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Full Length Review Paper

Efficiency of sugarcane bagasse inoculated with *Microsporum* and *Alternaria species* in the removal of Pb, Cr and Cd from refinery effluent

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Abstract

In this study, sugarcane bagasse was inoculated with single and co-cultures of *Microsporum* and *Alternaria* species to assess its efficiency in the removal of Pb, Cr and Cd from Kaduna refining and petrochemical company effluent. The heavy metal content in the refinery effluent was determined using absorption spectrophotometer and gave the following values; Pb: 1.294 mg/L, Cr: 11.31 mg/L and Cd: 1.9 mg/L. Sorption capacity of inoculated/uninoculated sugarcane bagasse was assessed and one-way Analysis of Variance was used to compare means. The removal rate of the heavy metals from the various treatments, were as follows: 89-92% of Pb, 86-94% of Cr and 99% of Cd. This corresponds to adsorption of: 0.9-1.0 mg/g of Pb, 0.7-0.9 mg/g of Cr and 1.8 mg/g of Cd. Inoculated bagasse with *Microsporum* species had the highest removal of Pb and the least was recorded for inoculated bagasse with *Alternaria* species; inoculated bagasse with *Alternaria* species had the least. However, there was no statistical difference (P>0.05) between inoculated and uninoculated sugarcane bagasse implying that the incorporation of fungal biomass as component of the sorbents did not result in any measurable difference in the percentage of the targeted metal ions removed as observed in this study.

Keywords: Sugarcane bagasse, Biosorption, Biomass, Fungi and eavy metal

INTRODUCTION

Effluents from petroleum refineries are contaminated with toxic organic compounds and heavy metal ions [1]. The harmful impacts of these toxic pollutants, on the recipient environment and on human health, have been widely reported in several studies [2-4]. Conventional methods of waste water treatment have proven to be costly, less eco-friendly and inefficient especially at low metal concentration [5].

Hence, recent studies on the adsorption of heavy metals have been centered on the use of biological materials which are available in large quantities as well as certain waste products from industrial and agricultural operations [6]. Based on the metal binding capacities of various biological materials, bio sorption is employed to separate heavy metals from wastewater through physico-chemical pathways [7]. Biomass of fungal origin have been of paramount interest, and therefore, given considerable attention out of the numerous microbial biomasses assessed [8]. Plant derived biomasses are made up of proteins, carbohydrates, phenolic compounds and other constituents, which contain functional groups, such as carboxyl, hydroxyl and amine, capable of binding metal ions in solution [9]. It is conceivable therefore, that the decomposition of plant derived biomasses such as agricultural wastes by fungi could enhance its capacity to bind and remove heavy metal ions from solution. Such enhancement in the sorption capacity of the biomass could be brought about by generating additional reactive functional groups on the surfaces of the biomaterials and providing substrate for the growth and accumulation of fungal biomass which serves as additional sorbent [8].

MATERIALS AND METHODS

Sample Collection and Handling

Sugarcane bagasse was collected in clean polythene bags from markets in Samaru, Zaria metropolis, Kaduna State, Nigeria. It was sundried, milled and preserved in clean polythene bags until needed.

The refinery effluent sample was collected in clean ten liter plastic gallon from waste retention pond of the Kaduna Refining and Petrochemical Company (KRPC). The sample collected was transported immediately to the Department of Microbiology, Ahmadu Bello University, Zaria.

Preparation of Fungal Spore Inoculum

Sporulated cultures of fungal isolate (*Microsporum* and *Alternaria* species) grown on potato dextrose agar slants for seven days were used. Spores of the fungal isolates were picked using a sterilized inoculating needle. The spores were transferred into sterilized bottles containing 50 mL sterile potato dextrose broth. The bottles were placed in a shaker and incubated at room temperature for a period of two days (Modified method [10]).

Preparation of Experimental Sorbents

Experimental sorbents were prepared by weighing five batches of 200 g each of milled sugarcane bagasse into separate Aluminum columns measuring 5 cm in diameter × 25 cm in depth to serve as fixed bed columns and as substrate for growth and accumulation of mycelia of Microsporum and Alternaria species. Four of the five fixed bed columns thus prepared were sterilized by autoclaving and the fifth was left unsterilized. Of the four sterile columns, two were inoculated with 50 mL of spore suspensions prepared from reactivated pure cultures of Microsporum and Alternaria species. A third column was inoculated with a mixture of spore suspensions of both fungal isolates and the fourth was left uninoculated. The unsterilized column was also left uninoculated. The uninoculated columns served as the controls. Inoculated columns were incubated aerobically under ambient laboratory conditions for 14 days to obtain the experimental sorbents [11].

Experimental Setup for Sorption Process

One liter of effluent sterilized by autoclaving was carefully dispensed into each of the sorption columns after two weeks of incubation. Twenty milliliter of filtrate was collected from each sorption column in sterile and well labeled plastic sample bottles one hour after introducing the effluent into the sorption columns. The residual concentrations of Pb, Cr and Cd in the filtrates were then determined using Atomic Absorption Spectrophotometer (Model AA240S Varian Technologies, USA) [12]. This exercise was repeated at hourly intervals for 6 hours as shown in Figure 1 [11].

