Areca nut as a Potential Bio-adsorbent for Remediation of Chromium

[1]Ankitha R, [2]Prakruthi S, [3]S Sneha, [4] Seema Tharannum
[1], [2], [3], [4] Department of Biotechnology, PES Institute of Technology, Bangalore-560085
[1] ank653@gmail.com, [2] prakruthishivakumar@gmail.com, [3] snehasrinivas162gmail.com, [4] seema@pes.edu

Abstract: **— Bioremediation is one of the leading sought after techniques for heavy metal removal and to prevent it's contamination in the effluent discharge hence avoiding various toxicological effects. Heavy metals such as chromium have a number of applications such as leather tanning, organochemicals, fertilizers etc. Once chromium is introduced into the environment, it exists in two different oxidation states, Cr (III) and Cr (VI). The trivalent form is relatively innocuous but hexavalent chromium is toxic, carcinogenic and mutagenic in nature, highly mobile in soil and aquatic system and is also a strong oxidant capable of being adsorbed by the skin. In our present study, areca nut husk powder of two different sieve sizes 250µm and 719µm were used in comparison with the same along with PES B (Bacillus amyloliquefaciens) at 37°C to form a biofilm. Various concentrations of chromium i.e., 0, 50, 100, 150, 200, 250 ppm were treated with the adsorbent components. The amount of chromium from the sample was estimated using DPC method. It was found to reduce chromium to >50% in case of adsorbent with biofilm and up to 50% in case of adsorbent without biofilm for 50 ppm concentration showing maximum capacity. Hence, adsorbent with biofilm has a greater potential compared to adsorbent without biofilm for Cr (VI) bioremediation from polluted effluents.**

Keywords**—Hexavalent chromium, PES B (Bacillus amyloliquefaciens), areca nut husk, DPC method, biofilm, bioremediation.**

I. INTRODUCTION

With increasing population there is an urgent need to meet the ends hence leading to rapid industrialization and urbanization and having deleterious effects on the environment hence affecting day to day activities. Huge discharge of chemicals especially from the industries into the water bodies has become the prime concern. Mainly these industrial discharges comprise of heavy metals. These heavy metals are improperly handled before discharging acting devastating effects on both human and environment (Chimezie Anyakora *et al*., 2011).

Heavy metals such as Lead, Mercury, Cadmium, Nickel, Chromium and other toxic organic chemicals or phenolic compounds discharged from pharmaceutical industries are known to affect the surface and ground waters. Chromium is a major pollutant present in the environment and also highly toxic in nature, it is mainly found in dyes, chromium plating industries, leather tanning industries, pigments and wood preserving units (Chimezie Anyakora *et al*., 2011).

Discharge from chromium containing effluents from electroplating, leather tanning, textile dyeing and a biocide and cooling waters of power plants contain approx. 100mg/L concentration which is much higher than the permissible limit 0.05-1.0mg/L (Bai *et al*., 2000).

Conventional physico-chemical processes which are used for the Cr (VI) removal include precipitation and caustic soda coagulation.

The other processes are reverse osmosis, ion-exchange, electrolysis which are expensive, ineffective removal of metal ions at lower concentration, high energy process and also result in generation of large amounts of sludge leading to their discharge problems hence resulting in secondary pollution (Bai *et al*., 2000).

The above mentioned methods have its own merits and demerits (Hima Karnika Alluri *et al*., 2007) the methods suggested are time consuming and needs expertise. Hence Eco-friendly processes therefore need to be developed to clean-up the environment without creating harmful waste by-products.

Bioremediation is a type of remediation technique in which toxic metals are removed using living organisms. Bioaccumulation is the widely used bioremediation technique in which heavy metals enter into living organisms through Food or proximity to emission sources. These Organisms tend to bio accumulate and are store faster than they excrete (Chimezie Anyakora *et al*., 2011). Though the technique is

widely used, has its own pros and cons.

