

CHROMIUM TOLERANT INDIGENOUS FUNGAL STRAINS FROM INDUSTRIAL EFFLUENTS OF ANUGUL DISTRICT, ODISHA, INDIA

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ABSTRACT

Industrial discharges, in the form of effluent or wastewater is one of the biggest problems causing serious environmental pollution. Bioremediation is an emerging technology for removing the heavy metals from the contaminated environment. In the present study, industrial effluent samples from five different sites of Bhusan Steel Plant (BSP) and National Thermal Power plant Corporation (NTPC) were collected. Heavy metal analysis of effluent samples showed the presence of a number of metals like Chromium(Cr), Zinc (Zn), Manganese (Mn), Lead (Pb), Cobalt (Co), Copper (Cu), Nickel (Ni), and Cadmium (Cd), from which Mn, Cr, Pb and Zn were beyond the permissible limit. Five different fungal strains were isolated and identified as *Penicillium* sp., *Aspergillus fumigatus*, *Aspergillus* sp., *Penicillium adametzi* and *Aspergillus niger*. Out of which, *Aspergillus fumigatus* was found to remove maximum Cr from the culture broth in comparison with other isolates. Hence, from the present study it can be concluded that indigenous fungi can be a novel tool for bioremediation. Further studies need to be carried out on biosorption of toxic heavy metals through fungi.

Key words: Bioremediation; heavy metals; indigenous fungal isolates; industrial effluents

1. INTRODUCTION

In the wake of industrialization, consequent urbanization and ever increasing population, the basic amenities of life viz. air, water and land are being polluted continuously. Industrial complexes have become the focus of environmental pollution Shukla *et al.* (2007). The main pollutant from these industrial effluent were heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg, Cd etc. and various organic compounds such as phenols, formaldehyde etc. Rajendran *et al.* (2003). The biomagnifications of these heavy metals in the effluents act as a major threat to human life Yigit and Altindag (2006); Hooda (2007).

Therefore, it is highly necessary to reduce and remove these heavy metals from the water sources in order to increase the water quality and maintain a healthy human life Ilhan *et al.* (2004). According to World Health Organization (1984), the metals like Cd, Cr, Pb, Cu, Ni, Zn, Hg etc. are of immediate concern. Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation. So in order to overcome the problems, an alternative and innovative biological treatment has been focused on.

Chromium is one of the major pollutants in the environment and is frequently present in

wastewaters from various industrial units. Several conventional physical and chemical treatment techniques are used for the removal of chromium but such processes are neither economical nor eco-friendly. Hence, potential utilization of microorganisms for the removal of Cr has been recognized as an alternative method. Microorganisms are advantageous for metal detoxification as they are easy to grow, resulting in a rapid production of biomass, and are part of the natural environment. Microbial treatment systems have the advantage of being simple in design and low in cost. The mechanism by which microorganisms remove heavy metals can be divided into three categories such as biosorption of metals ions on the cell surface, intracellular uptake of metals ion and chemical transformation of metal ions by microorganisms Pardo *et al.* (2003).

Various studies found that micro-organisms like *Bacillus* sp., *Pseudomonas* sp., *Desulphovibrio* sp., *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp. etc. have the ability to accumulate the metal nutrients in higher amounts. Fungi are the versatile groups

which can adapt and grow under various extreme conditions of pH, temperature and nutrient availability as well as high metal concentrations Anand *et al.* (2006). Heterotrophic fungi such as *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., and *Yarrowia* sp. can remove both soluble and insoluble metal species from solution and are able to leach metals cations from solid waste White *et al.* (1997). White rot fungus *Phanerochaete chrysosporium* isolated from soil samples enriched by continuous pulp and paper mill effluent irrigation was capable of 84% effluent decolourization along with 79% COD reduction Prabhu and Udayasoorian (2005).

The present investigation is primarily focused on the isolation of indigenous Cr tolerant fungi from the industrial effluent samples from different sites of Anugul district of Odisha and their heavy metal tolerating activity.

