taken up completely by leather and relatively large amount escapes into the effluent. Due to chrome leather tanning process, large quantities of chromium compounds are discharged through liquid, solid and gaseous wastes into the environment and can have significant adverse biological and ecological effects. Several reports have shown that the values for Chromium in tannery effluent are considerably higher than the safe limits prescribed by National and International standards.

The Environmental Protection Agency (EPA) has reported that Chromium levels in tannery effluents are considerably higher than the safe limits required by the National Environment Conservation Strategy (EPA, 1990).

Tannery wastewaters are mainly characterized by high salinity, high oxygen demand, high organic loading and specific pollutants such as chromium (Bajza and Vrcek, 2001; Colak *et al.,* 2005). Among the oxidation states of chromium, the hexavalent Chromium (Cr (VI)) and trivalent Chromium (Cr (III)) forms are of significance in aqueous systems. The hexavalent form is water soluble, toxic, mutagenic and carcinogenic to humans and mammals whereas the trivalent form is less soluble and less mobile and 100 times less toxic than Cr (VI) (DeFlora *et al.,* 1990; Ramakrishna and Philip, 2005).

The damage to the environment by the hazardous tannery effluent has become an acute problem in the country. The main objectives of the present study focused the isolation, enrichment and chromium reduction of the indigenous bacteria from the tannery contaminated soil.

II. MATERIALS AND METHODS

PREPARATION OF NUTRIENT MEDIA

The general growth nutrient medium for bacterial growth consisted of peptone 10g, beef extract 2g, yeast extract 1g and sodium chloride (NaCl) 5g in 1 L of distilled water. All media were autoclaved at 120^{o}C and 15 psi for 15 minutes and stored in room temperature for further use. The pH was maintained at 7±0.2 by using HCl or NaOH.

ISOLATION OF MICROORGANISMS

Bacterial strains were isolated from the contaminated soil. About 1g of soil sample was added into 100 ml of nutrient medium and incubated for 24 hrs in facultative condition. A loopful from the above culture was streaked on agar plate, incubated for 24 hrs and stored in the refrigerator at 4^{o}C for further use.

DETERMINATION OF MAXIMUM REDUCTION OF THE HEXAVALENT CHROMIUM USING INDIGENOUS BACTERIAL ISOLATES

About 1 ml of previously grown bacterial culture was transferred into 100 ml nutrient broth containing different concentrations of hexavalent chromium (100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 600ppm and 700ppm). These were incubated for 72 hrs in facultative conditions. The blanks were also maintained in same conditions without bacterial inoculation. From these cultures, 5 ml of sample was taken to evaluate hexavalent chromium at different time intervals (0hr, 12hrs, 24hrs, 36hrs, 48hrs, 60hrs and 72hrs).

A loopful from the media containing mixtures were streaked on agar slants, incubated for 24 hours and stored in the refrigerator at 4^{o}C for screening of the enriched cultures.

MEASUREMENT OF OPTICAL DENSITY (OD)

All the samples drawn at different time intervals (0hr, 12hrs, 24hrs, 36hrs, 48hrs, 60hrs and 72hrs) from the above mentioned experiments Optical Density (OD) was measured through UV-Visible Spectrophotometer, at 540 nm wavelength.

IDENTIFICATION OF MICROORGANISMS

The isolated microorganisms were identified by colony morphology, staining and biochemical tests. The tests that employed were Gram's stain, motility, Indole, methyl red, Voges-Proskaeur, citrate utilization tests, starch hydrolases, gelatin hydrolases, nitrate reduction test, oxidase, catalase and TSI (0.1% Glucose, 1% Sucrose, 1% Lactose and Iron) (Dubey and Maheshwari, 2002).

III. RESULTS AND DISCUSSION

MEASUREMENT OF OPTICAL DENSITY AND MAXIMUM REDUCTION OF HEXAVALENT CHROMIUM

Bacteria were grown on nutrient media which contained different concentrations of hexavalent chromium (100, 200, 300, 400, 500, 600 and 700ppm) (Plate 1A). At definite time intervals (0, 12, 24, 36, 48, 60 and 72 hrs) OD was measured in these samples.