According to Bai *et al*., 2000 biosorption refers to different modes of non-active metal uptake by microbial; biomass where metal sequestration of cells can take place through ion-exchange, adsorption, Complexation, co-ordination etc. Biosorption has provided an alternative treatment of industrial wastewaters. It involves the use of natural substrates such as agricultural residues forestry waste products, microorganisms (Senthil Kumar S *et al*., 2000).

Selection of a proper biosorbent is necessary for successful biosorption process. Pre-treatment and immobilization are done to increase the efficiency of metal uptake, desorption results in reuse of biosorbent (Hima Karnika Alluri *et al.*, 2007).

In our present study, biomass considered to be wastes were used as adsorbents because of its high availability and low-cost. Low cost adsorbent used was areca nut husk powder and the same with the bacterial strains forming a biofilm were used as adsorbents in order to compare the efficiency. Although areca nut has many uses its husk is not commercially used in large quantities hence it is considered a waste and disposed to the environment without any further usage**.**

II. MATERIALS AND METHOD

A. Glassware and Instruments

The glassware used were

- Volumetric flasks
- Conical flasks
- Pipettes
- Measuring cylinders
- Test tubes

All the glassware used was supplied by Borosil.

The instruments used were

- Hot air oven (Servewell Instruments Pvt. Ltd)
- Mixer (Kenstar Smart)
- Bacteriological incubator (Servewell Instruments Pvt. Ltd)
- Weighing balance (Sartorius)
- Rotary shaker (Secor India)
- Orbital shaker (Servewell Instruments Pvt. Ltd)
- Micropipette (Nichipet EX)
- Cooling centrifuge (Remi)
- Colorimeter (Labtronics)
- Autoclave

B. Chemicals and Reagents

- Potassium dichromate
- 1,5 diphenyl carbazide
- Ethanol
- Sulphuric acid

All the chemicals and reagents used were of analytical grade.

C. Sampling

The adsorbent, areca nut husks were brought from a village near Tumkur.

They were washed with tap water, later washed with distilled water, sun dried initially and then later dried in hot air oven at 60ºC for 1 day. These areca nut husks were cut into small pieces and later grounded into a powder using a mixer. Using two sieves, 250 microns and 719 microns, they were separated into two different particle sizes.

The sample was further washed with Luke warm water 2-3 times to remove any color imparted by these particles.

Fig. 1: Dried areca nut husk

Fig. 2: Powdered areca nut husks

D. Growth and Processing of the cells

All the glassware's were initially sterilized at 121 ^{0}C, 15 psi for 15mins. Glassware's were allowed to cool and used.

To grow the bacterial strains LB broth was prepared in four different sterilized flasks. The flasks were closed tightly using a cotton plug and wrapped using a newspaper and sterilized by autoclaving at 121 ^{0}C, 15 psi for 15mins. The contents were allowed to cool after sterilizing. A loopful of inoculum PES B: *Bacillus amyloliquefaciens* was inoculated into the broth under aseptic conditions.

The contents in the flask were thoroughly mixed and incubated on shaker at 120 rpm and 37°C for 2 days.

E. Chromium (VI) Estimation

Standard chromium solution of 100mg/L was prepared using potassium dichromate.

Optical density of known standards was recorded and a graph of absorbance at 540 nm v/s amount of chromium in micrograms was plotted.

Chromium standard was prepared by adding 0.283 mg potassium dichromate to 100ml of distilled water to get a concentration of 100mg/L. Different aliquot of standard chromium was taken in series of clean test tubes and was made up to 5ml with distilled water. 250 µl of DPC reagent was added and OD was read at 540nm.Graph of adsorption at 540nm v/s amount of chromium (µg) was plotted.

F. Batch Studies

The batch studies of different concentrations were carried out using 250ml conical flasks rotating continuously at 120 rpm at 37^{0}C in a rotary/orbital shaker.

Batch studies were divided into two categories, one with the biofilm layer and another without the biofilm layer. Twenty four 250mL conical flasks were taken and divided into two groups containing 12 conical flasks each for each sieve size (250 microns and 719 microns), further these 12 conical flasks were subdivided into 6 conical flasks for biofilm and rest 6 for without biofilm layer.