2. MATERIALS AND METHOD

2.1 Study area:

Figure-1: Map of study area

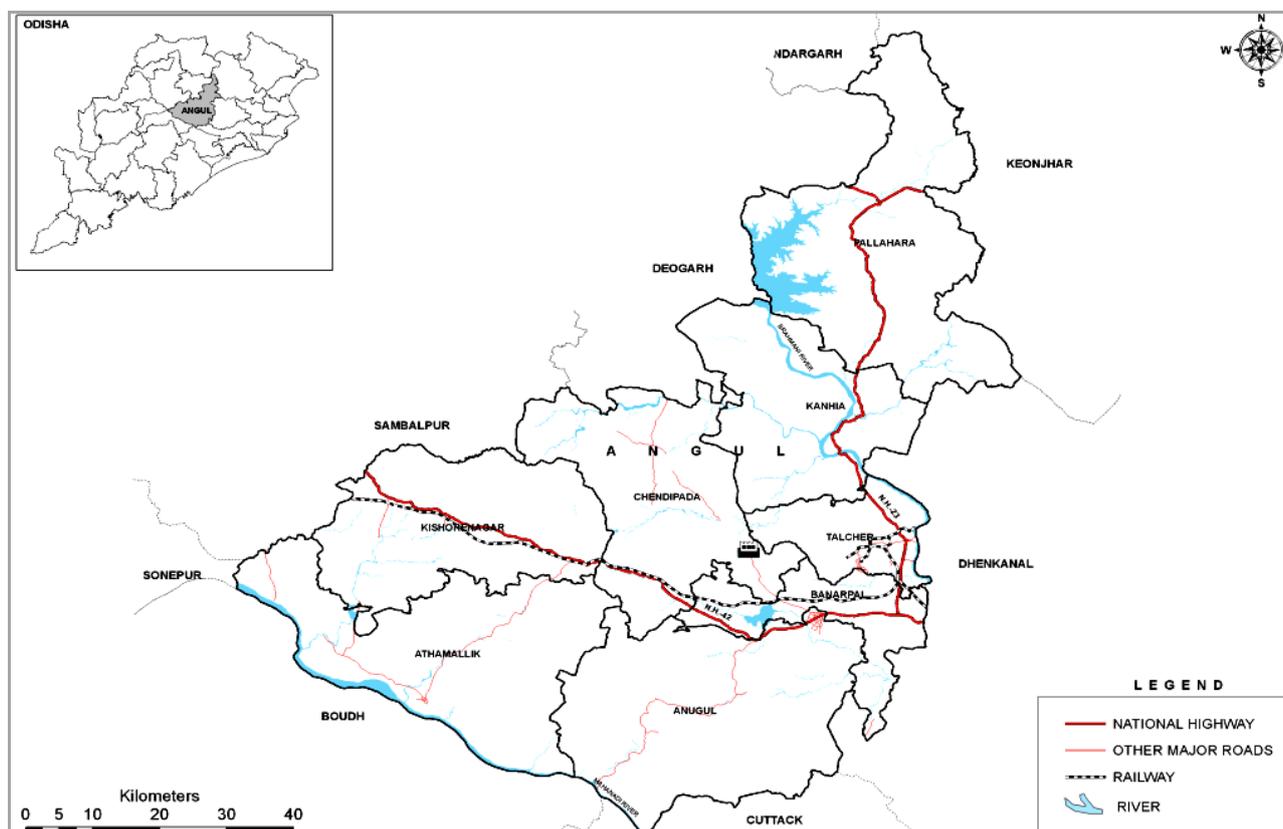


Table-1: Collection of effluent samples

Sl.No	Samples	Description	Color
1	IES-1	Discharge of industrial effluents into water	Turbid
2	IES-2	Nala site (flow of waste water)	Clear
3	IES-3	Before the meeting of discharge industrial effluents into water	Turbid
4	IES-4	Discharge of NTPC into water	Clear
5	IES-5	Waste water from Nandira river	Clear

The current study was carried out from industrial effluents discharged from five different areas of Bhusan Steel Plant (BSP) and National Thermal Power Corporation (NTPC) Ltd. located at Anugul district of Odisha, India. Anugul district lies between 20° 31' N and 21° 40' N latitude and 84° 15' E and 85° 23' E longitude where a large number of industries are present (Figure-1). The industrial effluents from various industries are released into the nearby water bodies as well as agricultural lands, making them highly polluted.

2.2 Collection of samples:

The industrial effluent samples (IES) were collected from five different sites of Anugul district of Odisha, India such as IES-1, IES-2, IES-3, IES-4 and IES-5 during the month of January, 2012 (Table 1). The effluent samples were collected in clean high density polythene (HDPE) bottles which were pre-cleaned with concentrated nitric acid (conc. HNO₃) and milli-Q water. During the collection of samples, the HDPE bottles were rinsed with the samples for inner surface absorption and three sets of bottles of each sample were collected from each site. The sample bottles were capped air-tightly and preserved by acidification with concentrated nitric acid (conc.HNO₃) and concentrated hydrochloric acid (conc. HCl) and brought to the laboratory of IMMT, Bhubaneswar, Odisha, India and stored at 4°C temperature for further analysis.