Among all the hexavalent chromium concentrations, a higher bacterial growth was observed from 100ppm to 500ppm. However, in 600ppm and 700ppm hexavalent chromium concentrations, bacterial growth showed a declining trend. Among all the time intervals a higher bacterial growth was observed at 48 hrs (Table 1).

The present study focused on the reduction of toxic hexavalent chromium into trivalent chromium by the native bacterial strain which showed very high-level of reduction up to 500ppm of potassium dichromate on nutrient broth, it was reduced at 90.40% (Table 2). The similar results were reported by several authors. Camargo *et al.* (2003) have reported that most of the dichromate contaminated isolates tolerated and reduced hexavalent chromium at lower than 1500mgL^{-1}. Faisal and Hasnain (2004) studied very high-level resistance against potassium dichromate both in nutrient broth (up to 25mg ml^{-1}) and on nutrient agar (40mg ml^{-1}) and also reported that when increases the initial hexavalent chromium concentration from 100 to 500µg ml^{-1} the chromium content in the cell become almost doubled.

Jeyasingh and Philip (2005) also reported that the hexavalent chromium reduction rate increased with the initial Cr (VI) concentration even at high initial Cr (VI) concentration of 400mg/l. The isolated microbes are able to reduce or remediate Cr (VI) even at higher concentrations though it takes a long time. Anaerobic microbes are usually more sensitive to toxic compounds like heavy metals. Chromium resistant bacteria isolated from effluent of tanneries could resist up to 250µg ml^{-1} of Cr (VI) in the medium (Basu *et al.*, 1997).

Garbisu *et al.* (1998) and Megharaj *et al.* (2003) isolated the bacterial isolates *Anthrobacter* sp. and a *Bacillus* sp., from the tannery waste contaminated soil that showed similar resistance to Cr (VI) and had the ability to reduce Cr (VI) to Cr (III).

Several authors used an initial hexavalent chromium concentration of 10, 20, 50 and 100ug/ml for the bioremediation experiment. Amongst these concentrations the indigenous strains resist up to 50ppm of chromate (Ganguli and Tripathi (2002); Megharaj *et al.* (2003) and Faisal and Hasnain (2004)).

Microorganisms with the ability to reduce Cr (VI) can be used for detoxification of tannery contaminated soil. In this study, the isolation and screening of Cr (VI) reducing bacterial isolates and characterization of Cr (VI) reduction by the selected isolates have been attempted. From the isolated colony (Plate 1B), morphology, staining and biochemical tests, the results inferred that the isolated microorganisms were *Pseudomonas fluorescens* and *Bacillus subtilis* (Table 3; Plate 2 and 3).

The results suggested that 90.40% reduction of hexavalent chromium into trivalent forms was due to the enzymatic chromium reductions by *Pseudomonas fluorescens* and *Bacillus subtilis* appear to be a form of respiration in intact cells and may have beneficial environmental effects. The similar results were observed by Rehman *et al.* (2007) and Parameswari *et al.* (2009). The biosystem developed in the present investigation is a viable alternate for chromium contaminated soil treatment. The system is environmental friendly, as it is utilizing only naturally available waste materials, cost effective and sustainable.

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AUTHORS

First Author – A. Senthil kumar, M.Sc., M.Phil., Ph.D., Assistant Professor, PG & Research Department of Zoology, Chikkaiah Naicker College, Erode – 638 004. Tamil Nadu, India. E-mail: moosen.ska@gmail.com
Second Author – K. Nagarajan, M.Sc., Ph.D., Associate Professor & Head (Rtrd.), PG & Research Department of Zoology, Sri Vasavi College, Erode – 638 316. Tamil Nadu, India., E-mail: jcdrkn@gmail.com
Third Author – Dr. M. Moorthi, M.Sc., M.Phil., Ph.D., Assistant Professor, PG & Research Department of Zoology, AVC College (Autonomous), Mannampandal, Mayiladudurai - 609 305. Tamil Nadu, India. E-mail: moovim24@gmail.com

Correspondence Author – A. Senthil kumar, M.Sc., M.Phil., Ph.D., Assistant Professor, PG & Research Department of Zoology, Chikkaiah Naicker College, Erode – 638 004. Tamil Nadu, India., E-mail: moosen.ska@gmail.com, Mobile: 09688226168

