Bioremediation of Cr^{3+} from Industrial Wastewater Using *Rhizobium* BJVr 12 Extracellular Polysaccharides

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ABSTRACT

The efficiency of extracellular polysaccharides (EPS) extracted from *Rhizobium* BJVr 12 in the adsorption of Cr^{3+} from industrial wastewater was investigated. Wastewater containing Cr^{3+} concentrations from a semiconductor company was obtained. It was then diluted to 1.0 ppm Cr^{3+} concentration. It was divided into three sets, each adjusted at different pH level – pH 3, 6 and 9. Two types of adsorbent were made – sand alone and sand with EPS. Several 250 mL Erlenmayer flasks containing 100 mL aliquot of wastewater samples were prepared. Each adsorbent was submerged in the flask containing wastewater solutions. Flasks were placed in a shaker and were observed at different time intervals. Collected samples were then acidified and analyzed using Atomic Absorption Spectrophotometric (AAS) method. General Factorial Design and Duncan's Multiple Range Test (DMRT) were used to evaluate the data. Results showed that adsorbent with EPS yielded significant reduction of Cr^{3+} concentrations from the wastewater samples. This suggests the efficiency of *Rhizobium* BJVr 12 EPS in the adsorption process. Maximum reduction of Cr^{3+} using EPS was achieved after 48 hours at pH 3. The lowest residual Cr^{3+} concentration obtained in the experiment was within the limit set by the Department of Environment and Natural Resources (DENR).

INTRODUCTION

A. Background of the study

With the increasing complexity of life comes the rapid progression towards industrialization. Hence, the emergence of numerous industrial establishments and the uncontrollable growth of their activities are inevitable. Accompanying this trend towards industrialization is the increased production of toxic wastes discharged to the environment.

Chromium is a naturally occurring element found in the environment. There are trace amounts of chromium in rocks and soil, in fresh water and ocean water and in the air (Dartmouth, 2003). Chromium is present in the environment in primarily three different chemical states. The most common forms are chromium-0 (Cr), chromium+3 (Cr^{3+}), and chromium+6 (Cr^{6+}) (ATSDR, 2003). Although Cr^{3+} is widely dispersed in the environment and needed for proper health, several studies have been made regarding its toxicity as an environmental pollutant. Such investigations include those made by Ferrer (2001), Friedman (1994), Mamaril, Paner and Alpante (1997). Although chromium is an essential trace element, it is considered toxic beyond the 0.1 ppm limit set by the Department of Environment and Natural Resources (DENR).

Major occurrences of environmental contamination by toxic metals discharged by industrial companies pose severe outcomes in the long run. Exposure to toxic metals may result in respiratory tract infection, high blood pressure, anemia, and other diseases

(Frei and Hutzinger, 1985). Hence, various institutions have undertaken measures to reduce environmental waste effluents released into the environment. One such method of biotechnology involves the treatment of wastes by microorganisms in a process referred to as bioremediation.

Bioremediation is a technological process whereby biological systems are harnessed to clean up environmental pollutants. This may involve either aiding the indigenous microbial populations in the affected area or adding new strains of microorganisms with particularly desirable degradative traits. Currently, microbial systems are most widely employed in bioremediation programs, generally in the treatment of soil and water contaminated with pollutants (Cobet, *et al.*, 1993).

Microorganisms are present everywhere in the environment and some are known for their waste-degrading capabilities. However, wastes that are produced are not easily degraded or eliminated. For this reason, measures to treat wastes have been implemented to clean up contaminated sites. It was in 1988 when scientists began using microbes to clean up pollutants and toxic wastes produced by various industrial processes (Tortora, *et al.*, 2001). Bioremediation was developed to accelerate the removal of environmental pollutants and has been studied for its potential use as an alternative to conventional remedial technologies (Buzea and DeStefanis, 1998).

The role of biological processes in metal transformation and concentration has a recent resurgence because of the concern over the accumulation of toxic metals (copper, cadmium, chromium, lead, mercury, zinc, gold, manganese, arsenic, selenium) in the environment. The use of microorganisms in the bioremediation of wastewater is at present being studied extensively as an alternative to costly treatment methods in

removing toxic metals in wastewater effluents. Furthermore, biotechnological approaches in solving these constraints are preferred over pure physical or chemical means due to higher efficiency and greater sustainability (Cheng, *et al.*, 1995).

One potential process scheme that is being utilized in bioremediation is adsorption. Adsorption can be the most versatile technique for the removal of toxic pollutants from aqueous solutions (Ferrer, 2001).

The National Institute of Molecular Biology and Biotechnology (BIOTECH) at the University of the Philippines Los Baños is currently conducting researches on the efficiency of extracellular polysaccharides (EPS) extracted from a nitrogen-fixing bacterium, *Rhizobium* sp., obtained from the roots of the mung bean plant (*Vigna radiata*), in the reduction of metals from effluents. By excreting mucilaginous EPS, *Rhizobium* BJVr 12 is able to survive in high concentrations of toxic metals (Gonzales, 2002). These EPS are said to prevent entry of toxic metals into the cell (Mamaril, *et al.*, 1997). To date, it has been shown that *Rhizobium* sp. is capable of producing greater yields of mucilaginous polysaccharides able to bind metals and removing them from wastes.

B. Statement of the problem

The primary concern of the study is to determine the efficiency of *Rhizobium* BJVr 12 EPS in reducing Cr^{3+} concentrations from industrial wastewater.

C. Objectives of the study

1. to determine the efficiency of *Rhizobium* BJVr 12 EPS in the reduction of Cr^{3+} in industrial wastewater at different pH levels
2. to reduce 1.0 ppm Cr^{3+} concentration of wastewater samples to the acceptable limit of 0.1 ppm set by the DENR

D. Significance of the study

Bioremediation is a less expensive biological treatment that can selectively achieve complete destruction of organic pollutants without collateral destruction of either the site material or its flora and fauna, and can be used in sites for pollutants that are at low but environmentally relevant concentrations (Rochanaroon, 1999 unpublished).

Several studies on the adsorption capacity of *Rhizobium* BJVr 12 EPS have been well documented by Mamaril (1989, 1991 and 1997). The process of adsorption is of low cost and low maintenance compared with other remediation methods. The accomplishment of this study will provide alternative technology for the reduction of pollutants, particularly toxic metals, for the treatment of contaminated sites.

E. Scope and limitations

1. The industrial wastewater sample containing Cr^{3+} was obtained from a semiconductor company that specializes in chrome plating.

2. Atomic Absorption Spectrophotometric Analyses of the samples were performed by the Analytical Services Laboratories of the Natural Sciences Research Institute and the Institute of Chemistry at the University of the Philippines in Diliman.
3. The wastewater sample was diluted from an initial Cr^{3+} concentration of 49, 400 ppm to 1.0 ppm only.
4. The diluted samples were adjusted at pH 3, 6 and 9.
5. *Rhizobium* BJVr 12 EPS were already extracted by the National Institute of Molecular Biology and Biotechnology (BIOTECH).
6. Wastewater samples were observed at longer time intervals.
7. Sand was the only immobilizer used.

A. Chromium as an environmental pollutant

Chromium, with the symbol Cr, is a gray metallic element that can take on a high polish. The atomic number of chromium is 24 and is one of the transition elements of the periodic table. It was discovered in 1797 by the French chemist Louis Nicolas Vauquelin, who named it chromium (Greek *chroma,* "color") because of the many different colors of its compounds. Chromium has an atomic weight of 51.996; the element melts at about 1857° C (about 3375° F), boils at about 2672° C (about 4842° F), and has a specific gravity of 7.2 (Microsoft Encarta, 2001).

Chromium is widely dispersed in the environment. It is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases (ATSDR, 2003). The primary form of chromium found in the environment is Cr^{3+}, which is stable. This common form of chromium is always found in a complex with other chemical partners such as oxygen or chlorine. In these compounds it is very "inert to substitution", that is, it is resistant to changing its form or exchanging its chemical partners (Dartmouth, 2003).