2.3 Chemical Analysis:

Physico-Chemical characterization of effluent samples:

pH and Electrical conductivity of the effluent samples were recorded by using a digital pH meter (Metzer Optical Instruments Pvt. Ltd) and Electrical conductivity meter (Hanna Instruments), respectively. Nitrate (NO₃), Nitrite (NO₂), Ammonia (NH₄), total nitrogen (N₂), total phosphorous (TP) and silicate (SiO₄) were analyzed to the standard methods of American Public Health Association APHA (2005).

Heavy Metal Analysis:

The concentration of different heavy metals present in the collected industrial effluents was analyzed by using Atomic Absorption Spectroscopy (Shimadzu, AA- 6300). All the five effluent samples were analyzed for the presence of different heavy metals like Cadmium (Cd), Lead (Pb), Zinc (Zn), Manganese (Mn), Copper (Cu), Nickel (Ni), Cobalt (Co) and Iron (Fe). Chromium (Cr) analysis was carried out by spectrophotometric method by using 1, 5 Di-Phenyle Carbazide APHA (2005).

2.4 Isolation of Fungi

Fungi were isolated from the industrial effluent samples by serial dilution using spread plate method on PDA (Potato Dextrose Agar) media plates. The media plates containing samples were incubated at 28° C for 48-72 hours. After the observation of growth the fungal isolates were further sub-cultured on PDA media to obtain pure culture and stored at 4°C in refrigerator for further study. The total fungal count was expressed in "Colony Forming Unit/ml" (CFU/ml) and the fungal isolates were labeled as AMTF (Anugul Metal Tolerant fungi) according to their collection sites and characteristics.

2.5 Screening of metal tolerance activity of the fungal isolates

Five different types of fungal strains were isolated from the five different effluent samples. Stock metal solution of 1000mg/L of Mn (II), Cr (VI), Zn (II) and Pb (II) were prepared by dissolving AR grade salt sources such as $MnSO_4 \cdot H_2O$, $K_2Cr_2O_7$, $ZnSO_4 \cdot 7H_2O$ and $Pb(NO_3)_2$ in double distilled water. The working metal solution was prepared from the stock solution.

The individual fungal isolates were inoculated into 100ml of specific Czapek's Dox growth medium and 10ppm of different heavy metal in a 250ml Erlenmeyer's flask. The inoculated flasks were incubated with control containing growth medium without fungal spores in rotary shaker at 180rpm at 30° C for 48-72 hours.

Chromium tolerance activity of the isolates:

For the Cr tolerance activity of the individual fungal isolates, Potassium dichromate ($K_2Cr_2O_7$) was used as a Cr source in the growth medium i.e. Czapek's Dox broth. The fungal isolates AMTF-1, AMTF-2, AMTF-3, AMTF-4 and AMTF-5 were inoculated into the individual liquid medium and incubated in a rotary shaker at 200rpm at 30° C for 48- 72 hours.

2.6 Optimization of Culture conditions:

Cr⁺⁶ concentrations:

The optimization of culture conditions was carried out for the potent fungal isolate at different concentrations of Cr such as 50ppm, 100ppm, 250ppm and 500ppm. The potent fungal isolate was inoculated into the liquid medium and incubated in a rotary shaker at 180rpm at 30° C for 72 hours. The Cr conc. left in the solutions was recorded at 0 hour, 24 hours, 48 hours and 72 hours, respectively.

pH:

The optimization study at different pH 5, 7 and 9 of the potent fungal isolate was carried out. Metal solutions were prepared from the stock solution and was added to the growth and

sterilized. The potent fungal isolate was inoculated into the liquid medium and incubated in a rotary shaker at 200rpm at 30°C for 72 hours. The optimization study was carried out by recording the chromium concentrations at 0 day, 1 day, 2 day and 3 day respectively.

2.7 Identification of fungal strains

The morphological and structural characteristics of the fungal colonies were studied using the standard microbiological methods. The microscopic study was carried out by the LPCB (Lacto Phenol Cotton Blue) staining and they were observed under microscope. Based on the morphological (upper and lower part of the culture plates) as well as microscopical (the conidial, hyphal and spore) structures, the fungal colonies were identified following the Key "Handbook of fungi" by Nagamani *et al.* (2006).

The most potent Cr tolerant fungal isolate was identified both by LPCB staining as well as Scanning electron microscopic (SEM) study.