The flasks containing biofilm had 2g of the adsorbent mixed with 25mL of the PES B (*Bacillus amyloliquefaciens*) culture and allowed to grow for 24hrs. Later this solution was drained out and the components were mixed with 100mL of different concentrations of standard chromium solution i.e., 0, 50,100,150,200,250 ppm concentration.

The flasks without biofilm contained 2g of the adsorbent mixed with 100mL of 0, 50,100,150,200,250 ppm concentration of standard chromium. These flasks were kept in an orbital shaker at 37 ^{0}C and 120 rpm. At regular intervals of 24, 48,72,96.hrs, about 1mL of solution was collected and centrifuged at 6000 rpm for 5min at 4 ^{0}C. This supernatant was used for estimation of chromium by DPC method.

G. Scanning Electron Microscopy (SEM) And Energy Dispersive Spectroscopy (EDS)

A small piece of sample was cleaned by ultra-sonication and alcohol washing followed by drying in front of an air heater. After drying the sample was stuck to the SEM setup using two sided conductive sticker.

After being coated with gold, the entire setup was placed in a pre-vacuum chamber. With the suitable magnification and position, the sample was viewed and corresponding images were captured. EDS, involving the generation of X-ray spectrum from the entire scan area of the SEM allows the identification of particular elements and their relative proportions.

Y-axis of EDS represents the number of X-rays received and processed by the detector while X-axis shows the energy level of those counts. SEM and EDS was obtained of Arecanut husk powder before and after adsorption of chromium (Seema Tharannum *et al.*, 2015).

III. RESULTS AND DISCUSSION

A. Estimation of Chromium

The hexavalent chromium is determined calorimetrically by reaction with diphenylcarbazide in acid solution. Absorbance at 540nm was found to increase with increase in the concentration of chromium.

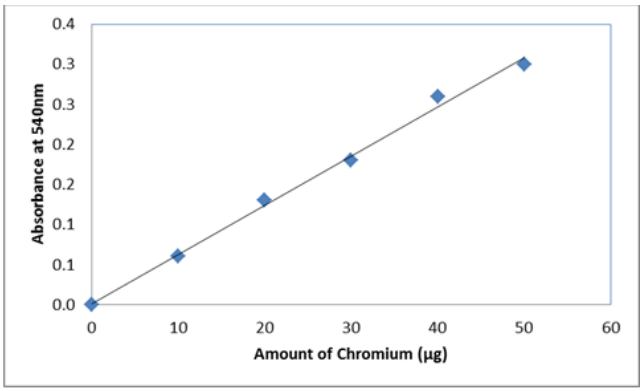


Fig. 4: Absorption at 540nm v/s Amount of Chromium

B. Metal Biosorption Studies

1. Batch Studies Without Biofilm

In the batch studies without biofilm, the ability of areca nut husk powder of different particle sizes i.e., 250µm and 719µm to adsorb chromium was evaluated. The amount of chromium decreased from day 1 to day 7.

*a.**Particle Size - 719µm***

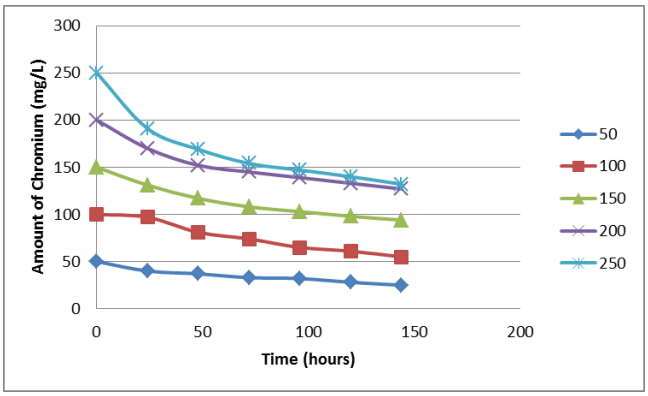


Fig. 5: Amount of Chromium v/s Time

The decrease in amount of chromium with respect to time is depicted above. The amount of chromium was seen to reduce from day 1 to day 7 in all the samples containing different concentrations of chromium. The sample containing 50ppm of chromium was seen to reduce to a concentration of 25ppm in 7 days. The other samples were seen to reduce to almost half of the concentration.

