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Removal of Cr (VI) from Tannery Effluent and Aqueous Solution by Sequential Treatment with Microfungi and Basidiomycete-Degraded Sawdust

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Abstract

Removal of Cr (VI) from aqueous solution and tannery effluent in sequence with Cr (VI) resistant microfungi (*Aspergillus niger*, *Penicillium chrysogenum*) and sawdust degraded by basidiomycete (*Gloeophyllum sepiarium*) was investigated in the laboratory. Initial or primary treatment with microfungi reduced 200 mg/l Cr (VI) in aqueous solution by 64.6% - 78.2% while a markedly lower 0.52 mg/l Cr (VI) in tannery effluent was reduced by 72.4% - 84.6%. However, the residual Cr (VI) in both aqueous solution and tannery effluent was reduced to a non-detectable level after secondary treatment by passage through basidiomycete-degraded sawdust column. The recovery of 65.4% - 87.7% of the Cr (VI) removed by treatment microfungi by elution indicated adsorption as the major mechanism for Cr (VI) removal. The microfungi reduced BOD in tannery effluent by 85.3 ± 5.6 - 92.7 ± 6.8 and concomitantly removed Cr (VI), hence it is hypothesized that non-Cr (VI) constituents of tannery effluent may have interfered with biosorption of Cr (VI) by treatment microfungi. It is concluded that the two-stage sequential treatment process may be of potential cost-saving stratagem for removal of chromium from industrial wastes.

Keywords

Chromium, Tannery Effluent, *Gloeophyllum*, *Aspergillus*, *Penicillium*, Sawdust

1. Introduction

The tanning industry generates wastes which include compounds such as chromium, phenol, chloride sulphide

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tannins and formaldehyde among others [1] [2]. Chromium especially the hexavalent form is very toxic [3], hence industrial wastes containing chromium are usually treated before discharge. Physical and chemical technology methods are the conventional means of removing chromium from wastes, but the cost is often unbearable for the low-income countries. Microorganisms have therefore been investigated for their ability to remove chromium and other metals from aqueous solutions or industrial wastes in several studies [3]-[7]. Succinctly, these reports show that microorganisms can mobilize or immobilize metals by biosorption, sequestration, production of chelating agents, chemoorganotrophic and autotrophic leaching, methylation and redox transformations. These mechanisms stem from prior exposure of microorganisms to metals which enable them to develop the resistance and tolerance useful for biological treatment [8]. It has also been shown that cells of plants, algae, fungi and bacteria, otherwise referred to as biomass, possess functional groups such as carboxyl, hydroxyl, sulphate, phosphate and amino that can bind metals [9].

Despite their tolerance of metals, microorganisms are not always able to remove the metals completely from industrial wastes. For example [10] reported that *Aspergillus* and *Micrococcus* isolated from soil samples of an electroplating industry tolerated Cr (VI) toxicity up to 10,000 and 8000 mg/l, respectively, but did not achieve 100% removal from a 100 mg/l solution despite the high tolerance. Some studies [11]-[15] have also shown that removal of Cr (VI) from tannery effluent by microorganisms was rarely 100% effective even with nutritional amendment. This can be attributed to limited binding sites on the microbial biomass or interference by other pollutants. It will therefore be desirable to find a second phase low cost treatment that can remove the residual chromium after initial treatment by microorganisms.

Aromatic compounds produced by some wood rot fungi during wood decomposition, include carboxyl, methoxyl and hydroxyl groups [16]-[18] that can also act as metal-binding agents. It is therefore hypothesized that introducing basidiomycete degraded sawdust as a second step to the treatment process began by microorganisms can remove the remaining Cr (VI) in the tannery effluent or aqueous solutions. This hypothesis was therefore tested using Cr (VI) resistant *Penicillium chrysogenum* and *Aspergillus niger* (microfungi) isolated from tannery effluent-contaminated soil, and obeche wood (*Triplochyton scleroxylon*) sawdust degraded by the basidiomycete *Gloeophyllum sepiarium*.