More than half the production of chromium goes into metallic products, and about another third is used in refractories. The chief use of chromium is to form alloys with iron, nickel, or cobalt. The addition of chromium imparts hardness, strength, and corrosion resistance to the alloy. Because of its hardness, an alloy of chromium, cobalt, and tungsten is used for high-speed metal-cutting tools. When deposited electrolytically,

chromium provides a hard, corrosion-resistant, lustrous finish. For this reason it is widely used as body trim on automobiles and other vehicles. The extensive use of chromite as a refractory is based on its high melting point, its moderate thermal expansion, and the stability of its crystalline structure (Microsoft Encarta, 2001). Cr^{3+} is also used for chrome plating, dyes and pigments, leather tanning, and wood preserving (ATSDR, 2003).

The most common health effect from exposure to chromium is contact dermatitis - skin inflammation or rash (Dartmouth, 2003). One can be exposed to chromium by eating food containing Cr^{3+}, drinking contaminated well water, breathing contaminated workplace air or skin contact during use in the workplace, or living near uncontrolled hazardous waste sites containing chromium or industries that use chromium (ATSDR, 2003).

Chromium is a metal widely used in industry and released in wastewaters hence its dispersion must be highly regulated. According to the Department of Environment and Natural Resources (DENR) Administrative Order No. 35 Revised Effluent Regulations of 1990, the maximum limit of chromium in industrial effluents is set depending on the body of water to which the effluent is to be discharged (Ferrer, 2001). Discharge of sewage and/or trade effluents are prohibited in certain public water supplies and waters suitable for the propagation, survival and harvesting of shellfish, tourist zones and national marine parks, coral reef parks and reserves. A maximum limit of 0.1 mg/L for old or existing industries is set by the DENR for selected public water supplies, for waters used for swimming bathing, skin diving and other recreations and

coastal and marine waters used as spawning areas of fishes. Inland water effluent is set to a maximum limit of 0.2 mg/L and 0.1 mg/L for old and new industries respectively.

The Environmental Protection Agency (EPA) has set a limit of 100 μg Cr^{3+} per liter of drinking water (100 μg/L). The Occupational Safety and Health Administration (OSHA) has set a limit of 500 μg water soluble Cr^{3+} compounds per cubic meter of workplace air (500 $\mu g/m^3$) (ATSDR, 2003).

B. Atomic Absorption Spectrophotometry (AAS)

Atomic Absorption Spectrophotometry (AAS) is used for the analysis of certain metals in water and wastewater. The major components of an AAS are the hollow cathode lamp, rotating chopper, vaporizing system, monochromater and photo detector. The solution containing the metal ions is aspirated into the plane where it is volatized, and many of the ions are reduced into atoms. The hollow cathode lamp emits energy at specific wavelengths absorbed by the atoms of the element being analyzed. The amount of light energy at one of these specific wavelengths absorbed by the sample is proportional to the amount of element vaporized in the flame (Tissue, 2000).

C. The principles of bioremediation

Bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism (Shmaefsky, 1999). It is a technique used to remove waste pollutants or toxic chemicals from contaminated sites using

biological agents such as bacteria, fungi or plants (Calolot and Po, 2002). Bioremediation attempts to harness the waste-degrading capability of microorganisms and use it to destroy toxic organic substances found in hazardous waste (EPA Journal, 1994).

Bioremediation utilizes several strategies to remove recalcitrant wastes from contaminated places. One of these involves the use of microorganisms that already exist and thrive in the environment and that which can be used to degrade certain pollutants. This type of method is referred to as natural bioremediation (Young and Cerniglia, 1995). However, this technique has one major downside. The problem of accumulation arises because pollutants are degraded very slowly by indigenous microorganisms. Hence, the condition of the microorganisms can be enhanced.

Creating an environment conducive for indigenous microbes can speed up the bioremediation process. The natural capabilities of microorganisms can be enhanced by adding oxygen, water, nutrients or chemicals to environments where microorganisms are found. The addition of oxygen, however, is an inefficient process due to its low solubility, losses associated with injection, and reaction with inorganic species (Ensley and DeFlaun, 1995).

Then again, other contaminated sites are not occupied by organisms necessary for the removal of particular pollutants. Thus, certain microorganisms with specific degradative functions can be added in an environment where wastes ought to be degraded. Addition of microbial degraders is commonly isolated and applied in bioremediation to hasten the natural biodegradation to clean up oil spill pollutants like oil, petroleum and wastes that contain biodegradable hydrocarbons (Salanitro, 1997).

D. *Rhizobium* EPS

Rhizobium Vigna radiata (L.) Wilezek belongs to the family Leguminosae and is commonly known as green gram, golden gram, chop suey bean, chickasaw pea and mungbean. Locally, it is known as *munggo* or *balatong* (Ilocano and Bicol) (Mupas, 1999).

Mamaril and colleagues (1989, 1991) have deduced in their studies that *Rhizobium* BJVr 12 (BIOTECH-Jaica *Vigna radiata* strain #12) located in the nodules of mungbean (*Vigna radiata*) were found to be heavy producers of mucilaginous polysaccharides that can sequester and reduce the concentration of metals in dilute aqueous solutions to a high degree. The capacity of these microorganisms to produce gummy polysaccharides is one of its external means of preventing the entry of toxic amounts of heavy metals into the cell where they can interfere with the organism's normal metabolic processes (Mamaril, *et al.*, 1989). Several studies were performed using this strain and the results obtained showed that it can adsorb metals including Cu^{2+}, Ag^{+}, Au^{3+}, Cd^{2+}, Hg^{+}, and Pb^{2+} (Mamaril *et al.*, 1997; Aguilar, 1996; Galan, 1996; Padolina, 1994; Paner *et al.*, 1999).

Rhizobium EPS have been studied for their role in plant-host specificity but only recently have their metal sorption capacity investigated. Researches performed on some *Rhizobium* isolates revealed that the bacteria were able to reduce radionuclide concentration and that they were able to tolerate and grow in an environment containing a relatively high concentration of lead (Cotoras, *et al.*, 1992).

In a study conducted by BIOTECH to determine the chemical properties of *Rhizobium* BJVr 12 EPS, it was found that based on the average CHO composition, the molecular

formula of the EPS is $C_6H_{12}O_7$. Glucose is the predominant sugar with mannose and galactose in lesser quantities. Spectroscopic analysis revealed that the functional groups of the EPS are hydroxyl (-OH), aldehyde (-CHO) and alcohol (-CO) (BIOTECH, unpub. data 1999).

E. Adsorption and biosorption

Adsorption is a process which involves the concentration of gases, vapors, liquids or solids (i.e. solids dissolved in a solvent) on a solid (Fernandez, 2002). Adsorption can also be defined as the taking up by the surface of a solid or liquid (adsorbent) of the atoms, ions, or molecules of a gas or other liquid (adsorbate) (Microsoft Encarta, 2001). The interaction between the adsorbed species and surface may either be chemical, physical or ion exchange and may be of more than one type, depending on the component's chemical structure (Perry and Green, 1997).

One type of adsorption process applied for the treatment of wastewater is called biosorption. Biosorption is the use of organically derived materials, which have properties that make them potential adsorbents (Junter, 2001).

Microorganisms can accumulate metals by precipitating or binding the metals onto cell walls and cell membranes because of the presence of carboxyl, hydroxyl, phosphoryl, and other negatively charged sites in anionic walls. Some microorganisms synthesize EPS, polymers extending from the outer membrane which also serve as sites of metal accumulation (El Aziz *et al.*, 1991). Other microorganisms adsorb metals metabolically. They actively take in metals and compartmentalize them into specific organelles such as

vacuoles or render them non-toxic by binding them to proteins or precipitation (Wilkins and Yang, 1996).

