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Method for the recovery of Cr and Co species from effluents using agricultural adsorbent – immobilized *E. coli*, *S. aureus* and *S. typhi* isolates and FAAS detection

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ABSTRACT

Microbial chromium and cobalt reduction was investigated for application in their recovery from industrial wastewater using flame atomic absorption technique. This paper presents the development of a routine method for the recovery of Cr and Co species in microbial-treated industrial wastewater using agricultural adsorbents and silica gel. *E. coli*, *S. typhi* and *S. aureus* were used in reducing these heavy metals. Results indicate that the palm kernel shell charcoal exhibited a good recovery capacity in the presence of the bacterial strains. Recovery rates of Cr in the activated charcoal and a bacterial optimum growth at pH 7.2 – 7.4 and 37 °C are 99.72% (*S. typhi*), 99.61% (*E. coli*) and 99.64% (*S. aureus*), while that of silica gel are 98.08% (*S. typhi*), 98.79% (*E. coli*) and 98.02% (*S. aureus*). The recovery of Co using the palm kernel shell charcoal is 99.71% (*S. typhi*), 99.58% (*E. coli*) and 99.60% (*S. aureus*). The results using the silica gel are 98.36% (*S. typhi*), 98.82% (*E. coli*) and 99.42% (*S. aureus*). In comparison to silica gel the palm kernel shell exhibited a higher recovery rate of Cr and Co in the presence of the bacterial strains.

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INTRODUCTION

The environmental effects of heavy metals is a global concern due to the fact that they are non-biodegradable and causes acute toxicity and threat to human life. Metal exploitation has lead to an alarming increase of metal pollution due to their discharge to the environment without proper treatment. This anthropogenic activity has increased metal pollution due to its high rate of dispersion, solubility, mobility in aquatic systems and biomagnifications through food chain. Over

exposure to high levels of heavy metals has been linked to inherent health effects such as birth defects, skin lesions, retardation of growth, lung infections, liver disabilities, kidney damage and cancer (Hughes and Poole, 1989; Poole and Gadd, 1989).

Cobalt and chromium are one of the most important trace nutrients since they are present in vitamin B₁₂ and involved in glucose metabolism respectively. As an essential metal Co functions as catalysts for biochemical reactions, stabilizers of protein

structures, cell walls and maintain osmotic balance in microorganisms (Ji and Silver, 1995). Cr as a transition metal has oxidation states from Cr(-II) to Cr(+VI). The two environmentally important valence states are Cr(III) and Cr(VI). They have contrasting impacts on the environment and health (Compton et al., 2004). Trivalent Cr is relatively harmless and is an essential trace element in mammalian metabolism. Hexavalent Cr on the other hand is much more toxic due to its high water solubility and mobility. Under normal physiological conditions, Cr⁶⁺ interacts spontaneously with the intracellular reductants (e.g. ascorbate and glutathione) to generate the short lived intermediates Cr⁵⁺, Cr⁴⁺, free radicals and end product Cr³⁺ (Costa and Klein, 2006). The process reduces reactive oxygen species (ROS) that easily combines with DNA-protein complexes (Cheung et al., 2006).

Cobalt is a plant bio-stimulant similar to molybdenum because it is required by nitrogen-fixing bacteria, especially on the root nodules of legumes. In root nodules, Co is associated with the production of cobamide compounds by the rhizobia. Cobamide co-enzymes are required for metabolic processes of the bacteroids (Peterson and Girling, 1981). Bacteria belonging to different taxonomic and physiological groups representing members of the genera *Pseudomonas*, *Brevibacterium*, *Rhodopseudomonas*, and *Lactococcus* have been reported to form intracellular Co – and Cr containing magnetic inclusions (Ariskina et al., 2004).

Staphylococcus aureus is a common human pathogen associated with a number of diseases. Resistance to cadmium, mercury, antimony and arsenic in staphylococci is plasmid encoded, while staphylococcal strains without plasmid show resistance to nickel and cobalt. This implies that a plasmid-independent chromosomal determinant might encode resistance to heavy metals such as zinc and cobalt. Xiong and Jayaswal (1998) have reported the cloning, sequencing, and genetic analysis of a determinant located on the

bacterial chromosome that codes for Zn and Co resistance in *S. aureus*. Microbial biomass derived from algae, fungi, yeasts and bacteria have the pronounced ability to bind and accumulate metal ions. Some investigators have examined the utility of dried, non-living microorganism for the removal of metal ions from aqueous solutions, while others have used the modified microbial biomass to improve biosorption capacity (Gadd, 1988; Brierly, 1990; Akhtar et al., 1996).