FIG. 1
GROWTH OF CHROMATE RESISTANT BACTERIAL STRAIN BIOMASS IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM (50, 100, 200, 300, 400, 500, 600 AND 700ppm) AT DIFFERENT TIME INTERVALS (0, 12, 24, 36, 48, 60 and 72 hours)

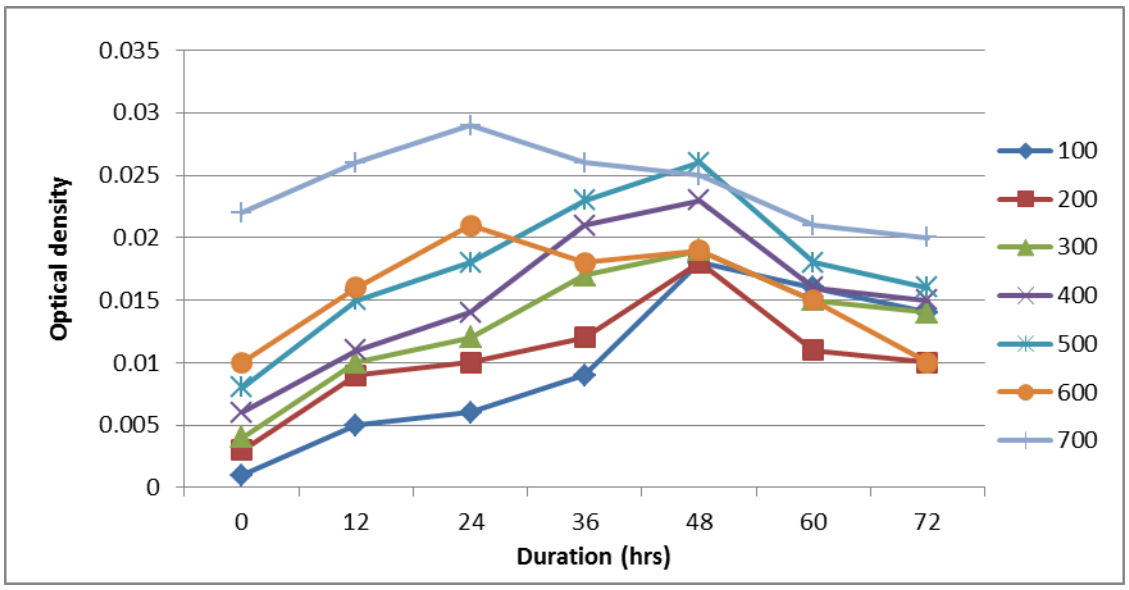


FIG. 2
Cr (VI) CONTENT (ppm) IN NUTRIENT BROTH COTANINTING DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM (100, 200, 300, 400, 500, 600 and 700ppm) AT DIFFERENT TIME INTERVALS (0, 12, 24, 36, 48, 60 and 72 hours)
AND % REDUCTION OF Cr (VI)