MATERIALS AND METHODS

A. Sources of materials

Wastewater effluent

Industrial wastewater was obtained from a semiconductor company. Chromium content of the sample was analyzed and determined by the Research and Analytical Services Laboratory of the Natural Sciences Research Institute at the University of the Philippines in Diliman using Atomic Absorption Spectrophotometry (AAS). Initial chromium analysis of the wastewater sample revealed a Cr^{3+} content of 49, 400 ppm.

Immobilizer

White sand was obtained from Boracay, Aklan in Visayas and was used as an immobilizer of *Rhizobium* BJVr 12 EPS.

Rhizobium EPS

Extracted *Rhizobium* BJVr 12 EPS were obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH) Microbial Culture Collection and Services Laboratory in UP Los Baños. *Rhizobium* BJVr 12 EPS were maintained on slants of Yeast Extract Mannitol Agar (YEMA). Coconut water was used for the mass production of biomass EPS.

B. Methodology

Extraction of EPS

EPS were precipitated from *Rhizobium* BJVr 12 culture using 95% ethanol. The volume ratio of *Rhizobium* BJVr 12 EPS and ethanol was 1:3, respectively based on the procedure provided by the BIOTECH. The precipitated polysaccharides were separated by decantation and were drained using cheesecloth.

Dilution and pH adjustment of wastewater sample

The wastewater sample was diluted from an initial concentration of 49, 400 ppm to 1.0 ppm using triple distilled water. The pH of the diluted wastewater sample was adjusted into three levels—pH 3, pH 6 and pH 9 by adding either concentrated sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) pellets. The pH level was determined using a pH meter.

Adsorbent preparation

White sand was homogenized using a strainer and washed with distilled water. It was subsequently drained using cheesecloth and oven dried at 70° C/gravity. The precipitated EPS were immediately combined with a fraction of the white sand in a ratio of 1:1 and were again subjected to drying. The dried material was then pulverized.

Two kinds of adsorbent were made – one set containing five grams of sand with EPS and another set containing five grams of sand alone wrapped in small pieces of cheesecloth.

Adsorption of Cr^{3+}

Several 250 mL Erlenmayer flasks were filled with 100 mL aliquot of prepared wastewater samples at pH level 3, 6 and 9. Adsorbents were then submerged simultaneously in each flask Flasks were positioned on a shaker where adsorption under regularly shaken conditions (120 rpm) took place.

Two set-ups were made, each with three replicates. One set-up was comprised of the controlled variable (sand without EPS) while the other set-up included a mixture of sand and EPS. Set-ups were observed and adsorption rates were recorded at time intervals of 0.5, 2, 6, 24, 48, 72 and 96 hours.

Acidification of samples

Adsorbents were removed from the flasks and 20 mL aliquot of each sample was collected and transferred using a pipette into 25 mL volumetric flasks. To create a solution of 1.16 normality (N), 2.5 mL concentrated HCl were added and the samples were diluted up to the 25 mL mark with triple distilled water. Acidified samples were transferred into labeled film canisters and refrigerated.

AAS analysis of the samples

The Cr^{3+} content of the samples were determined using Atomic Absorption Spectrophotometry (AAS). The analysis was conducted at the Analytical Services Laboratory at the Institute of Chemistry, University of the Philippines in Diliman, Quezon City.

C. Experimental design

General Factorial Design was used to test the variables of the study. Two types of adsorbent were used – sand alone and sand with EPS. Water samples were adjusted at three pH levels (pH 3, 6 and 9). Samples were collected at 7 different time intervals for Cr^{3+} analysis. Three replicates of each set-up were made. A total of 126 samples were analyzed by the AAS (2 types of adsorbent x 3 pH levels x 7 time intervals x 3 replicates). The factorial experiment was used to analyze the effect of the variables simultaneously. The Duncan's Multiple Range Test (DMRT) was used to determine which adsorbent, at what pH and fraction of time maximum reduction of Cr^{3+} occurred.

Adsorption of Cr^{3+}

At pH 3, maximum percent reduction of Cr^{3+} concentrations for the adsorbent composed of sand alone was 97.00% which occurred at 24 hours. On the other hand, adsorbent comprised of sand with EPS gave a maximum percent reduction of 98.00% at 48 hours (Table 2 and Figure 1).

At pH 6, adsorbent composed of sand alone obtained a maximum percent reduction of 97.00% of Cr^{3+} concentrations at 48 hours. A 97.33% maximum reduction of Cr^{3+} concentrations occurred at 48 hours for the adsorbent composed of sand with EPS (Table 4 and Figure 2).

At pH 9, maximum percent reduction of Cr^{3+} concentrations for the adsorbent composed of sand alone was 97.00% which occurred at 24 hours. On the contrary, adsorbent comprised of sand with EPS gave a maximum percent reduction of 97.00% at 2 hours (Table 6 and Figure 3).

Effect of adsorbents

Adsorbents comprised of sand alone yielded significant results in the adsorption process (Table 11). On the other hand, adsorbents with EPS showed greater adsorption of Cr^{3+} concentrations (Appendix G).

Effect of time

Duncan's Multiple Range Test (Appendix B) shows that greatest adsorption of Cr^{3+} was obtained after 48 hours of treatment. Subsequent time intervals also exhibited significant results although to a lesser degree. Statistical analysis of the data revealed that time produced significant reduction of Cr^{3+} in wastewater samples (Table 11).

Effect of pH

Duncan's Multiple Range Test revealed that the highest value of residual Cr^{3+} concentration was at pH 9. On the other hand, the lowest residual Cr^{3+} was obtained at a pH of 3 (Appendix D). After a period of 96 hours, wastewater samples at pH 3 treated with EPS adsorbed the highest amount of Cr^{3+} ions from the solution. An average concentration of 0.01700 ppm Cr^{3+} remained in the samples (Figure 4 and Appendix G).

ANOVA (Table 11) shows that pH has no significant effect on the reduction of Cr^{3+} in the wastewater samples.

Interaction effects

Statistical analysis of the data showed that the interaction of adsorbent with pH did not produce significant results. The combined effect of adsorbent and pH did not significantly lower the amount of Cr^{3+} in the wastewater samples. Interaction effects of adsorbent and time also did not give significant reduction of Cr^{3+} in wastewater. Interaction between pH and time did not produce significant results. In addition, the combined effect of all the factors in the experiment (adsorbent, pH and time) did not provide significant reduction of Cr^{3+} (Table 11).

DISCUSSION

Effect of adsorbents

Results showed that adsorbents comprised of sand alone were able to adsorb significant amounts of Cr^{3+} in the wastewater samples (Tables 1, 3 and 5; Figures 1, 2 and 3). The capacity of sand to adsorb significant quantities of Cr^{3+} is due to the presence of silicates which serve as binding sites for positively-charged metals. Conversely, greater adsorption was observed for sand with *Rhizobium* EPS due to the additional binding sites provided by the EPS (Tables 7, 8 and 9; Figures 1, 2 and 3). The mechanism involved for these adsorption processes may be by physical means arising from electrostatic attraction occurring between the adsorbent and the adsorbate possessing opposite charges (Ferrer, 2001).

Rhizobium BJVr 12 extracellular polysaccharides are known to possess negatively-charged functional groups like hydroxyl, sulfhydryl, and carboxyl groups, which are potential binding sites of cations like heavy metals (Gadd, 1990). Chemical characterization studies of the polysaccharide used in the experiment done by Dr. Veronica P. Amigo (1999) of BIOTECH, revealed the presence of the functional groups such as –OH, -COOH, -CHO, and –NH2.

Both adsorbents were effective in reducing Cr^{3+} content in wastewater samples. However, adsorbents with EPS showed a greater percentage reduction of Cr^{3+} concentrations.

Effect of time

After ½ hour, an average residual Cr^{3+} concentration of 0.03928 ppm was obtained (Appendix B). The short time it took to accomplish this result is a good indication of the efficiency of both adsorbents used in the experiment. At a short time of 0.5 hours, the adsorbents were already able to reduce Cr^{3+} concentrations from the wastewater samples to values lower than the 0.1 ppm limit.