3. RESULTS AND DISCUSSION

The results obtained from the study of industrial effluents from different sites of Anugul district, Odisha, India were as follows.

3.1 Chemical analysis of effluent samples

Physico-chemical analysis:

The effluent samples were clear in maximum samples except IES-1 and IES-3 which were turbid (Table-1). Various physico chemical parameters of industrial effluents such as pH, EC, total nitrogen, total phosphate, ammonia, nitrate, nitrite and silicate were analyzed (Table 2) . The pH of the all the effluents was within the permissible limit. Highest pH was recorded in the sample IES-1 (7.81) whereas lowest was in IES-4 (7.17). The electrical conductivity (EC) was in the range of 115- 332 ($\mu S/cm$). The total nitrogen content of the sample IES-3 was highest i.e. 1065.13 $\mu mol/L$ which may be due to the industrial wastes.

Table- 2: Physico-chemical properties of collected industrial effluent samples (Mean± SD)

Sample	pH	EC (µS/cm)	TN (µmol/L)	NH4 (µmol/L)	N03 (µmol/L)	N02 (µmol/L)	TP (µmol/L)	Si04 (µmol/L)
IES-1	7.81	332	585.20 ±0.36	63.27 ±0.97	0.25 ±0.005	0.05 ±0.007	4.73 ±0.30	25.49 ±0.29
IES-2	7.55	118	895.89 ±0.45	32.85 ±0.14	0.17 ±0.006	0.07 ±0.007	4.95 ±0.08	25.43 ±0.22
IES-3	7.58	321	1065.13 ±0.99	62.83 ±0.48	0.17 ±0.01	0.09 ±0.006	10.42 ±0.44	42.89 ±0.38
IES-4	7.17	115	1005.15 ±0.53	27.31 ±0.22	0.12 ±0.015	0.007 ±0.007	4.37 ±0.13	19.59 ±0.55
IES-5	7.35	162	488.19 ±0.27	14.35 ±0.47	20.92 ±0.06	0.01 ±0.003	2.43 ±0.188	61.43 ±0.26

Table- 3: Concentrations of different heavy metals present in the collected industrial effluent samples

Samples	Heavy metal concentrations in ppm								
	Cr ⁺⁶	Zn	Mn	Pb	Cd	Fe	Cu	Ni	Co
IES-1	2.0	5.091	2.325	0.427	1.375	2.325	2.608	2.547	0.501
IES-2	2.141	4.458	1.259	0.142	1.075	1.258	1.234	1.497	0.183
IES-3	2.361	5.762	2.521	0.665	1.49	2.521	2.853	2.697	0.648
IES-4	1.83	4.761	1.521	0.192	1.29	1.559	1.589	1.67	0.385
IES-5	1.92	4.879	2.104	0.332	1.35	2.104	2.538	2.033	0.476

The ammonia content varied from 14.35µmol/L to 63.27µmol/L. The nitrate content of IES-1 was 0.25µmol/L which was higher than other samples. The nitrite content of effluent samples varied from 0.007 to 0.9 µmol/L. The total phosphorous content was more in IES-3 as 10.50µmol/L. IES-5 contain 61.16µmol/L amount of silicate. IES-1 and IES-2 contain same 25µmol/L of silicate. IES-3 has high amount of silicate i.e. 42.60µmol/L and IES-4 was 19.56µmol/L of silicate.

Heavy metal analysis:

The collected industrial effluent samples contain different concentration of heavy metals as shown in Table 3. Among which most of heavy metals content was beyond the permissible limit. The concentration of all the heavy metals was maximum in IES-3 and minimum in IES-2.

Among all the heavy metals, four metals viz. Mn, Cr, Pb, Zn was present in higher concentration as well as beyond the permissible limit. The Zn concentration was in the range of 4.458 to 5.762 ppm. Highest Cr concentration was found in IES-3(3.361 ppm) sample whereas lowest in IES-2 (2.741 ppm). The concentration of Mn varied from 1.259 to 2.521 ppm whereas of that of Pb varied from 0.142 to 0.665 ppm.

3.2 Isolation and Screening of the fungal isolates for heavy metal tolerance

The total colony forming units (CFU/ml) of different samples were calculated. It was observed that the total CFU/ml was maximum, i.e. 4.5×10^2 CFU/ml in IES-5, whereas least in IES-3 i.e. 1×10^2 CFU/ml. IES-2 and IES-4 have almost same count, i.e. 3×10^2 CFU/ml

and 2.5×10^2 CFU/ml, respectively, and IES-1 has 1.5×10^2 CFU/ml (Figure-2).