Metabolically, inactive cells or dead biomass have been reported to be capable of accumulating same or greater amount of Co from aqueous solution. Kuyucak and Volesky (1989) screened the marine algal biomass for sequestering of Co and demonstrated that non-living biomass of seaweed; *Ascophyllum nodosum* has a high Co uptake capacity from solutions. The process involves ion exchange and the alginates of the cell wall bind Co ions rapidly, whereas penetration of Co into the cell occurs at a lower rate. Similarly, *Sargassum asperifolium*, *Cystoseira trinode*, *Turbinaria decurrens* and *Laurencia obtusa* collected from the beach of Red Sea at Hurghada in Egypt have also been screened for sequestering of Co along with other heavy metals like, Cr, Ni, Cu and Cd. Uptake of Co was maximum (86% of added metal) in *T. decurrens* (Hamdy, 2001).

The microbial reduction of Cr(VI) to Cr(III) has been discussed as a possible remediation technique in heavily contaminated environmental media or wastes (Chen and Hao, 1998). Factors affecting the microbial reduction of Cr(VI) to Cr(III) include biomass concentration, initial Cr(VI) concentration, pH, temperature, carbon source, oxidation-reduction potential, and the presence of both oxyanions and metal cations. Although high levels of Cr(VI) are toxic to most microbes, several resistant bacterial species have been identified that could ultimately be employed in remediation strategies (Chen and Hao, 1998). Previous study have suggested that lux (DABE-marked

Acinetobacter bacterium DF4/PUTK2 can be used to bioassay the ecotoxicity of wastewater and effluent samples contaminated with Co and Cr metals (Desouky et al., 2006). Other studies have indicated that bacterial isolates (*Klebsiella oxytoca*, *Citrobacter freundii* and *Bacillus anthracis*) can be exploited for bioremediation of arsenic containing wastes, since they seem to have the potential to reduce the arsenate into arsenite form (Farah et al., 2010; Bahig et al., 2008).

Additionally, several studies conducted with different strains of imperfecti fungi, *Penicillium* spp. have demonstrated their ability to degrade different xenobiotic compounds with low co-substrate requirements, and could be potentially interesting for the development of economically feasible processes for Cr and Co pollution transformation (Ana, 2009). Wasi et al. (2010) equally carried out a similar study with *Pseudomonas fluorescens* and showed that *Pseudomonas fluorescens* SM1 strain could be a good candidate for remediation of some heavy metals, phenolics and pesticides in heavily polluted sites.

The aim of this study is to investigate the effect of *E. coli*, *S. typhi* and *S. aureus* in the recovery of Cr and Co from water samples using palm kernel shell charcoal as adsorbent.

MATERIALS AND METHODS

Materials

The materials used for this work are aluminum foil, hand gloves, gas mask, cotton wool, glass rod, mortar and pestle, stopwatch, Bijoul bottle, paper tape, silica gel, activated charcoal, autoclave, incubator, fume cupboard, furnace, mechanical shaker, magnetic stirrer and Buck scientific flame atomic absorption spectrometer Model AVG 210 UK.

Reagents

All reagents used are analytical grade types and include $\text{Cr}_2(\text{SO}_4)_3$, $\text{CoCl}_2 \cdot \text{H}_2\text{O}$, MacConkey agar, nutrient agar, eosin methylene blue agar, peptone water, deoxycholate citrate agar, Salmonella-shigella agar, distilled water.

Preparation of media

All the glassware used for isolation are washed and rinsed with distilled water. They are further sterilized in an autoclave at 121 °C for 15 mins. The petridishes were equally sterilized at 121 °C for 2 hours.

All the media were prepared according to manufacturer instruction. They were weighed and dissolved in 1000 ml of distilled water. The mixture was vigorously stirred in order to obtain a homogenous mixture of the medium. The conical flask containing the medium was covered with cotton wool, and then wrapped with aluminum foil. This was then sterilized in an autoclave at 121 °C for 15 mins, removed and allowed to cool at 45 °C and finally poured into a disposable petridish.

Isolation of *E. coli*

The isolation of *E. coli* was carried out from stool samples. The media was dried in incubator and the sample was inoculated on it. Suspected colonies of *E. coli* were picked after 24 hrs of incubation at 37 °C and a pure culture of the organism was made. Identification of the organism was carried out using their biochemical reaction. Some of their biochemical test was done after their gram-staining reactions and these include indole reaction, lactose test, urase test, citrate reaction and triple sugar ion agar reaction.