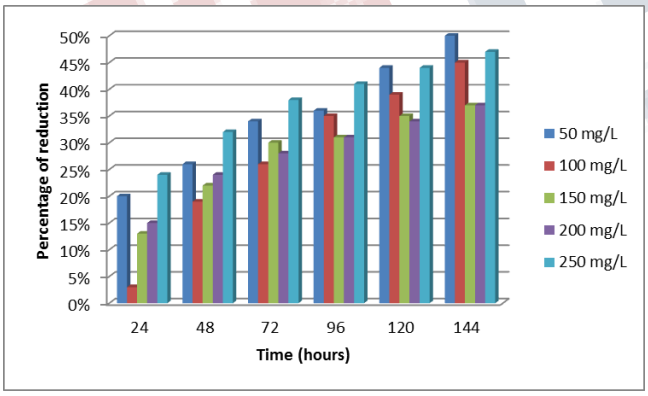


Fig. 6: Percentage of Reduction v/s Time

The percentage of reduction with respect to time is depicted above. The percentage of reduction was found to be 50% for 50ppm sample. The other samples showed percentage of reduction of <50%.

*b.**Particle Size - 250µm***

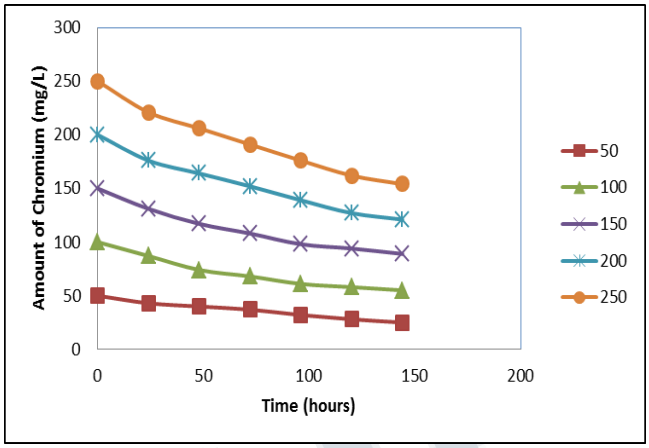


Fig. 7: Amount of Chromium v/s Time

The decrease in amount of chromium with respect to time is depicted above. The amount of chromium was seen to reduce from day 1 to day 7 in all the samples containing different concentrations of chromium. The sample containing 50ppm of chromium was seen to reduce to a concentration of 25ppm in 7 days. The other samples were seen to reduce to almost half of the concentration.

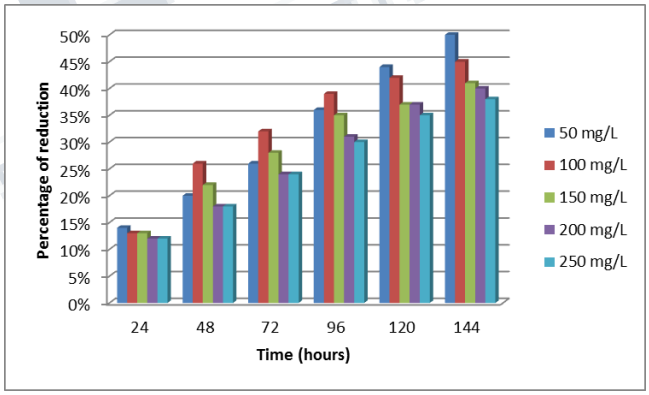


Fig. 8: Percentage of Reduction v/s Time

The percentage of reduction with respect to time is depicted above. The percentage of reduction was found to be 50% for 50ppm sample. The other samples showed percentage of reduction of <50%.

