Remediation of Cr(VI) and Ni(II) contaminated water by Metal Tolerant fungi isolated from Electroplating effluent

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Summary

Heavy metal pollution has magnetized the public concern due to negative consequences on human health and the environment. $Cr_{(VI)}$ and $Ni_{(II)}$ are two heavy metals have serious issues. These both metals are generated from different types of industrial and commercial activities such as electroplating, tannery, mining, paper-pulp, paint and pigment, etc. and discharged into the natural water bodies with their generating effluents without proper treatment. Discharged $Cr_{(VI)}$ and $Ni_{(II)}$ containing effluents disrupt the aquatic life by making number of alteration in their metabolic and physiological processes and get accumulated inside them as well as in plants too. Discharged effluents also contaminate the ground water through percolation from the discharged sites. Consumption of contaminated water and food is the way by which human being come in exposure of $Cr_{(VI)}$ and $Ni_{(II)}$. $Cr_{(VI)}$ and $Ni_{(II)}$ are toxic, carcinogenic and mutagenic and causes eye and skin irritation, severe diarrhoea, corrosion of skin and respiratory tract, kidney dysfunction, and probably lung carcinoma in human after coming in exposure via ingestion, inhalation and consumption.

Many physicochemical treatment systems are being used for the remediation of heavy metal contaminated water including $Cr_{(VI)}$ and $Ni_{(II)}$. But, their cost, insufficient treatment potency, generation of huge amount of sludge as a secondary pollutant, high energy and skilled man power requirement are the disadvantages that possess a need of new and advance treatment system for remediation of heavy metal polluted wastewater. Biological treatment system utilizes microbes and plants and can remove the disadvantages of the existing systems and may become more appropriate system for treatment purpose. In this regards many work has been done and several species of microbes and plants have been explored for the treatment perspective. Fungi possess several features such as easy in handle and use, adopted to stress environment such drought, saline, heavy metal, etc. They can be cultivated with low graded material and applied on sites as well engineered treatment systems without making hard efforts. Because, heavy metals are very toxic for living organisms, so only adopted fungi to heavy metal can be applied in the treatment system for the treatment of heavy metal polluted wastewater. The electroplating wastewater often contains different type of heavy metal such as Cr,

Ni, Pb, Cu, Mn, etc. The electroplating wastewater discharged site is contaminated with many metals. The microbes (bacteria and fungi) present in these contaminated sites may highly adopted to heavy metal including $Cr_{(VI)}$ and $Ni_{(II)}$ contaminated environment and may have high potency for reduction and removal of Cr(VI) as well as $Ni_{(II)}$. These heavy metals adopted organism which have the ability for reduction and removal of $Cr_{(VI)}$ and $Ni_{(II)}$ can be alternative for the bioremediation of heavy metal polluted wastewater. However, the tolerance and remediation mechanism of $Cr_{(VI)}$ and $Ni_{(II)}$ also not well explored presently in fungi and this is major gap to resolve for better applicability of fungi in remediation process. Keeping into mind these hypotheses, this work has been performed and some key finding of the entire study after critical evaluation and elucidation of the experimental data are as follows:

6.1. Physicochemical characterization of electroplating wastewater

- The electroplating wastewater was collected from the electroplating industrial area of Faridabad, India. This area is highly dense and clustered with different types of electroplating factories.
- The electroplating wastewater was highly laden with different types of heavy metal (Ni, Cr, Pb and Cu) and other pollutant which are presented in Table 6.1.

Table 6.1. Physicochemical characteristics of electroplating wastewater.

S. N.	Physicochemical Parameters	Recorded values*
1	pH	9.49 ± 0.12
2	Electrical Conductivity (mS/cm)	39.60 ± 3.3
3	BOD (mg/L)	670.00 ± 6.6
4	COD (mg/L)	1130.0 ± 11.12
5	Total Solid (mg/L)	3830.6 ± 13.13
6	Cr (mg/L)	54.84 ± 2.09
7	Cd (mg/L)	2.43 ± 1.20
8	Cu (mg/L)	23.76 ± 0.02

9	Ni (mg/L)	140.21 ± 1.2
10	Pb (mg/L)	0.83 ± 0.04
11	As (mg/L)	0.11 ± 0.02

*Values are mean of three replicates.

6.2. Isolation fungal isolate from electroplating wastewater

- Chromium tolerant fungal isolates were isolated on 50 mg/L of $Cr_{(VI)}$ amended potato dextrose agar plate.
- At 50 mg/L of $Cr_{(VI)}$ amended PDA plate four morphologically distinct microbes were recorded, that were further purified on PDA plate.