The fungal isolates (five) were then screened for metal tolerance activity with various heavy metals like Mn, Cr, Zn and Pb. The heavy metals (Mn, Cr, Pb and Zn) accumulation was observed (Figure 3). From the study, it was found that the Cr uptake by all the fungal isolates was higher as compared to the other metals. The Cr uptake was maximum i.e. 1.78 ppm by AMTF-2 followed by AMTF-5 (1.50 ppm). In comparison to Mn, Zn and Pb uptake was 0.0008ppm, 0.3521 ppm and 0.5417 ppm, respectively by AMTF-2.

Figure 2: Colony Forming Unit (CFU/ml)

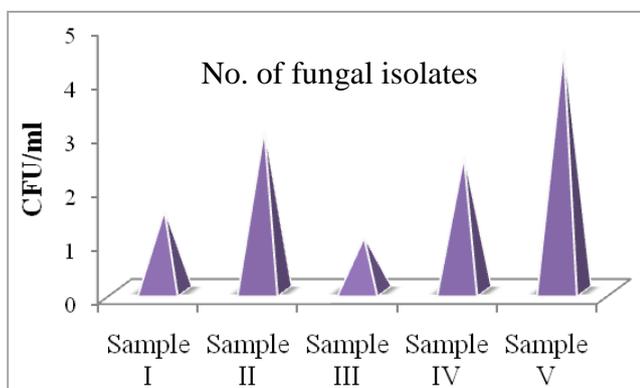
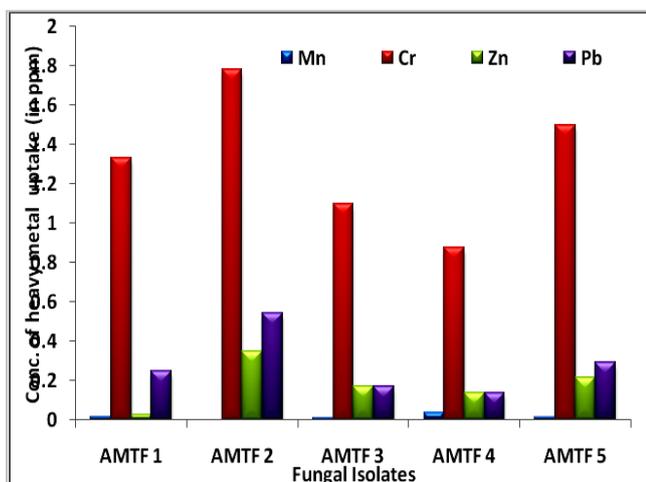


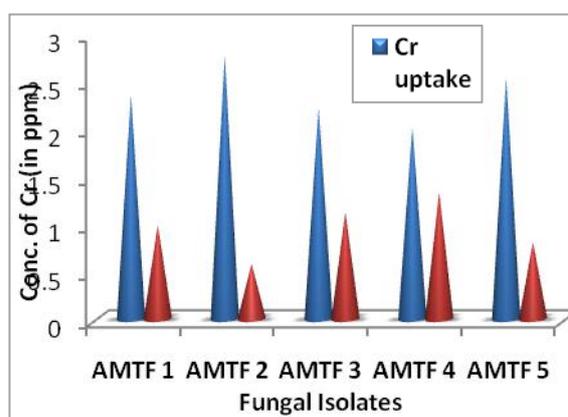
Figure 3: Metal tolerance activity of fungal isolates



3.3 Screening of the fungal isolates for Cr tolerance

The Cr tolerance activity was checked out with all the five fungal isolates. The concentration of Cr left out in the filtrate was minimum in case of AMTF-2 i.e. 0.577 ppm as compared to other fungal isolates (Figure-4). Results indicate that the ability of AMTF-2 was highest to accumulate or uptake Cr. The conc. of Cr left in the filtrate of AMTF- 4 was 1.3296 ppm whereas it was 1.1243 ppm in AMTF-3, 0.9875 ppm in AMTF-1, 0.8072 ppm in case of AMTF-5 (Figure-5). Therefore, this result indicates that the ability of AMTF-2 was highest i.e. 2.7679 ppm uptake of Cr in comparison to other isolates.

Figure 4: Cr tolerance activity of the fungal isolates



3.4 Optimization of culture conditions

Effect of various Cr concentrations:

The fungal isolate, AMTF-2 has higher potential to optimize Cr at various concentrations such as 50ppm, 100ppm, 250ppm and 500ppm (Figure 5). At 50ppm, the initial concentration was 2.756 ppm on day 0 and on day 3 it was reduced to 2.283 ppm.