2. Materials and Methods

2.1. Source of Cr (VI) Resistant Microfungi

The test *P. chysogenum* and *A. niger* were isolated from tannery effluent-contaminated soil in Kano, Northern Nigeria in preliminary studies. Both microfungi tolerated Cr (VI) concentrations of 500 mg/l in potato dextrose medium.

2.2. Inoculum Development

The test *P.chrysogenum*, *A. niger* and *G. sepiarium* were each propagated in 150 ml flasks containing potato dextrose broth made up to 50 ml level mark for uniformity. They were incubated statically at room temperature (30˚C ± 2˚C) till growth covered the entire surface of the medium in order to ensure that the same standard of mycelial inoculum was generated. The mycelia were subsequently harvested by filtration with Whatman filter paper No 1 and washed several times with sterile distilled water to remove any adhering growth medium. This was the standard inoculum used for the treatment tests.

2.3. Primary Treatment of Tannery Effluent and Aqueous Cr (VI) Solution

The effluent used for the tests was collected from a tannery located in Kano, Northern Nigeria. The level of Cr (VI) in the effluent was determined by the atomic absorption spectrophotometer method of [19] before treatment after filtration with Whatman No 1 filter paper to remove suspended solids. Thereafter 250 ml flasks containing 100 ml filtered and autoclave-sterilised effluent were inoculated with test fungal mycelia (developed as described earlier) and agitated on an incubator-shaker (150 rpm) at room temperature for up to 48 h. A total of 24 flasks were prepared and 3 were withdrawn at 6-hourly intervals, filtered to remove hyphal strands, and analysed for Cr (VI) and BOD by the procedure of [19]. The duration it took to achieve maximum removal of Cr (VI) was recorded and used for subsequent experiments. The procedure was repeated with 10 replicate 250 ml flasks incubated for the pre-determined duration and subsequently filtered to remove microfungal mycelia. The-

reafter the residual Cr (VI) was determined before the filtrates were set aside for secondary treatment. The concentration of Cr (VI) removed was the difference between the initial concentration in the tannery effluent and the residual Cr (VI) after treatment with the microfungi. This was expressed as percentage of the initial concentration. The above procedure was repeated using microfungi killed by autoclaving at 120˚C for 15 minutes or *G. sepiarium* as substitutes for the live treatment microfungi. The procedure outlined above was again repeated using 200 mg/l Cr (VI) prepared from $K_2Cr_2O_7$.

The ability of microfungal mycelia recovered from treated tannery effluent to remove Cr (VI) from aqueous solution was tested. The mycelia were recovered by filtration and washed with 0.1 M Tris-HCl buffer pH 7.8 [20] and sterile distilled water twice to remove adsorbed chromium and other tannery constituents. Thereafter, 10 replicate flasks containing 200 mg/l aqueous solution of Cr (VI) were inoculated with the mycelia and incubated as before. The Cr (VI) concentration was analyzed after the incubation period and the concentration of Cr (VI) removed was expressed as a percentage of the initial concentration (200 mg/l).

2.4. Secondary Treatment

Obeche wood (*Triplochiton scleroxylon*) sawdust was collected from sawmills, dried to constant weight at 105˚C, and dispensed at 50 g/250ml flask. The sawdust was moistened with 20 ml distilled water and autoclaved at 120˚C for 30 mins. On cooling, the flasks were inoculated with 10 ml Potato Dextrose Broth suspension of macerated mycelia of *Gloeophyllum sepiarium* (brown-rot basidiomycete) that was developed as described previously and manually turned over to effect sawdust/hyphal even mixture. The flasks were divided into 3 sets of 5 replicates bringing it to a total of 15 and set aside on the laboratory bench at room temperature for 3 months. They were moistened with 10 ml sterile distilled water/flask at weekly intervals to prevent desiccation. A set of 5 flasks were retrieved at intervals of 4 weeks, manually macerated to break the hyphae/sawdust entanglement and dried to 30% moisture content at 45˚C in a dessicator. This temperature was chosen to minimise any damage to potential Cr (VI) binding biomolecule that may be present. Thereafter they were aseptically packed into 5 replicate glass columns with an inner diameter of 4 cm and a height of 15 cm. The filtrates of effluents or aqueous solution from primary treatment were passed through the degraded sawdust column at a drop/second from a burette. The effluents collected from the outlet at the bottom of the column were analyzed for Cr (VI) concentration. For the purpose of control, the above experiment was repeated with un-degraded sawdust as substitute for the basidiomycete-degraded sawdust. The concentration of Cr (VI) removed was expressed as percentage of the residual concentration in the filtrates from the primary treatment.