6.3. Screening of Heavy metal tolerance of isolated fungi

- First, the isolated fungal species were tested for their tolerance towards different concentration of $Cr_{(VI)}$ only two (isolate A and C) of them were able to grow above 300 mg/L of $Cr_{(VI)}$. That's why only two species were selected for further study (as per the plan of wok).
- Isolate A was able to tolerate 800 mg/L of $Cr_{(VI)}$, while isolate C was able to tolerate 1000 mg/L of $Cr_{(VI)}$.
- Isolate A was renamed as isolate CR500 and isolate C as isolate CR700.
- The metal tolerance ability of both the isolates toward different concentration of some co-occurring metal contaminant was investigated.
- Isolate CR500 able to tolerate As(2000 mg/L), Mn(1600 mg/L), Ni (1600 mg/L), Pb (1200 mg/L), Cr(800 mg/L), Cu (200 mg/L) and Cd (100 mg/L).
- Isolate CR700 showed its tolerance towards As (2000 mg/L), Ni (1500 mg/L), Zn (1200 mg/L), Cu (1200 mg/L), Cr (1000 mg/L), and 100 mg/L of Pb and Cd.

6.4. Molecular Identification of fugal isolates

- For molecular identification, internal transcribed spacer region sequencing was done. For identification, blast tool, NCBI database was used to identify the fungal species using sequenced data.
- Isolate CR500 showed maximum similarities with *Aspergillus flavus* species.
- Isolate CR700 showed maximum similarities with *Trichoderma lixii* species.

6.5. *Aspergillus flvaus* CR500 and $Cr_{(VI)}$: Reduction, removal and biochemical response analysis

- For the reduction of $Cr_{(VI)}$ using *A. flavus* CR500, batch study was conducted and *A. flavus* CR500 showed high efficiency (almost 100%) for the reduction of $Cr_{(VI)}$ up to the concentration of 100 mg/L of $Cr_{(VI)}$. At lower $Cr_{(VI)}$ concentration, removal ability of $Cr_{(VI)}$ was also higher.
- Fungus also showed significant $Cr_{(VI)}$ reduction efficiency in the pH ranges from 6.0 to 8.0, temperature (28-35 °C) and under the presence of co-occurring different salts, anions, cations, metabolic inhibitor and heavy metal (Ni, Pb, Cu, Mn, Cd, As, etc.). The fungus also showed high $Cr_{(VI)}$ reduction efficiency in the Tannery wastewater.
- Intracellular Chromate reductase activity was recorded in the presence of $Cr_{(VI)}$ in *A. flavus* CR500 that played important role in intracellular reduction of $Cr_{(VI)}$ to $Cr_{(III)}$, while reduce form $Cr_{(III)}$ may exported to outside of the cell or accumulated inside the cell. Some amount of $Cr_{(III)}$ was also precipitated as Cr_2O_3 on the surface of cell wall.
- The removal of $Cr_{(VI)}$ happen through reduction, accumulation, adsorption and precipitation mechanism as explored in FTIR, SEM-EDX, XPS and Cr removal and adsorption experimentation.
- SEM results showed that in $Cr_{(VI)}$ exposure fungus showed different types of morphological responses (swelling in mycelia, protrusion less, ruptured, constricted mycelia). The functional group such as COO^-, O─H, N─H and C≡C are participated in adsorption of $Cr_{(VI)}$ on the surface of *A. flavus* CR500 evinced from FTIR analysis.
- $Cr_{(VI)}$ induced oxidative stress has been found which might reduce the growth of the fungus at higher concentration of $Cr_{(VI)}$.
- Enzymatic (CAT, SOD, POD, PPO and PAL) and non-enzymatic (total phenolic content, Proline, GSH and non-protein thiol content) antioxidants might play role in alleviation of ROS in *A. flavus* CR500 demonstrated from their increased activity and level under the presence of $Cr_{(VI)}$ in *A. flavus* CR500.
- In the phytoxicity assessment, *Vigna radiata* seeds showed good germination and shoot/root growth in fungal 100 mg/L of $Cr_{(VI)}$ solution as compared to

without fungal treated 100 mg/L of $Cr_{(VI)}$ solution confirming the reduction in the toxicity of $Cr_{(VI)}$ solution.

6.6. *Trichoderma lixii* CR700 and $Cr_{(VI)}$: reduction, removal and biochemical response

- In batch study, *T. lixii* CR700 showed 99% reduction of $Cr_{(VI)}$ to $Cr_{(III)}$ up to the concentration of 100 mg/L of $Cr_{(VI)}$.
- *T. lixii* CR700 also showed efficient $Cr_{(VI)}$ reduction capacity in the temperature of 28-35 °C and pH (6.0 to 8.0), under the presence of different heavy metal (Cr, As, Ni, Pb, Cu and Mn), salts, anions and metabolic inhibitor (EDTA) and showed efficient $Cr_{(VI)}$ reduction capability in tannery wastewater.
- Under the presence of hexavalent chromium, the activity of intracellular as well as extracellular chromate reductase enzyme was recorded in *T. lixii* CR700 that possibly involved in reduction of Chromium.
- *T. lixii* CR700 removed $Cr_{(VI)}$ via reduction, accumulation, surface adsorption and precipitation mechanism evinced from different investigation (SEM-EDX, XRD, FTIR, etc.).
- Elevated concentration of $Cr_{(VI)}$ induces ROS production in *T. lixii* CR700 that might be due the toxicity of $Cr_{(VI)}$ to fungus and is the possible cause for the reduction growth and biomass productivity of the fungus.
- At higher concentration of $Cr_{(VI)}$, activity of antioxidants enzyme (CAT, POD and SOD) and the level of non-enzymatic antioxidants (proline, total phenolic content, non-protein thiol content) was increased.
- Reduction in the toxicity of $Cr_{(VI)}$ solution was confirmed from good germination rate and shoot/root growth of *Cicer arietinum* and *Vigna radiata* in *T. lixii* CR700 treated $Cr_{(VI)}$ solution.