Hence from the result it can be concluded that the percentage of reduction of Cr was 17%. Similarly at 100, 250 and 500 ppm the percentage of Cr reduction was 26.02%, 19.11 % and 20.60% respectively (Figure-6). Hence, from this study it can be concluded that at higher concentration the rate of reduction of Cr through indigenous fungal isolates was less. Among all isolates, AMTF-2 has the highest

potential to uptake chromium from the growth medium at regular intervals of time.

Figure-5: Effect of different Cr concentrations

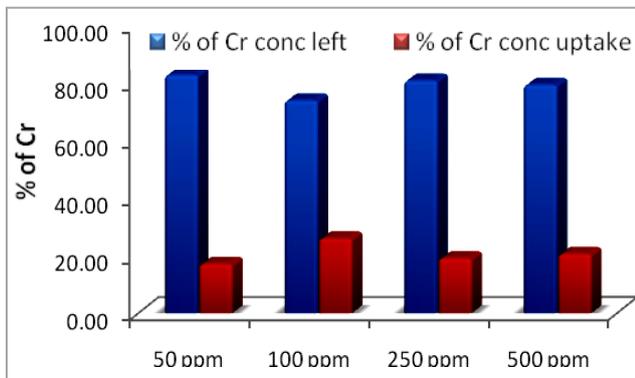
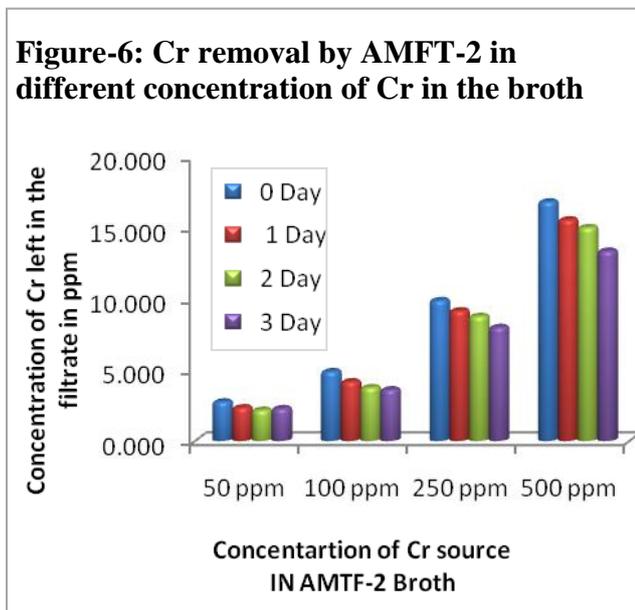


Figure-6: Cr removal by AMFT-2 in different concentration of Cr in the broth



Effect of pH:

The optimization study was carried out at different pH such as 5, 7 and 9 by shake flask experiment by the potent fungal isolate AMTF-2 (Figure 7). At pH 5 the conc. of Cr was 3.7904 and 2.1324 ppm at day 0 and 3 respectively, which was maximum in comparison with other isolates. Whereas at pH 7 it was 3.3431 & 2.0471 ppm and 3.9183 & 2.5706 ppm in pH 9 at day 0 and day 3 respectively. Hence, from the result, it can be concluded that the percentage of uptake of Cr was 43.74%. Similarly, at pH 7 and 9 the percentage of Cr reduction was 38.77 and 34.40% respectively (Figure 8). Hence, from this study it can be concluded that at higher concentration the rate of reduction of Cr through

indigenous fungal isolates was less. Among all isolates, AMTF-2 has the highest potential to uptake chromium from the growth medium at regular intervals of time.

3.5 Identification of fungal isolates

The fungal isolates were stained using LPCB (Lacto Phenol Cotton Blue) and observed under the microscope. The fungal isolates were identified according to their morphological and microscopically characteristics following the key by Nagamani *et al.*, (2006). The fungal isolates, AMTF-1, AMTF-2, AMTF-3, AMTF-4, AMTF-5 were identified as *Penicillium sp.*, *Aspergillus fumigatus*, *Aspergillus sp.*, *Penicillium adametzi* and *Aspergillus niger*, respectively.

From the scanning electron microscopic (SEM) study, the fungal isolate AMTF-2 was found to have globose conidia having rough surface. Hence it was identified as *Aspergillus fumigates* (Figure-9).