2.Batch Studies With Biofilm

In the batch studies with biofilm, the ability of areca nut

husk powder of different particle sizes i.e., 250µm and 719µm, along with the biofilm formed by the organism PES B (*Baccilus amyloliquefaciens*) to adsorb chromium was evaluated. The amount of chromium decreased from day 1 to day 7.

*a.**Particle Size -719µm***

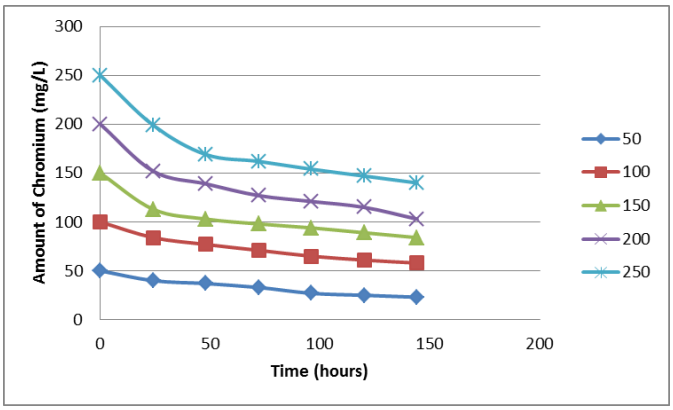


Fig. 9: Amount of Chromium v/s Time

The decrease in amount of chromium with respect to time is depicted above. The amount of chromium was seen to reduce from day 1 to day 7 in all the samples containing different concentrations of chromium. The sample containing 50ppm of chromium was seen to reduce to a concentration of 23ppm in 7 days. The other samples were seen to reduce to almost half of the concentration.

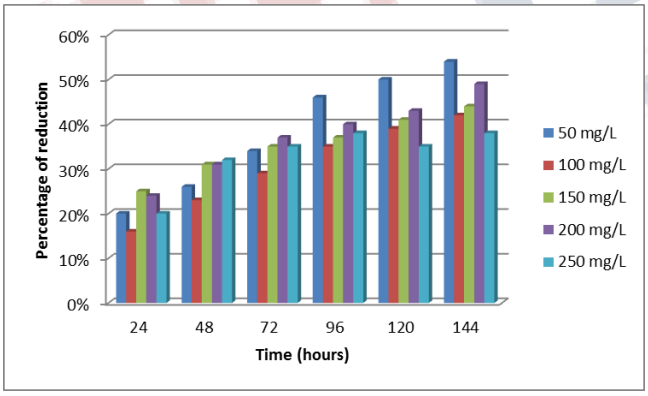


Fig. 10: Percentage of Reduction v/s Time

The percentage of reduction with respect to time is depicted above. The percentage of reduction was found to be 54% for 50ppm sample. The other samples showed percentage of reduction of <50%.

*b.**Particle Size - 250µm***

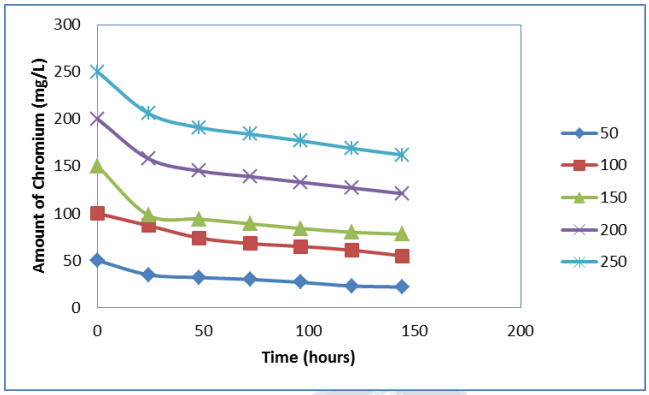


Fig. 11: Amount of Chromium v/s Time

The decrease in amount of chromium with respect to time is depicted above. The amount of chromium was seen to reduce from day 1 to day 7 in all the samples containing different concentrations of chromium. The sample containing 50ppm of chromium was seen to reduce to a concentration of 22ppm in 7 days. The other samples were seen to reduce to almost half of the concentration.