6.7. *A. flavus* CR500 and $Ni_{(II)}$: Removal, biochemical and morphological analysis

- *A. flavus* CR500 has high $Ni_{(II)}$ removal potential. The maximum removal (73 %) potential of *A. flavus* CR500 was recorded at lower concentration (10 mg/L) of $Ni_{(II)}$.
- *A. flavus* also exhibited removal efficiency under the presence of different salts, anions, metabolic inhibitor and co-occurring heavy metal contaminant of electroplating wastewater.
- SEM analysis revealed that morphology of the mycelia of *A. flavus* CR500 was changed from protrusion full, straight and cylindrical to protrusion less and swollen mycelia that might be due to toxicity response of fungi or due to accumulation of $Ni_{(II)}$ inside the cell of the fungus that results into swollen mycelia.
- FTIR study suggested the involvement of surface functional group in adsorption of $Ni_{(II)}$ ion the surface of fungal mycelia. However, removal $Ni_{(II)}$ is mostly driven by accumulation of $Ni_{(II)}$ inside the cell of the fungus as observed from determination of accumulation and adsorption study.
- Due to toxicity, $Ni_{(II)}$ induced ROS production was recorded that possibly reduces the growth of the fungus at elevated concentration.
- Increased SOD, CAT, POD activity and level of total phenolic content and proline was recorded in *A. flavus* CR500 in the response of $Ni_{(II)}$ that possibly reduces the effect of ROS and maintain the cellular homeostasis.
- *Vigna radiata* seeds showed high germination rate and shoot and root growth in fungal (*A. flavus* CR500) treated $Ni_{(II)}$ solution compared to without fungal treated solution in phytotoxicity test.

6.8. *T. lixii* CR700 and $NI_{(II)}$: Biochemical, morphological and $Ni_{(II)}$ removal investigation

- *T. lixii* CR700 has potential for effective removal of $Ni_{(II)}$. It has been investigated under the different stress condition and was able to remove $Ni_{(II)}$ under the presence of other co-occurring pollutants such heavy metal, mono and bi-anions and salts. The removal efficiency of *T. lixii* CR700 was high at low concentration of $Ni_{(II)}$ and was 64 % at 10 mg/L of $Ni_{(II)}$.
- The removal happen via surface adsorption and accumulation mechanism, confirmed from metal accumulation and surface sorption investigation. The

electrostatic attraction and hydrogen bond were the major mechanism of the surface functional group such as COO, OH, NH, etc. that played role in adsorption of $Ni_{(II)}$ onto the mycelia surface of the fungus.

- $Ni_{(II)}$ stress induces the ROS production that possibly diminished the fungal growth via causing oxidative damages. Increased CAT and POD activity and level of total phenolic content and proline may be participated in ROS reduction.
- In SEM investigation, it was found that mycelia surface of *T. lixii* CR700 after $Ni_{(II)}$ exposure swelled and enhanced than unexposed mycelia of *T. lixii* CR700 that might indicated the $Ni_{(II)}$ adsorption on the surface of mycelia as well as production extracellular polymeric substances.
- The toxicity of fungal treated $Ni_{(II)}$ solution was reduced as confirmed from high germination rate and growth of shoot/root in *T. lixii* CR700 treated $Ni_{(II)}$ as compared to untreated $Ni_{(II)}$ solution.

6.9. Multiple metal removal by *A. flavus* CR500 from simulated wastewater (SWW)

- The multiple removal ability of *A. flavus* CR500 was investigated in batch study. *A. flavus* CR500 was able to remove 97.5% of As followed by 93.3, 82.2, 46.6% of Pb, Cr, and Ni from SWW containing 5 mg/L of each metal respectively. Increasing metal concentration up to 20 mg/L of each in SWW significantly reduces the biomass and metal removal potential of *A. flavus* CR500.
- The pH of the medium was also optimized for multiple metal removal from SWW using 5 mg/L of each metal. *A. flavus* CR500 exhibited high metal removal efficiency at pH 7.0.
- The removal of efficiency of *A. flavus* CR500 was also investigated in real tannery wastewater to assess its applicability for real wastewater treatment. *A. flavus* could remove 76.8% of externally added Pb followed by 61.2, 58.2 and 38.6% of externally added Cr, As, Ni respectively from diluted tannery wastewater (in ratio of 1:2; v:v) and amended with PDB.