DISCUSSION

Removal of toxic heavy metals from the environment has become an important concern now a days. In the present study, industrial effluent samples were collected from different areas of Bhusan Steel Plant (BSP) and National Thermal Power Corporation (NTPC) ltd. located in Anugul district, Odisha, India. The industrial effluent samples showed the presence of various heavy metals like, Cr, Pb, Zn, Cu, Mn, Fe, Co, Ni, Cd and out of which four metals such as Mn, Cr, Pb, Zn were beyond the permissible limit. Similar study has been carried out in which, a variety of mechanisms exist for the removal of heavy metals from aqueous solution by bacteria, fungi, algae, ciliates, macrophytes and higher plants Holan and Volesky (1995).

A total of five indigenous fungal strains were isolated from the effluent samples. The sample, IES-2 was found to have highest fungal count i.e. 4.5×10^2 CFU/ml as compared to others. Then the fungal isolates were tested for metal resistance and they grew significantly at high

Figure 7: Effect of different pH

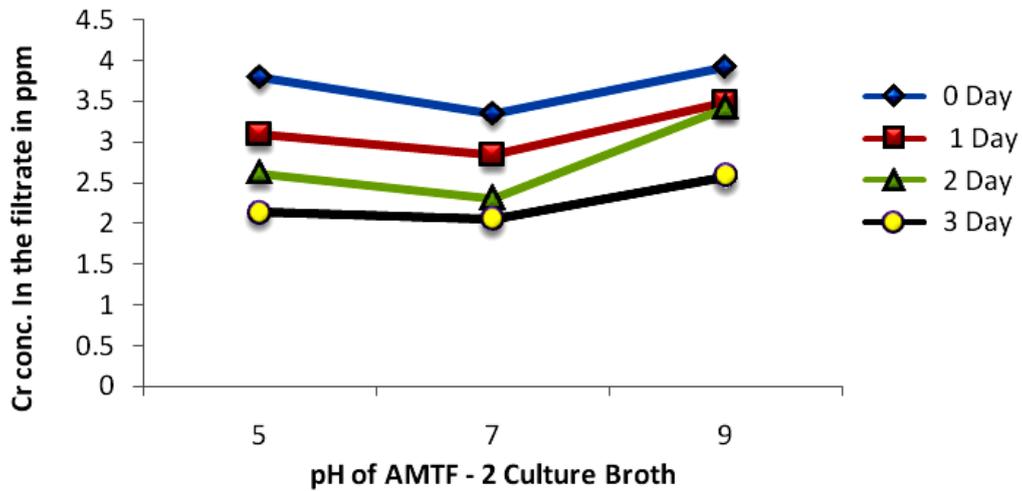


Figure 8: Cr removal by AMFT-2 in different pH

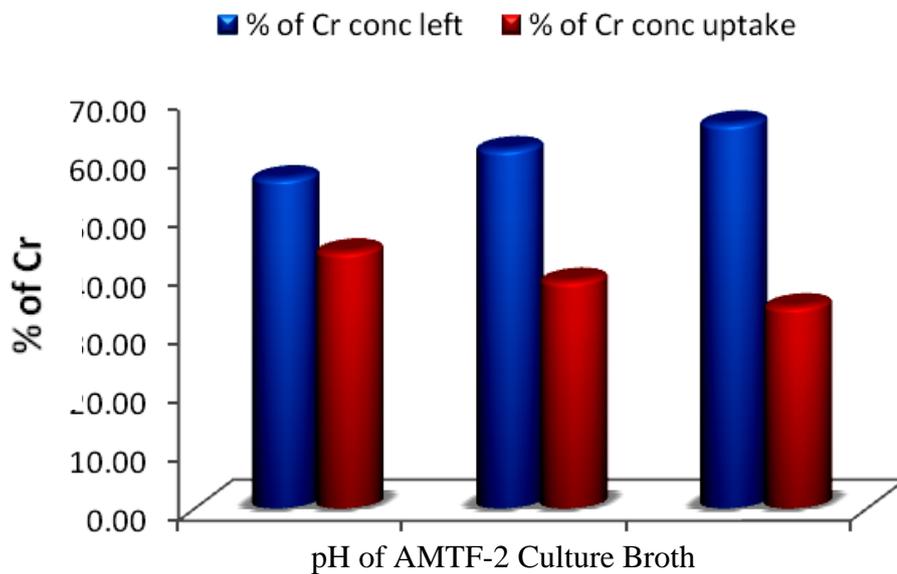
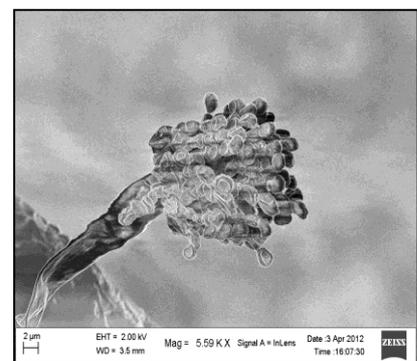
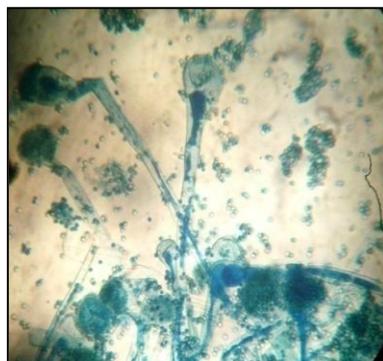