2.5. Biosorption Capacity Tests

Another set of microfungal mycelia were retrieved after primary treatment, dried overnight at 70˚C, and transferred to flasks containing 0.1 M Tris-HCl buffer solution (pH 7.8). They were shaken overnight at 200 rpm to allow desorption of Cr (VI) occur. The filtrates were subsequently analyzed for desorbed Cr (VI) and expressed as percentages of the concentrations adsorbed during primary treatment. The degraded sawdust in the glass column were also retrieved after secondary treatment and similarly desorbed.

3. Results and Discussion

The Cr (VI) removed from tannery effluent and aqueous solution by the microfungi peaked at the 36th hour of incubation (**Figure 1**). On the other hand Cr (VI) concentration removed by the basidiomycete was low and did not peak by the end of the incubation period (**Figure 1**). A 36 h incubation period was therefore adopted for subsequent tests.

The results of the primary treatment tests showed that the live microfungi removed over 70% of the chromium in the tannery effluent and aqueous solution and concomitantly reduced BOD by over 80% in the same effluent (**Table 1**). This was not unexpected because several reports have shown that microorganisms are capable of removing heavy metals including Cr (VI) from industrial wastes or aqueous solutions [3] [10] [12] [13] [21]. The basidiomycete removed less than 10% of the Cr (VI) and could only reduce BOD by less than 20% (**Table 1**). This suggests that the capacity of *G. sepiarium*, a brown-rot fungus, to adsorb or remove Cr (VI) is limited although research reports indicate that some basidiomycetes like the white-rot *Pleurotus* species can remove substantial concentrations of Cr (VI) from waste water [22] [23]. The dead mycelia were unable to remove Cr (VI) or reduce BOD in tannery effluent to the extent that the live mycelia did (**Table 1**). However, the dead mycelia

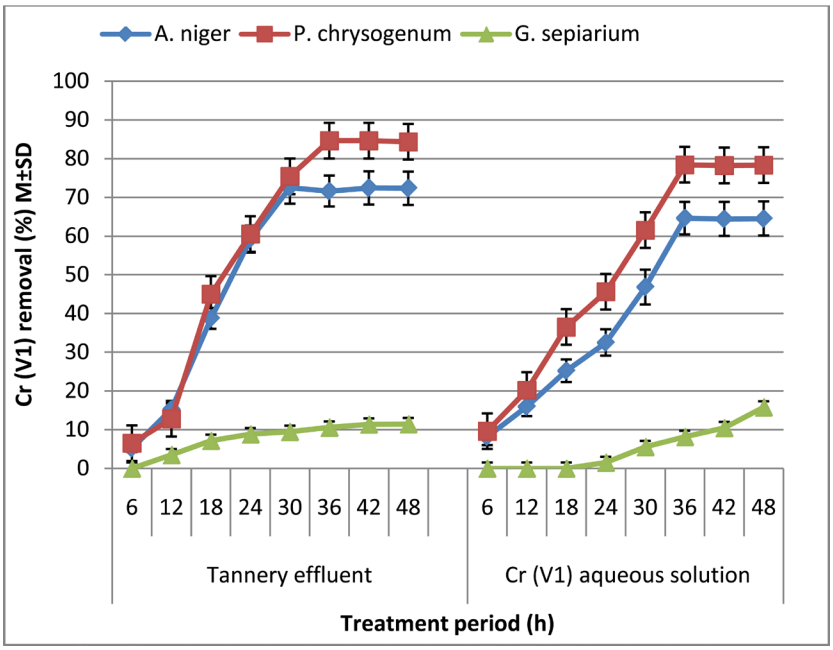

Figure 1. Removal of Cr (V1) from tannery effluent and aqueous solution. Cr (V1) concentration before treatment: tannery effluent 0.52 ± 0.08 mg/l; aqueous solution, 200 mg/l.