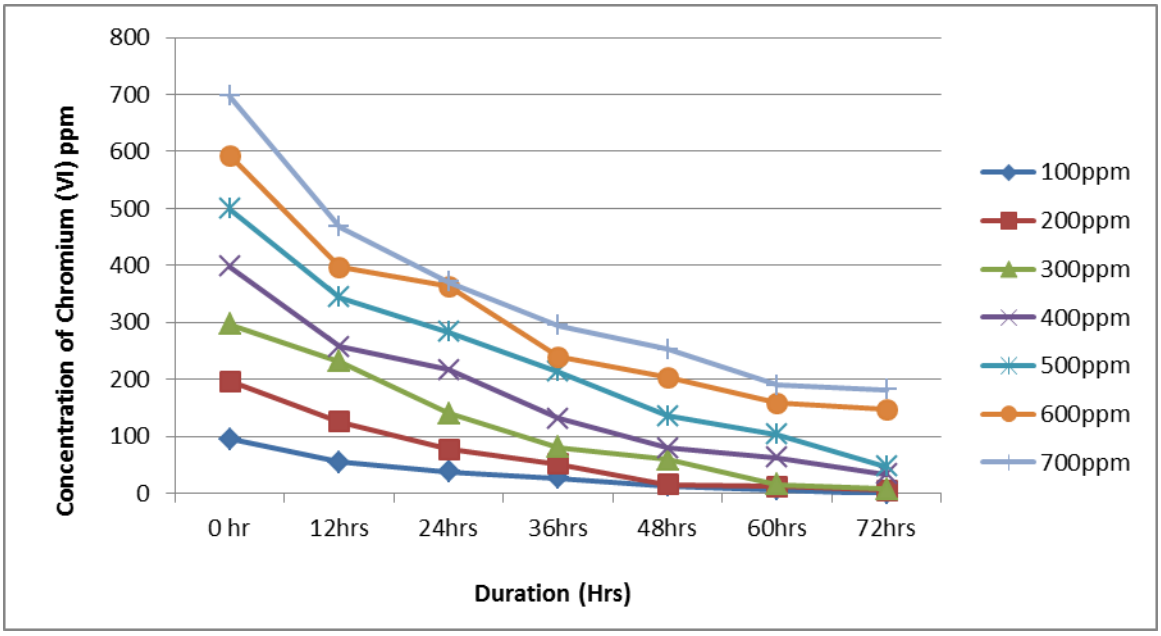
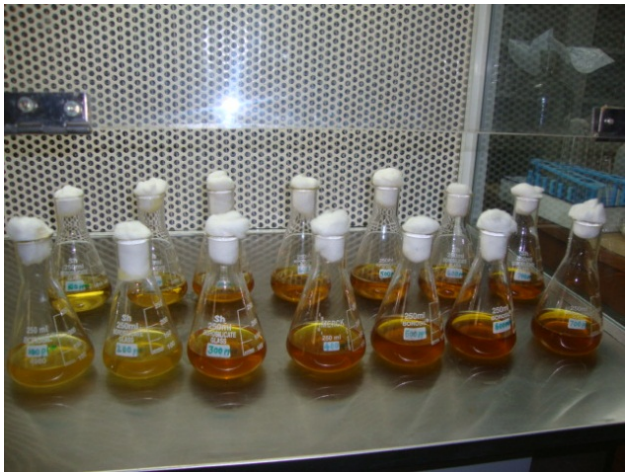


PLATE 1
A. BACTERIAL GROWTH IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM AND BLANK (WITHOUT MICROBIAL INOCULATION) IN LAMINAR AIRFLOW



B. ISOLATION OF BACTERIA FROM 500ppm CONCENTRATION OF HEXAVALENT CHROMIUM

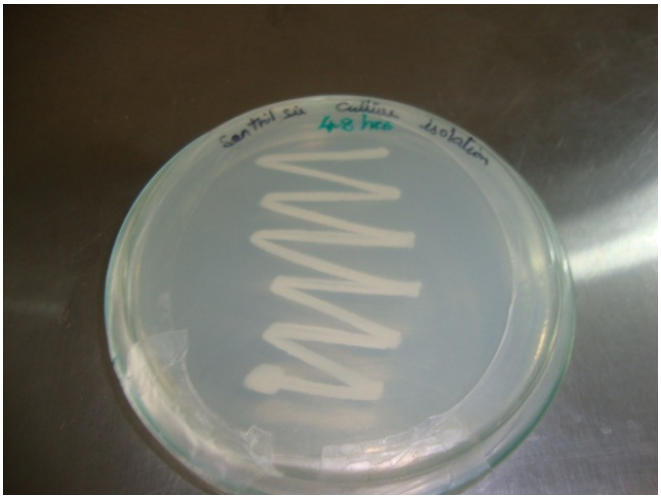
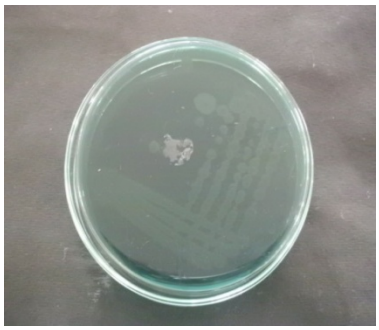
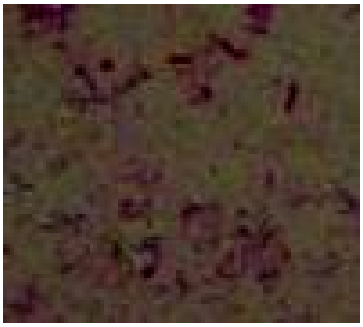