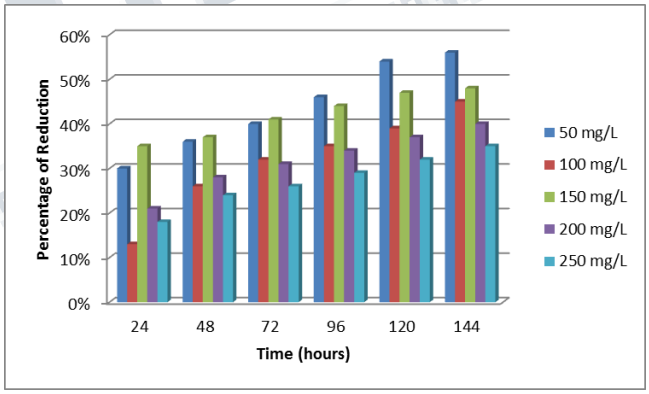
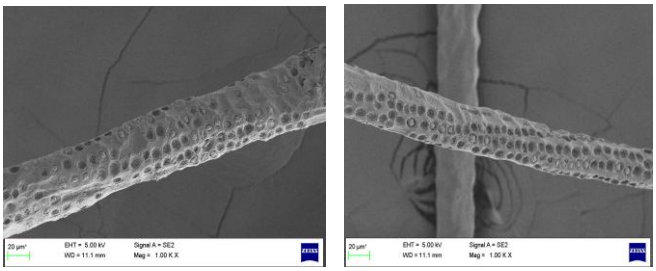


Fig. 12: Percentage of Reduction v/s Time

The percentage of reduction with respect to time is depicted above. The percentage of reduction was found to be 56% for 50ppm sample. The other samples showed percentage of reduction of <50%.

3. *SEM Images*

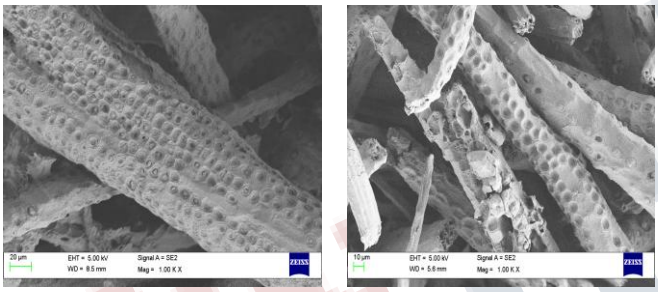


(13) (14)

Fig. 13: SEM Images of 250µm control sample without Biofilm

Fig. 14: SEM Images of 250µm test sample without Biofilm

The SEM image of the control sample shows fewer pores than the test sample. This indicates that the chromium uptake is due to the opening of the pores.



(15) (16)

Fig. 15: SEM Images of 250µm control sample with Biofilm

Fig. 16: SEM Images of 250µm test sample with Biofilm

The SEM images of the samples with biofilm show the presence of a thin layer around the husks. Further, the test sample has bigger pores than the control sample which suggests the uptake of chromium by the bio-adsorbent.

4. EDS Images

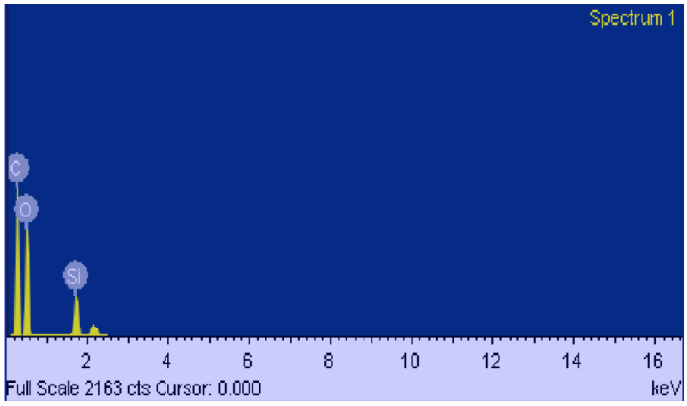


Fig. 17: EDS Image of biofilm control sample

Fig. 17 shows the presence of carbon, oxygen and silicon in the control sample.