Table 1. Removal of Cr (VI) in tannery effluent and aqueous solution by microfungi during primary treatment after 36 h.

Treatment organisms		After treatment	
Status	**Organism**	**Cr (VI) removal (%) M ± SD**	***BOD reduction (%) M ± SD**
Live in tannery effluent	*As. niger*	72.5 ± 4.2	85.3 ± 5.6
	P. chrysogenum	84.6 ± 4.7	92.7 ± 6.8
	G. sepiarium	9.6 ± 1.2	18.5 ± 3.3
Dead in tannery effluent	*As. niger*	25.3 ± 2.0	0.0 ± 0.0
	P. chrysogenum	32.1 ± 2.1	0.0 ± 0.0
	G. sepiarium	5.4 ± 1.0	0.0 ± 0.0
Live in aqueous solution	*As. niger*	64.6 ± 4.5	NA
	P. chrysogenum	78.2 ± 4.8	NA
	G. sepiarium	9.7 ± 1.5	NA
Dead in aqueous solution	*As. niger*	68.6 ± 3.4	NA
	P. chrysogenum	71.7 ± 4.3	NA
	G. sepiarium	6.3 ± 1.2	NA

Concentration of Cr (V1) before primary treatment: tannery effluent, 0.52 ± 0.08 mg/l; aqueous solution 200 mg/l. *BOD concentration in tannery effluent before treatment = 1240.0 mg/l. NA. Not applicable.

removed Cr (VI) from aqueous solution to an extent that was not markedly different from the level it was reduced to by the live mycelia (**Table 1**). While removal of metals by biomass is physical and depends on the chelating agents on biomass surface, removal of BOD is dependent on metabolic activity which dead cells cannot perform.

The BOD test results confirm the presence of compounds in the tannery effluent that can interfere with metal binding sites on the fungal mycelia. This inference is supported by the observation that despite the low concentration of Cr (VI) in tannery effluent, the microfungi were unable to achieve 100% removal rate. This contrasts sharply with the result showing the same fungal mycelia dead or alive, removing markedly higher concentrations of Cr (VI) in aqueous solution. Further evidence comes from the observation that the mycelia of *A. niger* and *P. chysogenum* recovered from primary treatment of tannery effluent was able to remove 115.4 ± 5.3 mg/l (57.7%) and 122.2 ± 5.8 mg/l (61.1%) Cr (VI), respectively from aqueous solution. This suggests that interfering tannery constituents may have been dislodged from the mycelia by the washings with Tris-HCL buffer and

distilled deionised water. The limited Cr (VI) removal ability of dead mycelia in tannery effluent can be attributed to lack of metabolic activity that can degrade some of the tannery constituents that may have interfered with Cr (VI) binding sites on the mycelia.

The secondary treatment with *G. sepiarium*-degraded obeche wood sawdust removed the residual Cr (VI) in the tannery effluent and aqueous solution after primary treatment (with microfungi) to the extent that Cr (VI) could not be detected (**Table 2**). The results in **Table 2** also show that this was achieved in obeche sawdust degraded for 8 weeks and above. Although basidiomycete-degraded sawdust was able to remove Cr (VI) from untreated tannery effluent, the concentration was markedly lower than that removed from treated effluent (**Table 2**).

On the other hand, removal of Cr (VI) in tannery effluent or aqueous solution (with or without primary treatment) by un-degraded obeche sawdust was marginal (**Table 3**). Two hypotheses arise from these two findings. The first is that compounds produced during wood degradation can act as metal chelating or binding agents that immobilised Cr (VI) hence Cr (VI) was not substantially removed in un-degraded sawdust. The second is that some constituents of the untreated tannery effluent may be blocking the potential Cr (VI) binding biomolecules released during decomposition of sawdust. Evidence for the first hypothesis comes from previous research findings that wood degrading basidiomycetes produce compounds during wood decomposition that can bind metals [16]-[18] [24]. For the second hypothesis, research needs to be directed towards interaction between tannery effluent constituents and fungal mycelia. The focus of future research will be to address this limitation.