Determination of Sorption Performance

The difference in residual heavy metal concentration before and after bio sorption process was used to assess the capacity of heavy metal removal from each column. The



Figure 1: Unsterilized and sterilized sugarcane bagasse **A**: unsterilized sugarcane bagasse; **B**: sterilized sugarcane bagasse; **C**: sterilized sugarcane bagasse inoculated with *Alternaria* species; **D**: sterilized sugarcane bagasse inoculated with *Microsporum* species and **E**: sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species.

heavy metal removal (R) from the effluent was calculated and expressed in percentages using the equation by [13].

% Heavy metal removal (R) =
$$\frac{Ci - Cf}{Ci} \times 100$$

Where, Ci=initial concentrations and Cf = final concentrations of heavy metal ions in the effluent, before and after bio sorption process respectively.

RESULTS

The mean percentage of Pb, Cr and Cd ions removed by the different sorbents ranged from 89%-92%, 86%-94% and 99% respectively. It was observed that the sorbents varied in their capacity to remove the targeted metal ions from the solution. It was however observed that sorbents exhibited similar efficiency in the removal of Cd as shown on Figure 2a.



Figure 2a: Percentage of Pb, Cr and Cd removed by sorbents (P>0.05). Each data represents the means \pm SE. A=Unsterilized and uninoculated sugarcane bagasse, B=Sterilized but uninoculated sugarcane bagasse, C=Sterilized sugarcane bagasse inoculated with *Alternaria* species, D=Sterilized sugarcane bagasse inoculated with *Microsporum* species, E=Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species.



Figure 2b: Quantity of Pb, Cr and Cd adsorbed by sorbents (P>0.05). Each data represents the means \pm SE. A=Unsterilized and uninoculated sugarcane bagasse, B=Sterilized but uninoculated sugarcane bagasse, C=Sterilized sugarcane bagasse inoculated with *Alternaria* species, D=Sterilized sugarcane bagasse inoculated with *Microsporum* species, E=Sterilized sugarcane bagasse inoculated with *Alternaria* and *Microsporum* species.

Quantity of Heavy Metal ions Adsorbed by Sorbents

The mean quantity of metal ions adsorbed per gram of the different sorbents ranged from 0.9-1.0 mg/g, 0.7-0.9 mg/g and 1.8 mg/g of Pb, Cr and Cd respectively as shown in Figure 2b. Sorbents varied in their capacity to adsorb the targeted metal ions from the solution. However, it was observed that sorbents exhibited highest and same level of efficiency with regards to sorption of Cd ions (1.8 mg/g).

DISCUSSION

The assessment of capacity of the five experimental sorbents in the removal of Pb, Cr and Cd ions from the refinery effluent expressed both in percentile and quantitative terms revealed the sorbent, as having performed equally well. All the sorbents investigated were able to remove 89% to 92% of Pb, 86% to 94% of Cr and 99% of Cd from the refinery effluent over a period of 6 hours. This corresponds with adsorption of 0.9 to 1.0 mg/g of Pb, 0.7 to 0.9 mg/g of Cr and 1.8 mg/g of the sorbents respectively. These observations could be attributed to high carbohydrates, hemicellulose, cellulose and lignin contents of the sugarcane bagasse with various functional groups such as carboxyl (COO⁻), hydroxyl (OH⁻) and phosphate (PO $_{4}^{3}$) serving as attachment sites to which the heavy metal ions are bound and removed from solution with varying degree of affinity [9,14-17]. Since sorbent A and B were exclusively composed of these constituents, their performance in the removal of Pb, Cr and Cd could be explained by the chemical and physical processes by which adsorption of metal ions is mediated [18,19]. Sorbents C, D and E on the other hand, consisted the bagasse and live fungal, whose performance is driven by physical and chemical processes along with metabolic energy dependent processes (bioaccumulation) mediated by the live cells of *Alternaria Microsporum* species present as constituents of these sorbents [13,20,21].

From the observations made in this study, inoculation of the bagasse with Alternaria species and Microsporum species did not result in measurable differences in the sorption capacity of sugarcane bagasse, i.e. no significant difference existed (P>0.05). This could be attributed to various factors such as, competition by other metals present in the effluent [22], which could have succeeded in binding to the available sorption sites on the cell walls of the fungi species, thereby leaving little or no room for the targeted heavy metals [23]. It could also be that the fungal species had higher affinity for other heavy metals than the targeted metals in the effluent. Metabolic activities of the fungi such as respiration, nutrient uptake, and metabolites released could have led to changes in the pH of the micro-environment around the cells which in turn affected removal of the targeted heavy metals from the effluent by the fungal cells [24,25]. Also, fungal cell growth could have been inhibited by high levels of metals which have been reported to affect bio sorption in living systems [26].

CONCLUSION

All the sorbents investigated were able to remove 89% to 92% of Pb, 86% to 94% of Cr and 99% of Cd from the refinery effluent over a period of 6 hours. Inoculation of the bagasse with the test fungi did not result in measurable differences in the sorption capacity of sugarcane bagasse, i.e. no significant difference existed (P>0.05). This could be attributed to various factors such as, competition by other metals present in the effluent, affinity of fungal species for other metal ions, metabolic activities of fungi (nutrient uptake, respiration, metabolite), inhibition or death of fungal growth by high levels of metal concentration.

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