Each adsorbent exhibited a leveling off in the charts (Figures 1-6), suggesting attainments of saturation points. Duncan's Multiple Range Test (Appendix B) shows that maximum adsorption of both adsorbents occurred at 48 hours. The ANOVA table clearly illustrates that time allowed for significant reduction of Cr^{3+}.

After 48 hours, the adsorbents were still able to adsorb significant quantities of Cr^{3+} although to a lesser extent owing to the increasing saturation of the binding sites provided by both sand and EPS.

Effect of pH

Based on analysis of the data, samples at pH 3 have the lowest residual Cr^{3+} concentrations at 0.03183 ppm. In contrast, samples at pH 9 have the highest residual Cr^{3+} concentrations at 0.03583 ppm (Appendix D). This can be attributed to the addition of sodium hydroxide (NaOH) to wastewater samples allowing lesser adsorption of Cr^{3+} concentrations, negatively charged OH^- groups react to the binding sites present in sand. These OH^- groups occupy the binding sites, hence, lesser binding sites become available

for metal ions such as Cr^{3+}. Bases such as NaOH react with metal oxides like SiO_2 (sand) such that sodium silicates are formed (Skoog, 1996).

However, statistical analysis of the data showed that pH did not significantly decrease Cr^{3+} content in wastewater. This implies that changing the pH level of the samples would have no major effect on the adsorption process.

Interaction effects

Interaction of adsorbent with pH did not produce significant results according to ANOVA, probably because pH alone did not yield significant reduction of Cr^{3+}. The insignificance of pH in the experiment might have had greater effect in the adsorption process compared with the adsorbent.

Interaction effects of adsorbent and time did not provide significant results due to the opposing effects of both factors against each other. The reduction of Cr^{3+} in wastewater by the adsorbents is independent of time and is not determined by the time intervals used in the experiment.

Interaction between pH and time did not yield significant reduction of Cr^{3+} in wastewater possibly due to their antagonistic effect against each other. pH might have had a stronger effect on the adsorption process compared with time.

The combined effects of adsorbent, pH and time did not give significant results suggesting absence of interaction effect among the factors.

CONCLUSIONS

Both adsorbents used yielded significant reduction of Cr^{3+} concentrations from the wastewater samples. At 0.5 hours, reduction of Cr^{3+} was already observed. However, maximum reduction was not observed until 48 hours. At longer time intervals, continuous reduction was still observable although to a lesser extent. At pH 3, maximum reduction of Cr^{3+} was obtained due to the presence of more binding sites compared to pH 9 where binding sites are made less available for Cr^{3+} ions.

Results of the experiment showed that EPS extracted from *Rhizobium* BJVr 12 cells have the ability to adsorb and significantly reduce Cr^{3+} ions from the solution, suggesting its efficiency in the bioremediation process. The lowest residual Cr^{3+} concentration obtained was within the 0.1 ppm limit set by the Department of Environment and Natural Resources (DENR).

RECOMMENDATIONS

Future studies on the adsorption process could include other suitable immobilizers aside from sand which was used in the experiment. Bioremediation of other metals and varying their concentrations is a good area for investigation to further support the efficiency of *Rhizobium* BJVr 12 EPS. Furthermore, adsorption isotherms can be constructed to determine the mechanism of adsorption and the extent of the adsorption capacity of the adsorbents utilized in the experiment.

The actual wastewater sample collected can be used directly without any variation in terms of its concentration and pH level. Continuous time intervals can be employed to closely monitor changes in the process of adsorption.

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TABLES

Table 1. Residual Cr^{3+} concentrations (ppm) of wastewater samples at pH 3

sample i.d.	time (hours)	residual Cr^{3+} (ppm)			Average residual Cr^{3+} (ppm)
		replicate 1	replicate 2	replicate 3	
sand	0.5	0.042	0.039	0.045	0.042
w/ EPS	0.5	0.047	0.045	0.047	0.046
sand	2	0.035	0.037	0.037	0.036
w/ EPS	2	0.033	0.036	0.031	0.033
sand	6	0.035	0.035	0.036	0.035
w/ EPS	6	0.035	0.032	0.029	0.032
sand	24	0.031	0.033	0.035	0.033
w/ EPS	24	0.028	0.029	0.026	0.028
sand	48	0.031	0.034	0.029	0.031
w/ EPS	48	0.021	0.023	0.025	0.023
sand	72	0.032	0.034	0.033	0.033
w/ EPS	72	0.022	0.024	0.025	0.024
sand	96	0.031	0.032	0.032	0.032
w/ EPS	96	0.018	0.017	0.015	0.017

Table 2. Percent (%) reduction of Cr^{3+} concentrations in wastewater samples at pH 3

sample i.d.	time (hours)	% reduction			Average % reduction
		replicate 1	replicate 2	replicate 3	
sand	0.5	96	96	96	96.00
w/ EPS	0.5	95	96	95	95.33
sand	2	96	96	96	96.00
w/ EPS	2	97	96	97	96.67
sand	6	97	96	96	96.33
w/ EPS	6	97	97	97	97.00
sand	24	97	97	97	97.00
w/ EPS	24	97	97	97	97.00
sand	48	97	97	97	97.00
w/ EPS	48	98	98	98	98.00
sand	72	97	97	97	97.00
w/ EPS	72	98	98	98	98.00
sand	96	97	97	97	97.00
w/ EPS	96	98	98	97	97.67

Table 3. Residual Cr^{3+} concentrations (ppm) of wastewater samples at pH 6

sample i.d.	time (hours)	residual Cr^{3+} (ppm)			Average residual Cr^{3+} (ppm)
		replicate 1	replicate 2	replicate 3	
sand	0.5	0.037	0.040	0.036	0.038
w/ EPS	0.5	0.032	0.035	0.030	0.032
sand	2	0.043	0.040	0.046	0.043
w/ EPS	2	0.029	0.034	0.024	0.029
sand	6	0.045	0.043	0.041	0.043
w/ EPS	6	0.033	0.033	0.035	0.034
sand	24	0.043	0.035	0.039	0.039
w/ EPS	24	0.033	0.030	0.034	0.032
sand	48	0.030	0.028	0.029	0.029
w/ EPS	48	0.028	0.025	0.029	0.027
sand	72	0.034	0.034	0.035	0.034
w/ EPS	72	0.035	0.033	0.033	0.034
sand	96	0.038	0.040	0.036	0.038
w/ EPS	96	0.035	0.030	0.028	0.031

Table 4. Percent (%) reduction of Cr^{3+} concentrations in wastewater samples at pH 6

sample i.d.	time (hours)	% reduction			Average % reduction
		replicate 1	replicate 2	replicate 3	
sand	0.5	96	96	96	96.00
w/ EPS	0.5	97	97	97	97.00
sand	2	96	96	95	95.67
w/ EPS	2	97	97	97	97.00
sand	6	96	96	96	96.00
w/ EPS	6	97	97	97	97.00
sand	24	96	96	96	96.67
w/ EPS	24	97	97	97	97.00
sand	48	97	97	97	97.00
w/ EPS	48	97	98	97	97.33
sand	72	97	97	97	97.00
w/ EPS	72	96	97	97	96.67
sand	96	96	96	96	96.00
w/ EPS	96	96	97	97	96.67

Table 5. Residual Cr^{3+} concentrations (ppm) of wastewater samples at pH 9

sample i.d.	time (hours)	residual Cr^{3+} (ppm)			Average residual Cr^{3+} (ppm)
		replicate 1	replicate 2	replicate 3	
sand	0.5	0.040	0.042	0.044	0.042
w/ EPS	0.5	0.035	0.037	0.033	0.032
sand	2	0.039	0.041	0.036	0.039
w/ EPS	2	0.034	0.029	0.035	0.029
sand	6	0.040	0.038	0.036	0.038
w/ EPS	6	0.033	0.035	0.034	0.034
sand	24	0.035	0.033	0.035	0.034
w/ EPS	24	0.034	0.034	0.035	0.032
sand	48	0.027	0.033	0.030	0.030
w/ EPS	48	0.042	0.035	0.030	0.027
sand	72	0.038	0.035	0.039	0.037
w/ EPS	72	0.026	0.029	0.029	0.034
Sand	96	0.055	0.050	0.054	0.053
w/ EPS	96	0.029	0.028	0.028	0.031