Isolation of *S. typhi*

This organism can be found in blood, stool and urine. The isolation of this organism was carried out on faeces. With a straight

wire, a portion of the suspected non-lactose fermenting isolated colony was picked into peptone water and incubated for 1hr. Identification of the organism was carried out using its biochemical reactions. These include motility test, indole test, citrate test, oxidase test and triple sugar ion agar reaction. The pure culture was prepared and used for experiment.

Isolation of *S. aureus*

The organism was isolated from skin swab inoculated on blood agar and incubated at 37 °C for 24 hrs. A colony was picked and emulsified in normal saline with addition of hydrogen peroxide. Biochemical reactions were used to identify this organism. The test used includes coagulase test, catalase test, and the coagulase test using plasma. The pure culture of the organism was prepared and used for subsequent experiment.

Preparation of adsorbent and stock standard solutions

Palm kernel shell charcoal was collected in a polythene bag and washed thoroughly to remove dirt and then sundried. It was then activated in a furnace at 600 °C for 3 hrs, washed and ground to particle size using mortar and pestle. 200 g of silica gel was weighed and ground to particle size using mortar and pestle. Stocks of the metal salts [CoCl₂.H₂O and Cr₂(SO₄)₃] were prepared in distilled water to 10 g/L each.

Procedure

A 20 g of silica gel with particle size <40 µm was weighed and transferred to six different beakers and made to the mark with distilled water. The mixture was vigorously stirred and decanted. Using a 5 mL syringe,

Escherichia coli, *Salmonella typhi* and *Staphylococcus aureus* were inoculated into two beakers each containing silica gel.

A 20 g of <40 µm palm kernel shell charcoal was weighed into six different beakers and made to the mark of 40 mL with distilled water. Inoculation of the organisms into the six beakers was done as above at two beakers each. These beakers were all kept in a fume cupboard for a contact time of 1hr. 20 mL of standard solution of CoCl₂.H₂O was measured and transferred differently to each of these beakers containing silica gel and activated charcoal inoculated with *S. typhi*, *S. aureus* and *E. coli*. This was allowed to contact for 1hr in a fume cupboard. The same procedure was applied for Cr₂(SO₄)₃ standard into the remaining six beakers containing silica gel and activated charcoal inoculated with the organisms. The mixture was allowed to contact for 1hr in a fume cupboard.

A replica of the above procedure was performed this time without the inoculation of the organisms. This serves as a control.

After a contact time of 1 hr, the solutions were individually filtered using a filter paper. The filtrates were then analysed using Flame Atomic Absorption Spectrometer.

RESULTS

The results of the biochemical characteristics of the bacterial isolates and the adsorption of chromium and cobalt species on the adsorbents immobilized with bacterial isolates are presented in Tables 1 and 2 respectively, while the results of the adsorption of chromium and cobalt on silica gel and palm kernel shell charcoal in the absence of bacterial isolates is presented in Table 3.

Table 1: Biochemical characteristics of the bacterial isolates.

Biochemical test	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>
Gram staining	negative	negative	positive
Catalase test	negative	positive	positive
Urase test	negative	negative	negative
Lactose test	positive	negative	negative
Citrate test	negative	negative	negative
H ₂ S production test	positive	positive	positive
Indole test	positive	negative	negative
MacConkey agar test	positive	negative	negative
Motility test	positive	positive	negative
Oxidase test	negative	negative	negative
Coagulase test	negative	negative	positive

Table 2: Results of the adsorption of chromium and cobalt species on the adsorbents immobilized with bacterial isolates.

Substrate	Conc. of Cr in substrate µg/mL	Conc. of adsorbed Cr in µg/mL	Conc. of Co in substrate µg/mL	Conc. of adsorbed Co in µg/mL
Silica gel (<i>S. typhi</i>)	0.192	9.808	0.164	9.836
Silica gel (<i>E. coli</i>)	0.121	9.879	0.118	9.882
Silica gel (<i>S. aureus</i>)	0.198	9.802	0.018	9.942
Palm kernel charcoal (<i>S. typhi</i>)	0.028	9.972	0.029	9.971
Palm kernel charcoal (<i>E. coli</i>)	0.039	9.961	0.042	9.958
Palm kernel charcoal (<i>S. aureus</i>)	0.036	9.964	0.040	9.960

Table 3: Results of the adsorption of chromium and cobalt on silica gel and palm kernel shell charcoal in the absence of bacterial isolates.

