International Journal of Scientific Research in Multidisciplinary Studies
Vol.4, Issue.12, pp.01-06, December (2018)

Research Paper
E-ISSN: 2454-9312
P-ISSN: 2454-6143

Synergy Effect of *Aspergillus Terreus* and *Aspergillus Fumigatus* in Bioremediation of Chromium from Tannery Effluent

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Available online at: www.isroset.org

Received: 20/Nov/2018, Accepted: 26/Nov/2018, Online: 31/Dec/2018

Abstract- Large volume of wastewater is generated by tannery industry, which contains heavy metals such as Chromium, Cadmium, Lead, Copper, Zinc, Mercury and Arsenic. Chromium (Cr) is one of the common and toxic heavy metal pollutants released by industries in their effluent. In this study, tannery effluent was collected from Challawa industrial estate, Kano, Nigeria. Physico-chemical parameters of the effluent were determined; two fungal isolates Aspergillus terreus and Aspergillus fumigatus were obtained from the wastewater. The industrial effluent was supplemented with minimal salts medium: 3 mg/L NH_4Cl, 3 mg/L K_2HPO_4, 5 mg/L KH_2PO_4, 1 mg/L NaCl, 1mg/L $MgSO_4$ and tyndallized. The Cr content in effluent were measured before inoculation of the fungal isolates and after using Atomic Absorption Spectrophotometer (Model GBC 932). The co-isolates were found to absorb 97.32% of Cr in the effluent. Scan Electron Microscopy (SEM) was also carried out. Bioremediation is an eco-friendly and economically feasible process.

Keywords- Synergy, *Aspergillus terreus*, *Aspergillus fumigatus* Bioremediation, Chromium

I. INTRODUCTION

Tannery industry is an important activity in many developing countries, it has been estimated that about 18 feet square of leather are made annually around the world with huge amount of many of money involved. This industry uses large quantities of water and in turn produces large amount of effluents [1]. In leather tanning, a number of processes are performed using large number chemicals such as acids, surfactants, dyes, natural or synthetic tanning agents, sulfonated oils and salts to convert animals skins into leather products [2]. The effluent contain low biodegradable chemicals, tannery wastewaters cause serious environmental problems. In addition, the effluent contains high concentrations of chemical oxygen demand (COD), dissolved oxygen (DO), biological oxygen demand (BOD_5) and total suspended solids (TDS), which leads to persistent of the pollutants in the effluent [3]. Chromium salts have a high rate of penetration into the interfibrillar spaces of the skins. In spite of that, only a fraction of the salts used in the tanning process yields the desired reaction with the skins [4]. This happens because of the limited access to reactive sites for the tanning molecules, and/or the presence of low affinity species in the tanning materials. The rest of the salts remain in the tanning effluents and are subsequently sent to a treatment plant where the Chromium salts end up in the waste Chromium is the second most abundant inorganic groundwater contaminant at hazardous waste sites. It was observed that Cr (III) readily oxidizes to Cr(VI) when typical oxidation conditions are present [5]. The toxicity of Cr depends primarily on its chemical form. Trivalent Chromium compounds are much less toxic than those of hexavalent Cr, since hexavalent Chromium is known to be very mobile and hazardous to human health through inhalation, skin contact and ingestion. It is highly toxic, carcinogenic and mutagenic to living organisms, even when at very low concentrations in water [6]. The conventional methods of treatment such as coagulation, flocculation, advanced oxidation, ozonation and activated adsorption are not economically feasible and efficient for the heavy metal removal. The biological method of treatment of wastewater was found more effective and less costly, in which microbes are used for pollution control technology, the biological system are used to degrade or transform of various toxic chemicals to less harmful forms [7]. It mainly shows interest towards the pollution control using bacteria, fungi in combination with physicochemical methods [8, 9]. The fungal biomass has more advantage in absorbing heavy metals due their nature of mycelial growth and this makes them to reduce the

levels of metal ion in wastewater. The biological treatment is cost effective and cleanup process of the pollutants with no harmful effect of secondary metabolites [7].

II. MATERIALS AND METHODS

In the preparation of reagents chemicals of analytical Analar grade were used with deionised water. All glass ware were cleaned and rinsed with detergents and immersed in 25% nitric acid and finally rinsed with de ionized water as conducted by [10]. Samples were collected from Global and Harmatan tanneries industries in the Challawa industrial estate, Kano State, Nigeria. The Samples were collected in triplicate in sterile bottles for physicochemical parameters, heavy metal and microbiological analyses. The pH of the effluent was measured at the point of collection; samples were stored at 4^0C and transported to the laboratory for further investigations [11].