Table 6. Percent (%) reduction of Cr^{3+} concentrations in wastewater samples at pH 9

sample i.d.	time (hours)	% reduction			Average % reduction
		replicate 1	replicate 2	replicate 3	
Sand	0.5	96	96	96	96.00
w/ EPS	0.5	97	96	97	96.67
Sand	2	96	96	96	96.00
w/ EPS	2	97	97	97	97.00
Sand	6	96	96	96	96.00
w/ EPS	6	97	97	97	97.00
Sand	24	97	97	97	97.00
w/ EPS	24	97	97	97	97.00
Sand	48	97	97	97	97.00
w/ EPS	48	96	96	97	96.33
Sand	72	96	97	96	96.33
w/ EPS	72	97	97	97	97.00
Sand	96	97	96	96	96.33
w/ EPS	96	97	97	97	97.00

Table 7. Adsorption capacity (in mg Cr^{3+}/g adsorbent) of sand with EPS and sand without EPS at pH 3

sample id	time (hours)	Adsorption Capacity (in mg Cr^{3+}/g adsorbent)		
		replicate 1	replicate 2	replicate 3
sand	0.5	0.958	0.961	0.955
w/ EPS	0.5	0.953	0.955	0.953
sand	2	0.965	0.963	0.963
w/ EPS	2	0.967	0.964	0.969
sand	6	0.965	0.965	0.964
w/ EPS	6	0.965	0.968	0.971
sand	24	0.969	0.967	0.965
w/ EPS	24	0.972	0.971	0.974
sand	48	0.969	0.966	0.971
w/ EPS	48	0.979	0.977	0975
sand	72	0.968	0.966	0.967
w/ EPS	72	0.978	0.976	0.975
sand	96	0.969	0.968	0.968
w/ EPS	96	0.976	0.975	0.973

Table 8. Adsorption capacity (in mg Cr^{3+}/g adsorbent) of sand with EPS and sand without EPS at pH 6

sample id	time (hours)	Adsorption Capacity (in mg Cr^{3+}/g adsorbent)		
		replicate 1	replicate 2	replicate 3
sand	0.5	0.963	0.960	0.964
w/ EPS	0.5	0.968	0.965	0.970
sand	2	0.957	0.960	0.954
w/ EPS	2	0.971	0.966	0.976
sand	6	0.955	0.957	0.959
w/ EPS	6	0.967	0.967	0.965
sand	24	0.957	0.965	0.961
w/ EPS	24	0.967	0.970	0.966
sand	48	0.970	0.972	0.971
w/ EPS	48	0.972	0.975	0.971
sand	72	0.966	0.966	0.965
w/ EPS	72	0.965	0.967	0.967
sand	96	0.962	0.960	0.964
w/ EPS	96	0.965	0.970	0.972

Table 9. Adsorption Capacity (in mg Cr^{3+}/g adsorbent) of sand with EPS and sand without EPS at pH 9

sample id	time (hours)	Adsorption Capacity (in mg Cr^{3+}/g adsorbent)		
		Replicate 1	replicate 2	replicate 3
sand	0.5	0.960	0.958	0.956
w/ EPS	0.5	0.965	0.963	0.967
sand	2	0.961	0.959	0.964
w/ EPS	2	0.966	0.971	0.965
sand	6	0.960	0.962	0.964
w/ EPS	6	0.967	0.965	0.966
sand	24	0.965	0.967	0.965
w/ EPS	24	0.966	0.966	0.965
sand	48	0.973	0.967	0.970
w/ EPS	48	0.958	0.965	0.970
sand	72	0.962	0.965	0.961
w/ EPS	72	0.974	0.971	0.971
sand	96	0.965	0.964	0.962
w/ EPS	96	0.971	0.972	0.972

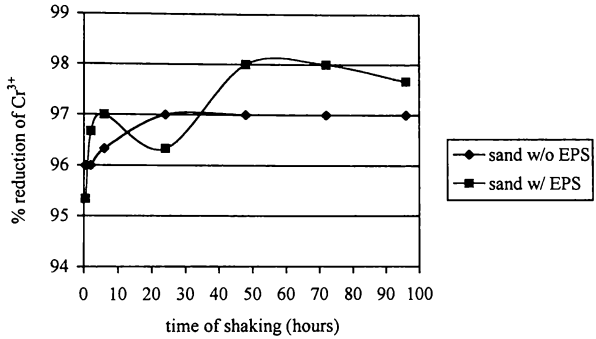

Figure 1. Average % reduction of Cr^{3+} concentrations using sand with EPS and sand alone vs. the time of shaking in hours at pH 3

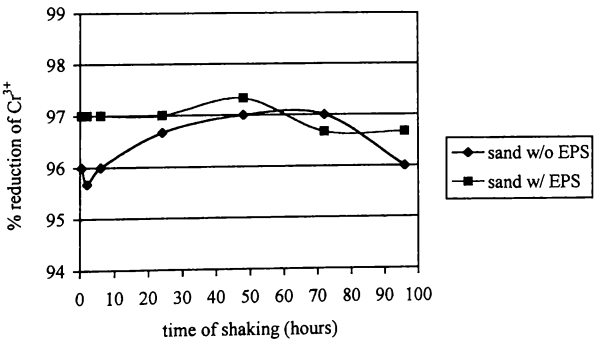

Figure 2. Average % reduction of Cr^{3+} concentrations using sand with EPS and sand alone vs. the time of shaking in hours at pH 6

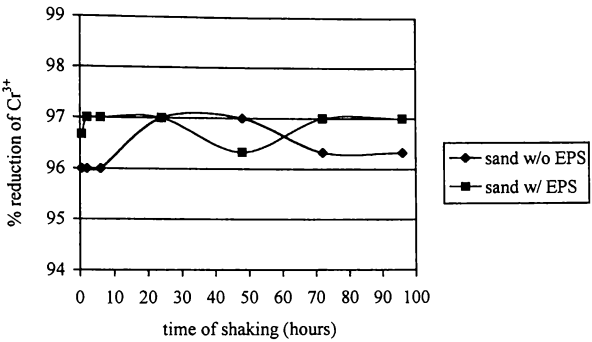

Figure 3. Average % Reduction of Cr^{3+} concentrations using sand with EPS and sand alone vs. time of shaking in hours at pH 9

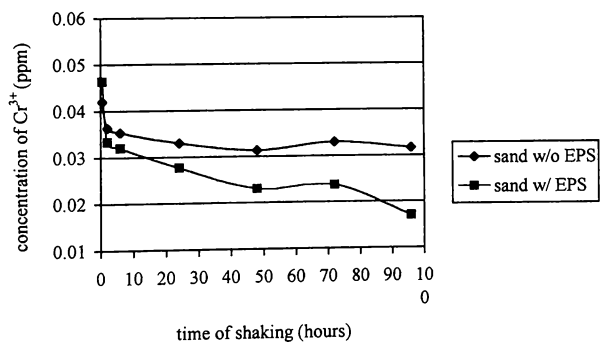

Figure 4. Average residual Cr^{3+} concentration in wastewater samples using sand with EPS and sand alone vs. time of shaking in hours at pH 3

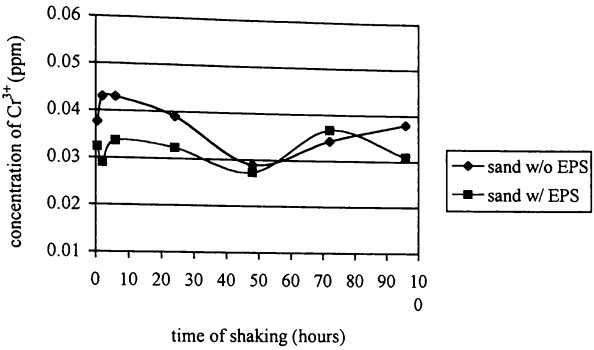

Figure 5. Average residual Cr^{3+} concentration in wastewater samples using sand with EPS and sand alone vs. time of shaking in hours at pH 6