Substrate	Conc. of adsorbed Cr in µg/mL	Conc. of adsorbed Co in µg/mL
Silica gel	9.019	9.011
Palm kernel shell charcoal	9.122	9.231

DISCUSSION

In the identification of the bacterial isolates as indicated in Table 1, the most suitable temperature for growth of *E. coli*, *S. typhi* and *S. aureus* was found to be 37 °C while the optimum growth shown by these bacterial isolates were at pH 7.2 – 7.4.

The adsorption of chromium on silica gel adsorbate immobilized with *S. typhi*, *E. coli* and *S. aureus* are 9.808 µg/mL, 9.879

µg/mL and 9.802 µg/mL respectively while the adsorbed Cr species on palm kernel charcoal are 9.972 µg/mL, 9.961 µg/mL and 9.964 µg/mL respectively. The adsorption of cobalt on silica gel adsorbate immobilized with *S. typhi*, *E. coli* and *S. aureus* are 9.836 µg/mL, 9.882 µg/mL and 9.982 µg/mL respectively. The values obtained for palm kernel shell charcoal are respectively 9.971 µg/mL, 9.958 µg/mL and 9.960 µg/mL. The

high adsorption of Co species may be attributed to the fact that metabolically active cells or dead biomass are capable of accumulating same or greater amount of Co from aqueous solution (Kuyucak and Volesky, 1989). Another factor that may be ascribed to this high recovery rate is that gram negative bacteria, particularly the constituents of their cell walls are known to bind strongly with metal ions (Beveridge and Fyfe, 1985). Thus Co species were recovered in all the substrates more than the recovery at the control experiment. This is an indication that the bacterial isolates have metal accumulating properties in aqueous solutions. Other factors that may have contributed to this high adsorption of Co includes physical adsorption, ion exchange, complexation and precipitation (Kuyucak and Volesky, 1989). Modified microbial mass have also been investigated and found to improve Co biosorption capacity (Gadd, 1988; Brierly, 1990; Akhter et al., 1996).

Microorganisms are known to play an important role in the biochemical cycle of heavy metals through its conversion to species with different solubility, mobility, bioavailability and toxicity (Silver and Phung, 2005).

The recovery of Cr using the two different adsorbates immobilized with the bacterial isolates follows a similar trend with that of Co. Such high recovery rate may be attributed to the transport of the Cr species across the cell membrane yielding intracellular accumulation which is dependent on the cells metabolism. In some cases, the metals first bind with some surface or extracellular ligands. These ligands in turn transfer the metals slowly inside the cell surface, and may become incorporated in enzymes, take part in biochemical pathways or trapped in an inactive form by binding with another intracellular ligand (Wood and Wang, 1985; Sigg, 1987). The magnesium transport system in *E. coli* have been found to transport Co and Cr and are likely processes of adsorption of Cr in the adsorbent matrix

(Hughes and Poole, 1989). The palm kernel shell charcoal had a higher adsorption than the silica gel adsorbent. This may be ascribed to the carbon source and the presence of oxyanions and metal cations (Chen and Hao, 1998). The gram-negative strain of *E. coli* may have had a significant effect in Cr biosorption (Churchhill et al., 1995).

Conclusion

The use of *S. typhi*, *E. coli* and *S. aureus* for bioremediation of Co and Cr species using palm kernel shell charcoal and silica gel showed that these bacterial isolates contributed well to the total recovery of these metals from aqueous solutions. Of particular importance is that palm kernel shell charcoal is readily available as a waste product in sub Saharan Africa, therefore it can be used as a raw material for the recovery of these metals in industrial effluents. Co and Cr are essential elements which play certain crucial roles in biological functions; however exposures to high levels of these metals are known to result in variety of health hazards. The unique features like bioaccumulation, biosorption and bioremediation of these Co and Cr resistant microorganisms can be utilized for detoxification and removal of Co and Cr from the environment using the locally available palm kernel shell charcoal as adsorbent. These results described how profuse biological resources could be utilized to remove at a very low cost, even minute amount of toxic metals from industrial effluents. Having shown good adsorbing ability, the bacterial strains represent good candidates for wastewater remediation processes of heavy metals in aqueous media. In comparison to silica gel adsorbent, these bacterial strains aid well in the adsorption of these metals using palm kernel shell charcoal.

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