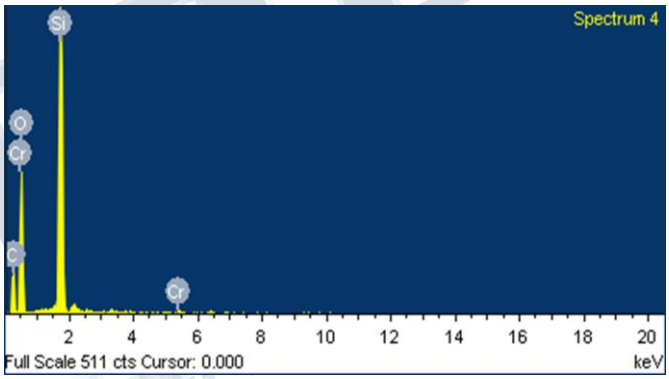


Fig. 18: EDS Image of biofilm test sample

Fig. 18 shows the presence of carbon, oxygen, silicon and chromium in the control sample. Hence indicating that carbon, oxygen, silicon forms the native elements as it is visible in both the cases i.e, before and after adsorption mechanism, presence of chromium apart from the native elements indicates that areca nut husks have adsorbed chromium from the solution.

IV. CONCLUSION

Heavy metal such as chromium is known to cause irreversible damage to the ecosystem and various serious problems such as carcinogenicity and immunogenicity in humans. Hence a bioremediation technique was adopted to overcome conventional chemical treatment as well as treatment of industrial effluents containing heavy metal.

The research substantiates the use of low cost adsorbent like areca nut husk powder and the combination of biofilm with the areca nut husk powder in adsorbing and

hence remediating chromium (VI). Therefore areca nut husk powder can be termed as a potent low cost adsorbent for chromium (VI) removal.

A batch study was performed under two parameters, one to study the influence of particle size and another to study the influence of a biofilm formation using PES B (*Bacillus amyloliquefaciens*) on the adsorption of chromium (VI) on to the adsorbent.

Two particle sizes (250 and 719 µm) were considered under study, it was shown that there was increase in chromium adsorption with decrease in particle size. And also there was increase in chromium adsorption in the presence of biofilm.

About 50% adsorption was shown in 7days with areca nut husk and about 56% of chromium adsorption was shown in 7 days with areca nut husk in combination with the biofilm.

The result was analyzed using SEM, in which a slight slimy layer developed was visible. Images also showed that the pores appear to be open after the chromium adsorption in comparison to the one before adsorption.

The result analyzed using EDS suggests that there is chromium adsorption by the sample.

ACKNOWLEDGEMENT:

The authors extend gratitude to the management of PES Institute of Technology, Bangalore for encouragement and research facilities to carry out this Project

REFERENCES

[1] Ahalya N, T V Ramachandra, R D Kanamadi (2003), "Biosorption of heavy metals: Review", *Research journal of chemistry and environment*, Vol 7(4), 71-79.

[2] APHA: Standard methods for the examination of water and wastewater, 21st Edn, Washington D C, (2005)

[3] Asha Latha Singh (2007), "Removal of Chromium from waste water with the help of microbes", *e- Journal of science and technology*, Vol 21, 361-370.

[4] Bai, R S, Abraham, T E, (2001), "Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigrificans"*, *Bioresources Technology*, Vol 79, 73-81.

[5] Chimezie Anyakora, Kenneth Nwaeze, Olufunsho Awovedele, Chinwe Nwadike, Mohsen Arbabi and Herbert Coker (2011), "Concentrations of heavy metals in some pharmaceutical effluents in Lagos, Nigeria", *Journal of Environmental Chemistry and ecotoxicity*, 3(2), 25-31.