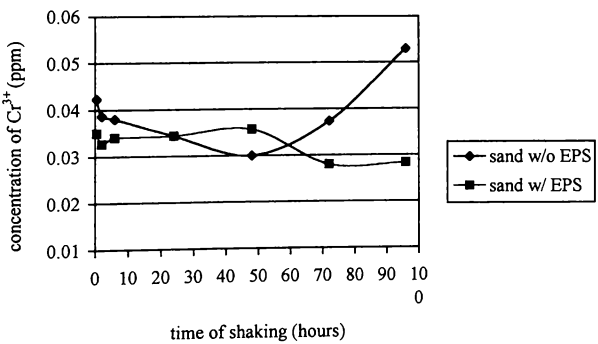

Figure 6. Average residual Cr^{3+} concentration in wastewater samples using sand with EPS and sand alone vs. time of shaking in hours at pH 9

PLATES

Plate 1. (above): 1.0 ppm wastewater samples at pH 3 and pH 6, respectively
(below): 49, 400 ppm wastewater sample and 1.0 ppm wastewater sample at pH 9

Plate 2. (from L-R): hydrochloric acid (HCl), triple distilled water, sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4)

Plate 3. Canisters containing 25 mL acidified treated wastewater samples for Atomic Absorption Spectrophotometry (AAS) analysis

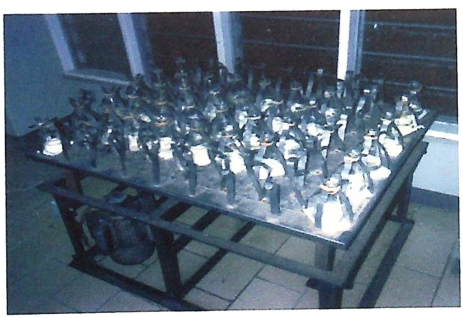

Plate 4. Shaker (120 rpm)

Plate 5. Erlenmayer flasks containing 100 mL wastewater samples and adsorbents

APPENDIX A

Statistical Analysis

General Factorial Design

Function: FACTOR

Experimental Model:
General Factorial Design

Date case no. 1 to 126

Factorial ANOVA for the factors:
Replication (Var 4: replicates) with values from 1 to 3
Factor A (Var 1: adsorbent) with values from 1 to 2
1 – sand alone
2 – sand with EPS

Factor B: (Var 2: pH) with values from 1 to 3
1 – pH 3
2 – pH 6
3 – pH 9

Factor C: (Var 3: time in hours) with values from 1 to 7
1 – 0.5 h
2 – 2 h
3 – 6 h
4 – 24 h
5 – 48 h
6 – 72 h
7 – 96 h

Variable 5: Residual Cr^{3+}

Grand Mean – 0.034 Grand Sum – 4.292 Total Count – 126

Table 10. Table of Means

4	1	2	3	5	total
1	*	*	*	0.034	1.429
2	*	*	*	0.034	1.448
3	*	*	*	0.034	1.415
*	1	*	*	0.037	2.341
*	2	*	*	0.031	1.951
*	*	1	*	0.039	0.707
*	*	2	*	0.036	0.639
*	*	3	*	0.036	0.648
*	*	4	*	0.033	0.602
*	*	5	*	0.029	0.529
*	*	6	*	0.032	0.570
*	*	7	*	0.033	0.597
*	1	1	*	0.041	0.366
*	1	2	*	0.039	0.354
*	1	3	*	0.039	0.349
*	1	4	*	0.035	0.319
*	1	5	*	0.030	0.271
*	1	6	*	0.035	0.314
*	1	7	*	0.041	0.368
*	2	1	*	0.038	0.341
*	2	2	*	0.032	0.285
*	2	3	*	0.033	0.299
*	2	4	*	0.031	0.283
*	2	5	*	0.029	0.258
*	2	6	*	0.028	0.256
*	2	7	*	0.025	0.229
*	*	*	1	0.032	1.337
*	*	*	2	0.035	1.450
*	*	*	3	0.036	1.505
*	1	*	1	0.035	0.728
*	1	*	2	0.038	0.792
*	1	*	3	0.039	0.821
*	2	*	1	0.029	0.609
*	2	*	2	0.031	0.658
*	2	*	3	0.033	0.684

4	1	2	3	5	total
*	*	1	1	0.044	0.265
*	*	1	2	0.035	0.210
*	*	1	3	0.039	0.232
*	*	2	1	0.035	0.209
*	*	2	2	0.036	0.216
*	*	2	3	0.036	0.214
*	*	3	1	0.034	0.202
*	*	3	2	0.038	0.230
*	*	3	3	0.036	0.216
*	*	4	1	0.030	0.182
*	*	4	2	0.036	0.214
*	*	4	3	0.034	0.206
*	*	5	1	0.027	0.163
*	*	5	2	0.028	0.169
*	*	5	3	0.033	0.197
*	*	6	1	0.028	0.170
*	*	6	2	0.034	0.204
*	*	6	3	0.033	0.196
*	*	7	1	0.024	0.146
*	*	7	2	0.034	0.207
*	*	7	3	0.041	0.244
*	1	1	1	0.042	0.126
*	1	1	2	0.038	0.113
*	1	1	3	0.042	0.127
*	1	2	1	0.036	0.109
*	1	2	2	0.043	0.129
*	1	2	3	0.039	0.116
*	1	3	1	0.035	0.106
*	1	3	2	0.043	0.129
*	1	3	3	0.038	0.114
*	1	4	1	0.033	0.099
*	1	4	2	0.039	0.117
*	1	4	3	0.034	0.103
*	1	5	1	0.031	0.094
*	1	5	2	0.029	0.087
*	1	5	3	0.030	0.090
*	1	6	1	0.033	0.099
*	1	6	2	0.034	0.103
*	1	6	3	0.037	0.112
*	1	7	1	0.032	0.095
*	1	7	2	0.038	0.114
*	1	7	3	0.053	0.159
*	2	1	1	0.046	0.139

4	1	2	3	5	total
*	2	1	2	0.032	0.097
*	2	1	3	0.035	0.105
*	2	2	1	0.033	0.100
*	2	2	2	0.029	0.087
*	2	2	3	0.033	0.098
*	2	3	1	0.032	0.096
*	2	3	2	0.034	0.101
*	2	3	3	0.034	0.102
*	2	4	1	0.028	0.083
*	2	4	2	0.032	0.097
*	2	4	3	0.034	0.103
*	2	5	1	0.023	0.069
*	2	5	2	0.027	0.082
*	2	5	3	0.036	0.107
*	2	6	1	0.024	0.071
*	2	6	2	0.034	0.101
*	2	6	3	0.028	0.084
*	2	7	1	0.017	0.051
*	2	7	2	0.031	0.093
*	2	7	3	0.028	0.085

Table 11. Analysis of Variance

Source of variation	Sum of squares	Degrees of freedom	Mean Square	F Value	F_{INV}
Adsorbent (A)	1.207142×10^{-3}	1	1.207142×10^{-3}	20.95587	3.954568
pH (B)	3.50285×10^{-4}	2	1.75142×10^{-4}	3.04018	3.105157
Time (C)	1.103603×10^{-3}	6	1.83933×10^{-4}	3.19278	2.208552
Adsorbent vs. pH (AB)	3.62×10^{-6}	2	1.81×10^{-6}	0.03142	3.105157
Adsorbent vs. time (AC)	5.85413×10^{-4}	6	9.7568×10^{-5}	1.69362	2.208552
pH vs. time (BC)	1.117826×10^{-3}	12	9.3152×10^{-5}	1.61696	1.869289
Adsorbent vs. pH vs. time (ABC)	7.32491×10^{-4}	12	6.104×10^{-5}	1.05955	1.869289
Error	4.839223×10^{-3}	84	5.7609×10^{-5}		
Total	5.571714×10^{-3}	125			

APPENDIX B

Case Range: 138 – 144
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 18

Duncan's Multiple Range Test
LSD value = 0.0006631
$s_{\bar{x}}$ = 0.0002357 at alpha = 0.050

Residual Cr^{3+} vs. time (hours) [Factor B]

Original Order			Ranked Order		
Mean	1 = 0.03928	A	Mean	1 = 0.03928	A
Mean	2 = 0.03550	B	Mean	3 = 0.03600	B
Mean	3 = 0.03600	B	Mean	2 = 0.03550	B
Mean	4 = 0.03344	C	Mean	4 = 0.03344	C
Mean	5 = 0.02939	E	Mean	7 = 0.03317	C
Mean	6 = 0.03167	D	Mean	6 = 0.03167	D
Mean	7 = 0.03317	C	Mean	5 = 0.02939	E

Note: A, B, C, D and E are codes indicating significant differences of the data, with A being the highest rank.