PLATE 2
Pseudomonas fluorescens

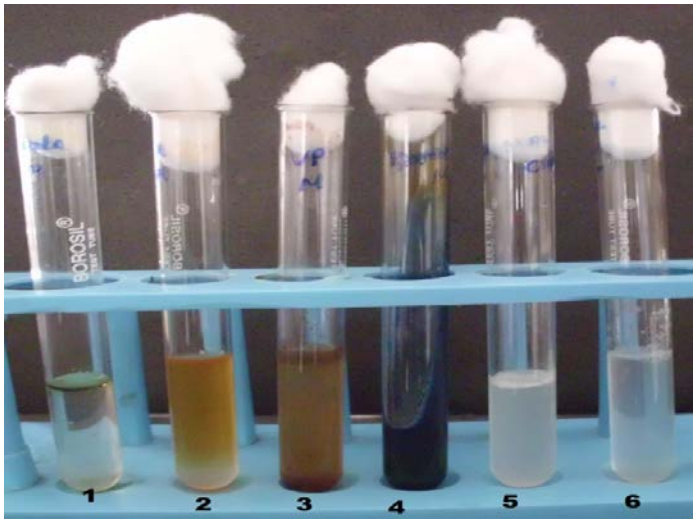
Pure culture of the isolated colony



Gram's Staining (-)

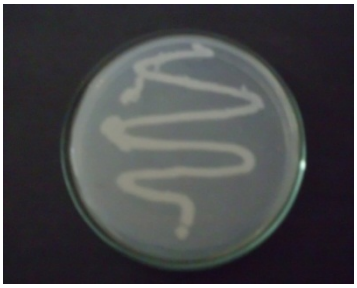


Biochemical characterization

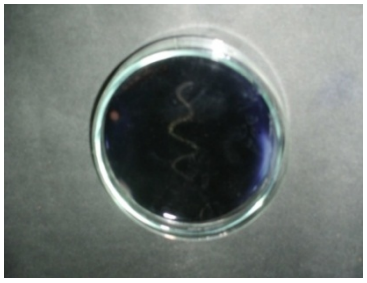


1.Indole (-), 2.Methyl red (-), 3. VP (+), 4. Citrate utilization (+), 5.Nitrate reduction (-) and 6.Catalase (+)

Gelatin hydrolases (+)



Starch hydrolases (-)



Oxidase test (+)

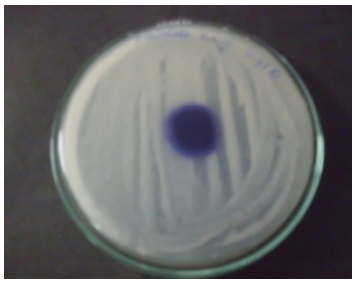
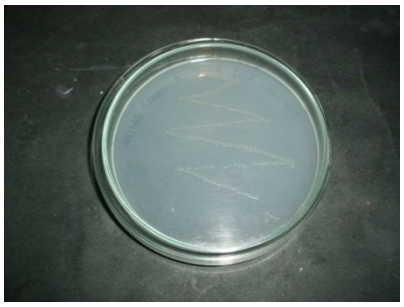


PLATE 3

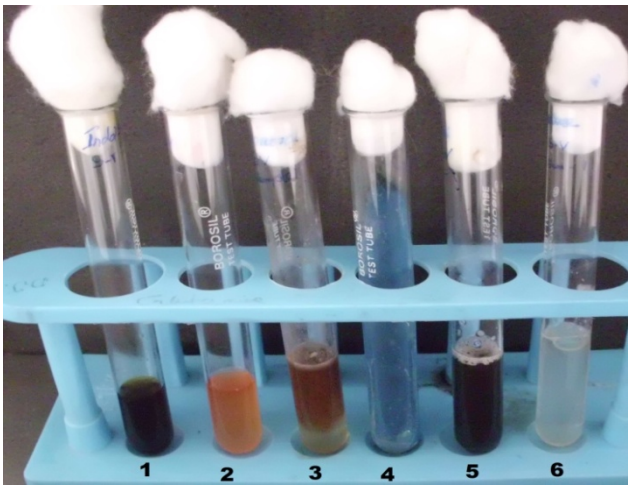
Bacillus subtilis

Pure culture of the isolated colony



Gram's Staining (+)