[6] Directorate of Arecanut and Spices development, Ministry of Agriculture, Government of India. [online] Available at: http://dasd.gov.in/index.php/arecanut.html?start=7

[7] Hima Karnika Alluri, Srinivasa Reddy Ronda, Vijaya Saradhi Settalluri, Jayakumar Singh (2007), "Biosorption: an ecofriendly alternative for heavy metal removal", *African journal of biotechnology*, 6(25), 2924-2931.

[8] Holmes A L, Wise S S and Wise J P (2008), "Carcinogenicity of hexavalent chromium", *Indian journal of medical research*, Vol 128, 353-378.

[9] Jianlong Wang, Can Chen (2009), "Biosorbents for heavy metals removal and their future", *Journal of biotechnology advances*, Vol 22, 196-222.

[10] Mehra R, Juneja M, (2003), "Adverse health effects in workers exposed to trace / toxic metals at workplace", *Indian Journal of Biochemistry and Biophysics*, 40, 131-135.

[11] Mohammad Arifur Rahman, Md. Abu Hasan, Md. Abdus Salam, Abdus Salam, Nur-E-Alam Siddique and A. M. Shafiqul Alam (2012) "Betel-nut Peel as an Adsorbent in the Removal of Cd, Cr and Pb from Aqueous Solutions", *Pak. J. Anal. Environ. Chem.*, Vol. 13, 2137– 2147.

[12] Nilanjana Das, P Kartika, R Vimala and V Vinodhini (2007), "Use of natural products as biosorbents of heavy metals", *Journal of environmental biology*, Vol 3, 55-64.

[13] Nilanjana Das, Laskshmi V, Geetanjali Basak, Jaseetha Abdul Salam, Evy Alice Abigail M (2012), "Application of biofilms on remediation of pollutants-An overview", *Journal of Microbiology and Biotechnology Research*, 2(5), 783-790.

[14] Paresh Chakravarthy, N Sen Sharma, H P Sharma (2010). "Biosorption of cadmium (II) from aqueous solution using heartwood powder of *Areca catechu"*, *Chemical Engineering Journal*, 162(3), 949–955.

[15] Sarabjeet Singh Ahluwalia (2014), "Microbial removal of Hexavalent and scale up potential", *International Journal of Current Microbiology and Applied Sciences*, 3(6), 383-398.

[16] Seema Tharannum, Nandini.V, Shruthi.P.T, V. Krishna Murthy (2015), "Study of Eggshell Membrane as a Potent Adsorbent of Chromium", *International Journal of Research*, 2(2), 1262-1269.

[17] Senthil Kumar S, Bharathi S, Nithyanandhi D, and Subburam V (2000), "Biosorption of toxic heavy metals from aqueous solutions", *Bioresource Technology*, Vol. 75, 163-165.

[18] Shankar Congeevaram, Sridevi Dhanarani, Joohong Park, Michael Dexilin, Kaliannan Thamaraiselvi (2007)," Biosorption of Chromium and nickel Heavy metal resistant fungal and bacterial isolates", *Journal of hazardous material*, Vol 146, 270-277.

[19] Suranjana Arora Ray and Manas Kanti Ray (2009), "Bioremediation of heavy metal toxicity-with special reference to chromium", *Al Ameen J Med Sci*., 2(2), 57-63.

[20] Wendy-Louise Smith (2001), "Hexavalent Chromium reduction and precipitation by sulphate–reducing bacterial biofilms", *Environmental Geochemistry and Health*, Vol. 23, 297-300.

[21] Xiao-ming Li, Wei Zheng, Dong-bo Wang, Qi Yang, Jian-bing Cao, Xiu Yue, Ting-ting Shen, Guang-ming Zeng (2010), "Removal of Pb (II) from aqueous solutions by adsorption onto modified areca waste: Kinetic and thermodynamic studies", *Desalination* 258, 148–153.