Legend:
1 = 0.5 hours
2 = 2 hours
3 = 6 hours
4 = 24 hours
5 = 48 hours
6 = 72 hours
7 = 96 hours

APPENDIX C

Case Range: 147 – 160
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 9

Duncan's Multiple Range Test
LSD value = 0.0009378
s_x = 0.0003333 at alpha = 0.050

Residual Cr^{3+} vs. sample and time (hours) [Factor A x Factor B]

Original Order

Mean	1 = 0.04067	A
Mean	2 = 0.03933	B
Mean	3 = 0.03878	BC
Mean	4 = 0.03544	D
Mean	5 = 0.03011	G
Mean	6 = 0.03489	D
Mean	7 = 0.04089	A
Mean	8 = 0.03789	C
Mean	9 = 0.03167	F
Mean	10 = 0.03322	E
Mean	11 = 0.03144	F
Mean	12 = 0.02867	H
Mean	13 = 0.02844	H
Mean	14 = 0.02544	I

Ranked Order

Mean	7 = 0.04089	A
Mean	1 = 0.04067	A
Mean	2 = 0.03933	B
Mean	3 = 0.03878	BC
Mean	8 = 0.03789	C
Mean	4 = 0.03544	D
Mean	6 = 0.03489	D
Mean	10 = 0.03322	E
Mean	9 = 0.03167	F
Mean	11 = 0.03144	F
Mean	5 = 0.03011	G
Mean	12 = 0.02867	H
Mean	13 = 0.02844	H
Mean	14 = 0.02544	I

Note: A, B, C . . . I are codes indicating significant differences of the data, with A being the highest rank.

Legend:

1 = sand w/o EPS at 0.5 hours
2 = sand w/o EPS at 2 hours
3 = sand w/o EPS at 6 hours
4 = sand w/o EPS at 24 hours
5 = sand w/o EPS at 48 hours
6 = sand w/o EPS at 72 hours
7 = sand w/o EPS at 96 hours
8 = sand w/ EPS at 0.5 hours
9 = sand w/ EPS at 2 hours
10 = sand w/ EPS at 6 hours
11 = sand w/ EPS at 24 hours
12 = sand w/ EPS at 48 hours
13 = sand w/ EPS at 72 hours
14 = sand w/ EPS at 96 hours

APPENDIX D

Case Range: 163 – 165
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 42

Duncan's Multiple Range Test
LSD value = 0.0004341
$s_{\bar{x}}$ = 0.0001543 at alpha = 0.050

Residual Cr^{3+} vs. pH [Factor C]

Original Order			Ranked Order		
Mean	1 = 0.03183	C	Mean	3 = 0.03583	A
Mean	2 = 0.03452	B	Mean	2 = 0.03452	B
Mean	3 = 0.03583	A	Mean	1 = 0.03183	C

Note: A, B and C are codes indicating significant differences of the data, with A being the highest rank.

Legend:
1 = pH 3
2 = pH 6
3 = pH 9

APPENDIX E

Case Range: 168 – 173
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 21

Duncan's Multiple Range Test
LSD value = 0.0006139
s_x = 0.0002182 at alpha = 0.050

Residual Cr^{3+} vs. sample and pH [Factor A x Factor C]

Original Order		Ranked Order	
Mean 1 = 0.03467	C	Mean 3 = 0.03910	A
Mean 2 = 0.03771	B	Mean 2 = 0.03771	B
Mean 3 = 0.03910	A	Mean 1 = 0.03467	C
Mean 4 = 0.02900	F	Mean 6 = 0.03257	D
Mean 5 = 0.03133	E	Mean 5 = 0.03133	E
Mean 6 = 0.03257	D	Mean 4 = 0.02900	F

Note: A, B, C, D, E and F are codes indicating significant differences of the data, with A being the highest rank.

Legend:
1 = sand w/o EPS at pH 3
2 = sand w/o EPS at pH 6
3 = sand w/o EPS at pH 9
4 = sand w/ EPS at pH 3
5 = sand w/ EPS at pH 6
6 = sand w/ EPS at pH 9

APPENDIX F

Case Range: 176 – 196
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 6

Duncan's Multiple Range Test
LSD value = 0.001149
$s_{\bar{x}}$ = 0.0004082 at alpha = 0.050

Residual Cr^{3+} vs. time (hours) and pH [Factor B x Factor C]

Original Order		Ranked Order	
Mean 1 = 0.04417	A	Mean 1 = 0.04417	A
Mean 2 = 0.03500	DEF	Mean 21 = 0.04067	B
Mean 3 = 0.03867	C	Mean 3 = 0.03867	C
Mean 4 = 0.03483	DEFG	Mean 8 = 0.03833	C
Mean 5 = 0.03600	D	Mean 5 = 0.03600	D
Mean 6 = 0.03567	DE	Mean 9 = 0.03600	D
Mean 7 = 0.03367	GHI	Mean 6 = 0.03567	DE
Mean 8 = 0.03833	C	Mean 11 = 0.03567	DE
Mean 9 = 0.03600	D	Mean 2 = 0.03500	DEF
Mean 10 = 0.03033	J	Mean 4 = 0.03483	DEFG
Mean 11 = 0.03567	DE	Mean 20 = 0.03450	EFG
Mean 12 = 0.03433	FG	Mean 12 = 0.03433	FG
Mean 13 = 0.02717	K	Mean 17 = 0.03400	FGH
Mean 14 = 0.02817	K	Mean 7 = 0.03367	GHI
Mean 15 = 0.03283	HI	Mean 15 = 0.03283	HI
Mean 16 = 0.02833	K	Mean 18 = 0.03267	I
Mean 17 = 0.03400	FGH	Mean 10 = 0.03033	J
Mean 18 = 0.03267	I	Mean 16 = 0.02833	K
Mean 19 = 0.02433	L	Mean 14 = 0.02817	K
Mean 20 = 0.03450	EFG	Mean 13 = 0.02717	K
Mean 21 = 0.04067	B	Mean 19 = 0.02433	L

Note: A, B, C . . L are codes indicating significant differences of the data, with A being the highest rank.