II.I LOCATION OF THE STUDY AREA

Challawa industrial estate is located in Kumbotso Local Government Area of Kano State. It is located in the northern Nigeria covering an area extending between latitude 12° 40' and 10° 30' and longitude 7° 40' and 90° 40'. The industries in the Challawa industrial estate range from tanneries and textiles to food and packaging / processing [12, 13].

II.II ANALYSIS OF PHYSICOCHEMICAL PARAMETERS

The physicochemical parameters were measured before and after treatment with the fungal isolates. The parameters include the following, pH, DO, COD, BOD_5, TSS, TDS, Turbidity and Electrical Conductivity [11].

II.III ISOLATION AND IDENTIFICATION OF FUNGAL ISOLATES

Serial dilution of the effluent was carried out and 1 mL was put on the Sabouraud Dextrose Agar (SDA), incubated at 37^0C for 4 - 5 days. Working solution of 100 - 400 mg/L of K_2CrO_4 was prepared. The solutions were enriched with Sabouraud Dextrose Broth (SDB), sterilized and inoculated, kept at 37^0C in the orbital shaker at 100 rpm for 5 days [14]. The two isolates were able to grown at 400 mg/L concentration of K_2CrO_4 were picked as the potential isolates for bioremediation. The morphology of isolate was studied by lacto phenol cotton blue staining [15, 16, 17]. Also the fungal isolates were confirmed as *Aspergillus terreus* and *Aspergillus fumigatus* at Department of Pathology, Indian Agricultural Research Institute (Pusa), New Delhi, India.

The tannery effluent collected was poured into 500 mL conical flask with minimal salts medium: 3 mg/L NH_4Cl, 3 mg/L K_2HPO_4, 5 mg/L KH_2PO_4, 1 mg/L NaCl, 1mg/L $MgSO_4$, covered and heated at about 80^0C for 15 min and allowed to cool. The process was carried out for five (5) consecutive days in order not to denature some vital components of the effluent [18].

II.IV BIOREMOVAL OF CR FROM EFFLUENT

100 mL of tyndallized effluent with minimal salt in 250 mL Erlenmeyer flask inoculated with spore of *Aspergillus terreus* and *Aspergillus fumigatus* in separate and co-culture, at 37^0C in the orbital shaker at 100 rpm for 120 h, after every 24 h 10 mL of the effluent dawn and filtered with filter paper, then determined the Cr removed [19].

The filtrate samples of the treated effluents were centrifuged at 10,000 rpm for five (5) minutes and the supernatants were again filtered using 0.25µm micro filter membrane, in order to remove fungal biomass in it. The fungal biomass was dried in the incubator at 55^0C and weight by using digital weigh balance. While residual amount of Cr in the effluent in each sample was analysed by Atomic Absorption Spectrophotometer (Model GBC 932) [20]. The percentage removal was calculated as follows:

$$\% \text{ Removal} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial Concentration}} \times 100$$

III. RESULTS AND DISCUSSION

The tannery industries released large volume of wastewater, due to the processing of animal hide and skin to produced leather. The physicochemical parameters e.g. pH, DO, COD, BOD_5 etc of the tannery effluent shown to be reduced after treatment with individual isolates, but remarkable reduction was observed with treatment of co-culture of *Aspergillus terreus* and *Aspergillus fumigatus* table 1. This similar to the findings of Balakrishnan *et al*. [21] which reported the reduction of values of physico-chemical parameters of tannery and textile effluents by microbial consortium. *Aspergillus flavus* played significant role in the reduction of TSS, TDS and BOD_5 in the tannery effluent [22]. In another finding higher DO was observed at initial stage in the textile effluent, which after treatment with the *Bacillus* and *Pseudomonas* spp for five days shows remarkable change in the BOD_5 in the effluent.

Table 1: Physico-chemical Parameters of Tannery effluent

Parameters	Unit	Before treatment	After treatment
pH		9.1	7.8
DO	mg/L	5.6	1.4
COD	mg/L	375.1	56.3
BOD_5	mg/L	82.5	31.2
TSS	mg/L	262.5	46.7
TDS	mg/L	287.3	55.2
Turbidity	NTU	64.8	21.7
Electrical Cond	µs/cm	10.4	4.2

DO –Dissolved oxygen, Chemical oxygen demand, BOD- Biological oxygen demand, TSS –Total suspended solids, TDS –Total dissolved solids, Electrical conductivity.