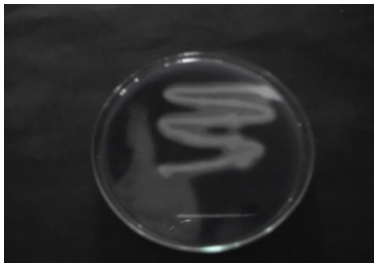
Biochemical characterization



1. Indole (-), 2. Methyl red (-), 3. VP (-), 4. Citrate utilization (+), 5.Nitrate reduction (+) and 6. Catalase (+)

Gelatin hydrolases (+)

Starch hydrolases (+)



Oxidase test (-)

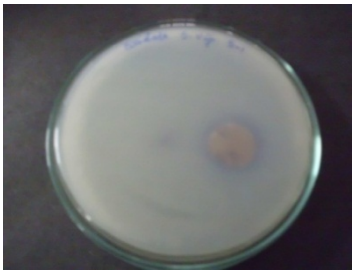


TABLE 1
OPTICAL DENSITY OF BIOMASS IN DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM DURING DIFFERENT TIME INTERVALS

Duration	CONCENTRATION OF HEXAVALENT CHROMIUM						
	100ppm	200ppm	300ppm	400ppm	500ppm	600ppm	700ppm
0 hr	0.001	0.003	0.004	0.006	0.008	0.01	0.022
12 hrs	0.005	0.009	0.01	0.011	0.015	0.016	0.026
24 hrs	0.006	0.01	0.012	0.014	0.018	0.021	0.029
36 hrs	0.009	0.012	0.017	0.021	0.023	0.018	0.026
48 hrs	0.018	0.018	0.019	0.023	0.026	0.019	0.025
60 hrs	0.016	0.011	0.015	0.016	0.018	0.015	0.021
72 hrs	0.014	0.01	0.014	0.015	0.016	0.01	0.02

TABLE 2
Cr (VI) CONTENTIN NUTRIENT BROTH COTANINTING DIFFERENT CONCENTRATIONS OF HEXAVALENT CHROMIUM (100, 200, 300, 400, 500, 600 and 700ppm) AT DIFFERENT TIME INTERVALS AND % REDUCTION OF Cr (VI)

Duration	CONCENTRATION OF HEXAVALENT CHROMIUM						
	100ppm	200ppm	300ppm	400ppm	500ppm	600ppm	700ppm
0 hr	95.77	197.1	297.03	398.36	498.97	591.98	696.78
12hrs	54.83	125.61	231.79	257.47	344.22	396.97	467.76
24hrs	38.17	77.73	140.19	217.22	283.15	362.96	370.6
36hrs	27.02	51.36	80.5	131.17	213.75	239.43	294.95
48hrs	13.19	15.96	59	79.12	135.33	203.34	253.31
60hrs	6.94	11.79	15.96	62.46	103.41	158.23	189.46
72hrs	0	5.55	7.63	33.31	46.97	147.13	182.52
% Reduction	100%	95.77%	96.46%	91.26%	90.40%	74.14%	73.47%

TABLE 3

THE IDENTIFICATION OF MICROORGANISMS USING COLONY MORPHOLOGICAL AND BIOCHEMICAL TEST

S.No.	Biochemical tests	*Pseudomonas fluorescens*	*Bacillus subtilis*
1	Gram's Staining	-	+
2	Motility test	+	+
3	Indole Test	-	-
4	Methyl red Test	-	-
5	VP Test	+	-
6	Citrate Test	+	+
7	Starch hydrolases	-	+
8	Gelatin Hydrolases	+	+
9	Nitrate reduction Test	-	+
10	Oxidase Test	+	-
11	Catalase Test	+	+
12	Glucose Test	A	A
13	Lactose Test	NG	A
14	Sucrose Test	A	A

(+ Positive, - Negative, A-Acid Production, NG-No Gas production.