The results presented in **Table 4** indicate biosorption as the predominant mechanism for the removal of Cr (VI) by both the microfungi and in degraded sawdust, because over 50% of the Cr (VI) taken up was recovered

Table 2. Removal of Cr (VI) in filtrates from primary treatment by obeche wood sawdust degraded by basidiomycete G. sepiarium.

Source of filtrate	Primary treatment organism	Removal of residual Cr (VI) (%) by degraded sawdust after:		
		4	8	12 weeks
Tannery effluent	[a]*A. niger*	96.5 ± 5.3	ND	ND
	[b]*P. chrysogenum*	97.4 ± 5.5	ND	ND
Aqueous solution	[c]*A. niger*	89.2 ± 4.7	ND	ND
	[d]*P. chrysogenum*	92.5 ± 5.0	ND	ND
Untreated tannery effluent	Control	59.3 ± 3.6	72.8 ± 4.5	75.2 ± 4.5

ND, Not detected. Cr (VI) in filtrate from primary treatment: [a]0.14 ± 0.03 mg/l; [b]0.08 ± 0.01 mg/l; [c]70.8 ± 3.6, mg/l; [d]44.5 ± 3.0 mg/l. Cr (VI) in: untreated tannery effluent, 0.52 ± 0.08 mg/l; aqueous solution, 200 mg/l.

Table 3. Removal of residual Cr (V1) in filtrates from primary treatment by un-degraded obeche wood sawdust.

Source of Cr (VI)	Primary treatment fungus	Removal of Cr (VI) in un-degraded sawdust (%) M ± SD
Tannery effluent	[a]*A. niger*	2.5 ± 0.10
	[b]*P. chrysogenum*	2.8 ± 0.10
	Not treated	1.6 ± 0.10
Aqueous solution	[c]*A. niger*	5.4 ± 0.15
	[d]*P. chrysogenum*	5.8 ± 0.15
	Not treated	2.3 ± 0.10

Cr (V1) in filtrate from primary treatment: [a]0.14 ± 0.03 mg/l; [b]0.08 ± 0.01 mg/l; [c]70.8 ± 3.6, mg/l; [d]44.5 ± 3.0 mg/l.

Table 4. Cr (VI) desorbed from fungal mycelia and *G. sepiarium*-degraded sawdust after treatment of tannery effluent and aqueous Cr (VI) solution.

Source of Cr (VI)	[*]Fungal mycelia	Desorbed Cr (VI) (%) M ± SD
Tannery effluent	*Aspergillus niger*	86.5 ± 4.0
	Penicillium chrysogenum	87.7 ± 4.2
Aqueous solution	*Aspergillus niger*	65.4 ± 3.6
	Penicillium chrysogenum	69.6 ± 3.7
Biodegraded sawdust	NA	52.5 ± 3.3

NA, Not applicable; [*]after primary treatment.

by desorption. However the percentage of Cr (VI) recovered from degraded sawdust was markedly lower than that recovered from fungal mycelia (**Table 4**). This notwithstanding, it is an indication that the degraded wood can be re-used to reduce the cost of the treatment process.

4. Conclusion

The main objective was to complement chromium biosorption capacity of microfungi with biosorption capability of brown-rot wood decomposition products in a sequential arrangement. This was achieved because Cr (VI) was reduced to non-detectable level at the end of the two-phase treatment sequence. The live mycelium possesses a better potential for initial treatment of tannery effluent than the dead mycelia. The reason was that in addition to removing Cr (VI) better than the dead mycelia, it also metabolized other tannery constituents as indicated by BOD reduction which was not possible with dead mycelia. This strategy may prove useful in a cost-effective treatment stratagem for chromium removal in industrial wastes if further tests with other wastes prove successful. Low-income countries that cannot afford the cost of the conventional treatment technology may find it attractive especially as sawdust is an abundant, but often wasted resource in many developing countries like Nigeria.

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