Figure 9 (A-C): (A) Pure culture of the fungal isolates AMTF-2, (B) Microscopic photograph of fungal isolates AMTF-2, (C) Scanning Electron Microscopic study of fungal isolate AMTF-2,



levels of Cr. The effect of heavy metals on fungal growth was depended on metal and its conc. in the medium. Biosorption of metals by the fungal isolates was determined as $\text{Cr}^{6+} > \text{Pb}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+}$. The fungal isolate AMTF-2 has a higher potential to accumulate a high amount of Cr from the medium. The present study showed that the fungal isolate could tolerate and uptake up to 26.02% of Cr at 100 ppm from the filtrate. Some hyper tolerant strains of *Aspergillus* sp. isolated from industrial effluents have the ability to remove chromium very effectively from liquid medium Mukherjee *et al.* (2009). In the present study, maximum Cr accumulation by AMTF-2 was found at pH 5 i.e. 43.74% after day 3 (72 hrs). Similar type of study was carried out by Aoyama and Tsuda (2001) in which maximum adsorption was recorded at pH 3. Optimal pH range for adsorption of Zn by *Aspergillus flavus* and *Aspergillus fumigatus* was 5.0 to 6.0 Faryal *et al.* (2006). Fungi are recognized for their superior aptitudes for the treatment of different waste water and metal from the contaminated samples Galun *et al.* (1983); Say *et al.* (2003); Leitao (2009). Filamentous fungi such as *Aspergillus*, *Rhizopus* and *Penicillium* species are frequently used in bioremediation processes GOMES *et al.* (1998) due to their metal uptake variation Saxena And Bhattacharyya (2006); BAJWA *et al.* (2010). In the present study, *A. fumigatus* (AMTF-2) is one of the potent strains for removal of Cr as earlier reported by Balakrishnan *et al.* (1994); Anita *et al.* (2013) and Niyogi *et al.* (1998) for removal of 82–100% of Ni from industrial waste in reactors. These fungal strains are a novel addition in the mycoflora of industrial effluents from Odisha. These fungal isolates can be further studied for bioaccumulation of heavy metal ions from different industries and other metal containing waste waters, and have a potential for use in a bioreactor for industrial discharge treatment, through the application of biotechnology.

CONCLUSIONS

Industrial effluents not only alter the surrounding soil chemistry, but also affect the micro and macro flora existing in such

environments. On the other hand, bacteria and fungi, due to exposure to a high load of metals and other contaminants become resistant and developed a potential to accumulate different heavy metals. The presences of toxic heavy metals in the water bodies make it highly polluted and affect the aquatic life. Various methods like ion-exchange, chemical reduction, reverse osmosis, etc are expensive and are not effective. Therefore, an alternative method of biosorption by using micro organisms has been carried out to remove the heavy metals from the water bodies. In the present study, the effluent samples were collected from five different areas of Bhusan Steel Plant (BSP) and National Thermal Power Corporation (NTPC) Ltd. The effluents were found to contain different heavy metals such as Cr, Zn, Mn, Pb, Cd, Cu, Ni and Co, which is the main cause of water pollution. Five types of fungi such as *Aspergillus* sp., *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* sp. and *Penicillium adametzi* were isolated from the effluents. Out of the five isolates, *Aspergillus fumigatus* have shown an excellent ability to accumulate chromium (Cr) up to 26.02% after 72 hrs. From the study, it can be concluded that fungi have high potential to remove the heavy metals from the toxic environment. Therefore, large no. of fungi can be isolated and cultured in order to reduce the high toxic concentration of heavy metals. So, the fungal isolates have a great potential to remediate not only industrial effluents, but can also be used for bioremediation of other waste waters and Cr contaminated sites as well. These serve as an eco- friendly tool in major aspects of bioremediation by which the environment can be cleaned up and ultimately leads to maintain a healthy life.

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