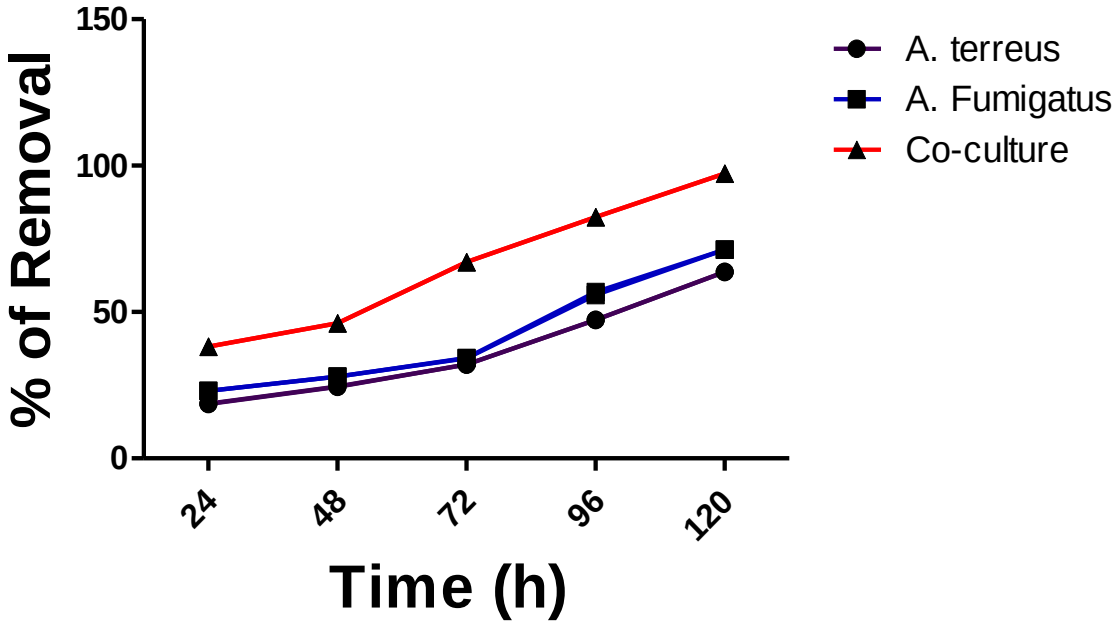


Fig**1: Percentage of removal of Chromium (Cr)** ***Aspergillus terreus, Aspergillus fumigatus*** **and their co-culture in**

120 h.