Legend: 1, 4, 7, 10, 13, 16, 19 = samples at pH 3 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96
2, 5, 8, 11, 14, 17, 20 = samples at pH 6 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96
3, 6, 9, 12, 15, 18, 21 = samples at pH 9 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

APPENDIX G

Case Range: 199 – 240
Variable 5: Residual Cr^{3+}
Function: RANGE

Error Mean Square = 1.000e-006
Error Degrees of Freedom = 82
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 0.001624
$s_{\bar{x}}$ = 0.0005774 at alpha = 0.050

Residual Cr^{3+} vs. sample, time (hours) and pH [Factor A x Factor B x Factor C]

Original Order

Mean	1 = 0.04200	C	
Mean	2 = 0.03767	DE	
Mean	3 = 0.04233	C	
Mean	4 = 0.03633	EFG	
Mean	5 = 0.04300	C	
Mean	6 = 0.03867	D	
Mean	7 = 0.03533	GHI	
Mean	8 = 0.04300	C	
Mean	9 = 0.03800	DE	
Mean	10 = 0.03300	KLMNO	
Mean	11 = 0.03900	D	
Mean	12 = 0.03433	HIJK	
Mean	13 = 0.03133	OPQ	
Mean	14 = 0.02900	RS	
Mean	15 = 0.03000	QR	
Mean	16 = 0.03000	KLMNO	
Mean	17 = 0.03433	HIJK	
Mean	18 = 0.03733	DEF	
Mean	19 = 0.03167	NOPQ	
Mean	20 = 0.03800	DE	
Mean	21 = 0.05300	A	
Mean	22 = 0.04633	B	
Mean	23 = 0.03233	LMNOP	
Mean	24 = 0.03500	GHIJ	
Mean	25 = 0.03333	JKLMN	
Mean	26 = 0.02900	RS	
Mean	27 = 0.03267	KLMNOP	

Mean 28 = 0.03200	MNOP
Mean 29 = 0.03367	IJKLM
Mean 30 = 0.03400	HIJKL
Mean 31 = 0.02767	S
Mean 32 = 0.03233	LMNOP
Mean 33 = 0.03433	HIJK
Mean 34 = 0.02300	T
Mean 35 = 0.02733	S
Mean 36 = 0.03567	FGH
Mean 37 = 0.02367	T
Mean 38 = 0.03367	IJKLM
Mean 39 = 0.02800	S
Mean 40 = 0.01700	U
Mean 41 = 0.03100	PQ
Mean 42 = 0.02833	RS

Ranked Order

Mean 21 = 0.05300	A
Mean 22 = 0.04633	B
Mean 8 = 0.04300	C
Mean 5 = 0.04300	C
Mean 3 = 0.04233	C
Mean 1 = 0.04200	C
Mean 11 = 0.03900	D
Mean 6 = 0.03867	D
Mean 9 = 0.03800	DE
Mean 20 = 0.03800	DE
Mean 2 = 0.03767	DE
Mean 18 = 0.03733	DEF
Mean 4 = 0.03633	EFG
Mean 36 = 0.03567	FGH
Mean 7 = 0.03533	GHI
Mean 24 = 0.03500	GHIJ
Mean 17 = 0.03433	HIJK
Mean 33 = 0.03433	HIJK
Mean 12 = 0.03433	HIJK
Mean 30 = 0.03400	HIJKL
Mean 29 = 0.03367	IJKLM
Mean 38 = 0.03367	IJKLM
Mean 25 = 0.03333	JKLMN
Mean 10 = 0.03300	KLMNO
Mean 16 = 0.03000	KLMNO
Mean 27 = 0.03267	KLMNOP

Mean 23 = 0.03233	LMNOP
Mean 32 = 0.03233	LMNOP
Mean 28 = 0.03200	MNOP
Mean 19 = 0.03167	NOPQ
Mean 13 = 0.03133	OPQ
Mean 41 = 0.03100	PQ
Mean 15 = 0.03000	QR
Mean 26 = 0.02900	RS
Mean 14 = 0.02900	RS
Mean 42 = 0.02833	RS
Mean 39 = 0.02800	S
Mean 31 = 0.02767	S
Mean 35 = 0.02733	S
Mean 37 = 0.02367	T
Mean 34 = 0.02300	T
Mean 40 = 0.01700	U

Note: A, B, C . . U are codes indicating significant differences of the data, with A being the highest rank.

Legend:

1, 4, 7, 10, 13, 16, 19 = sand w/o EPS at pH 3 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

2, 5, 8, 11, 14, 17, 20 = sand w/o EPS at pH 6 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

3, 6, 9, 12, 15, 18, 21 = sand w/o EPS at pH 9 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

22, 25, 28, 31, 34, 37, 40 = sand w/ EPS at pH 3 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

23, 26, 29, 32, 35, 38, 41 = sand w/ EPS at pH 6 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

24, 27, 30, 33, 36, 39, 42 = sand w/ EPS at pH 9 with time intervals of 0.5, 2, 6, 24, 48, 72, and 96

APPENDIX H

Dilution of wastewater sample

Initial concentration: 49, 400 ppm Cr^{3+}
dilute to 1.0 ppm Cr^{3+}

$$C_1V_1 = C_2V_2$$

where:

C_1 = initial Cr^{3+} concentration of the wastewater
C_2 = final concentration of desired wastewater sample
V_1 = volume of wastewater with initial Cr^{3+} concentration needed to attain 1.0 ppm Cr^{3+} concentration
V_2 = volume of triple distilled water needed to attain 1.0 ppm Cr^{3+} concentration

sample computation:

$$C_1V_1 = C_2V_2$$
$$(49, 400 \text{ ppm } Cr^{3+})\ V_1 = (1.0 \text{ ppm } Cr^{3+})\ (1000\text{mL})$$
$$V_1 = 0.02 \text{ mL}$$

APPENDIX I

STANDARD ATOMIC ABSORPTION CONDITIONS

Cr DETERMINATION BY FLAME ATOMIZATION

Cr
CHROMIUM

INSTRUMENTAL PARAMETERS

Light Source:	Hollow Cathode	IL Lamp No.:	62934	
Lamp Current:	6	mA		
Wavelength:	357.9	nm		
Slit Width:	160	µm	Bandpass:	0.5 nm
Burner Head:	Single Slot	IL No.:	43005-02	

Flame Description:

Nitrous Oxide-Acetylene

Fuel Rich, Red Cone 20 mm High

Photomultiplier Voltage (HV):

Once the lamp current, wavelength, and slit width have been set, adjust the HV control until the log intensity meter reads between 0.2 and 0.8 Volt.

NOTE:
1. For a more thorough discussion of the above instrumental parameters, please consult the appropriate heading in this manual.
2. The recommended lamp current is for a single element lamp.

Sensitivity

The sensitivity (at 0.0044 Absorbance = 1% Absorption) is about 0.06 µg/ml for the instrumental parameters described above.

A standard containing 1 µg/ml of Cr will give a reading of approximately 0.1 A.

Linear Range

The working range for Cr is linear up to a concentration of approximately 5 µg/ml (when using an aqueous solution and the instrumental parameters described above).

STANDARD ATOMIC ABSORPTION CONDITIONS

Cr DETERMINATION BY FLAME ATOMIZATION

Preparation of Standard Solution:

Dissolve 1.0000 gram of metallic chromium in 50 ml of 1:1 hydrochloric acid with gentle heating. Cool and dilute quantitatively to a volume of 1 liter. Final concentration is 1000 µg/ml Cr.

Alternate Analytical Lines:

Wavelength (nm)	Approximate Sensitivity (µg/ml)	Slit Width (µm)/SBW (nm)
357.9	0.06	160/0.5
359.4	0.10	160/0.5
360.5	0.13	160/0.5
425.4	0.20	160/0.5
427.5	0.23	160/0.5
428.9	0.50	160/0.5
520.8	12.0	40/0.15
520.5	30.0	40/0.15

Interferences:

Chromium absorption is suppressed by cobalt, iron and nickel in an air-acetylene flame, especially in the presence of perchloric acid. The signal suppression can also be overcome by use of a nitrous oxide-acetylene flame. No ionization suppressant is necessary.

In addition, the iron interference can be minimized by the addition of 2% (w/v) NH_4Cl to the samples and standard solutions.

Several investigators have found interference from copper, barium, aluminum, magnesium, and calcium in an air-acetylene flame. The extent of this interference is strongly dependent on flame stoichiometry. Use of a nitrous oxide-acetylene flame will eliminate the interference.

Flame Emission:

The most sensitive emission wavelength for Cr is 425.4 nm. A nitrous oxide-acetylene flame having a red feather height of 8 mm is recommended. Alternate emission wavelengths for Cr are 429.0 nm, 427.5 nm, 360.5 nm, 359.4 nm and 357.9 nm, respectively.

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