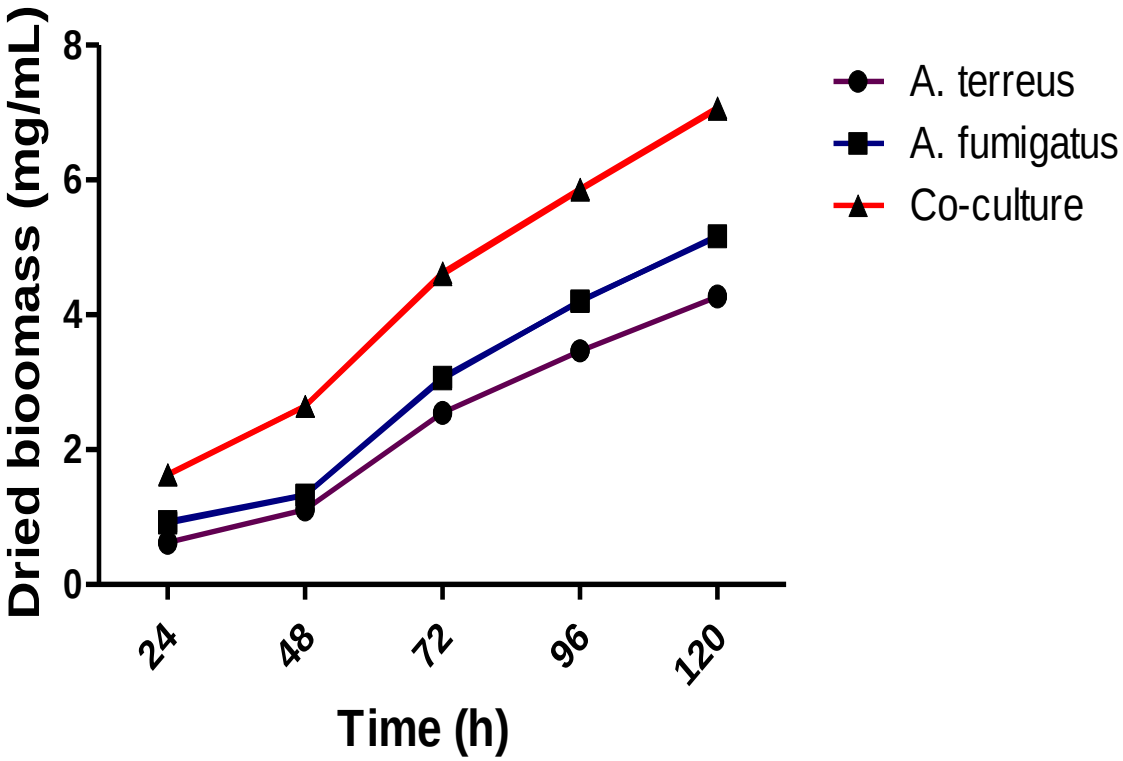
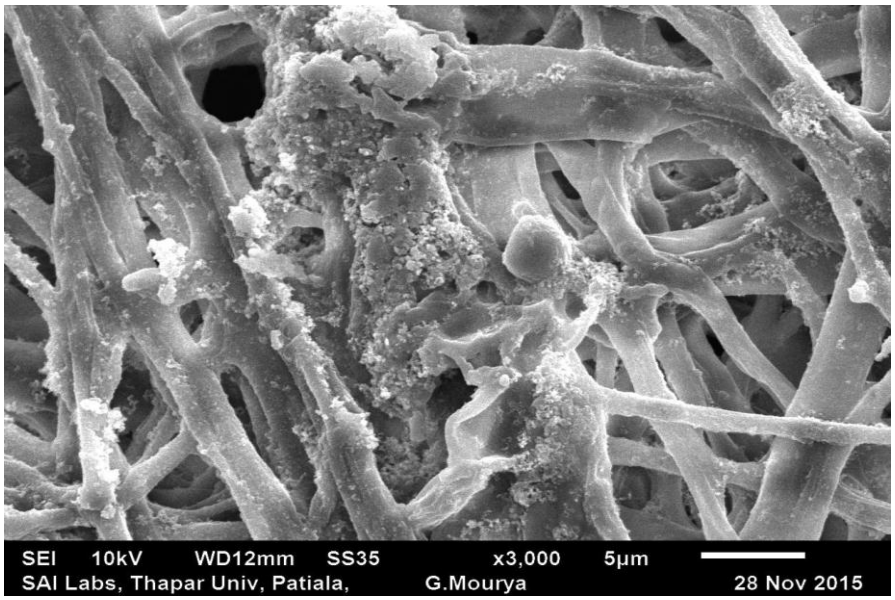


Fig. 2: Dried biomass produced by co-culture of *Aspergillus terreus* and *Aspergillus fumigatus* in



120

Fig. 3: Micrograph of co-culture of *Aspergillus terreus and Aspergillus fumigatus* treated tannery effluent

In this study, *Aspergillus fumigatus* and *Aspergillus terreus* recovered 71.34% and 63.72% within 120 h respectively. This corresponds to the findings of Murugesan *et al*., [23] in which high amount uptake was observed by the fungal isolates at 120 h, could be attributed to time contact time needed by the fungal biomass with metal ions and their synergy effect of the fungal isolates. And the contact time have role in the metal uptake by fungal biomass, has divided into two phases: primary rapid phase which account for the major part of metal biosorption and second slow phase that contribute relatively small portion of the process. For the combination of the two fungal isolates there was optimum removal of Cr with 97.32% within 120 h. Dong *et al*., [24] reported uptake of 96.71% of Cd from industrial effluent under agitation condition by mycelia of by mycelium of *Penicillium* spp and *Aspergillus niger*. The dried fungal biomass co-culture of *Aspergillus fumigatus* and *Aspergillus terreus* was fond to have 7.05 mg/mL; it could be attributed to the ability of the fungal isolates to with stand high concentrations of metal ions and in doing so more mycelial biomass are produced which enhanced uptake [24].

IV. CONCLUSION

Leather industries released effluents which contain heavy metals that can affect the quality of both surface and underground water. Physical and chemical methods of treatment were found insufficient, but biological treatment is found efficient and less costly for pollution control including bioremediation of heavy metals by microbes, especially fungal species which have ability to grow in wide habitat. Environmental regulation guidelines need to be strictly fallow, in order minimize pollution by industries through release of copious chemicals into the ecosystems.

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