Effect of Chromium on *Mucor* species and optimization of growth conditions

Bijay Kumar Sethi[1], Satyajit Kanungo[1,*], Jyoti Ranjan Rout[1], Prativa kumari Nanda[2], Santi Lata Sahoo[1]
[1] Microbiology Laboratory, P.G Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Pin-751004, Orissa, India.
[2] Saila Bala Women's College, Cuttack, Orissa, India.
satya_9bt@yahoo.com, santi_bot_uu@yahoo.co.in

Abstract: Czapek Dox broth medium is frequently used for the culture of fungal species like *Mucor*. The influences of incubation period, pH, Cr (VI) concentration, temperature on the concentration of biomass were also evaluated. At pH 5.5, the fungi *Mucor* species yields maximum biomass and the fungus can be able to degrade chromium to a particular concentration but at higher concentration growth reduces. From a practical viewpoint, this abundant and inexpensive fungal biomass has potential application in the conversion of toxic Cr (VI) into less toxic or nontoxic Cr (III). Maximum biomass weight was observed which is about 0.33±0.01mg/20ml at a constant temperature of 35^0C with an incubation period of 8 days. The protein content of the fungus was estimated and it was found that maximum yield of protein was recorded in the presence of 0.005 mM of chromium. [Nature and Science 2010;8(4):29-32]. (ISSN: 1545-0740).

Key words: Biomass; *Mucor* species; Czapek Dox medium; incubation period.

1. Introduction

The majority of toxic metal pollutants are waste products of industrial and metallurgical processes. Their concentrations have to be reduced to meet ever increasing legislative standards according to World Health Organisation (WHO). The metals of most immediate concern are cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc. The effluent from metal finishing processes may contain up to 10 mg/L of metal dusts. Usually, when using methods such as chemical precipitation, reverse osmosis for the removal of metal ions from dilute aqueous stream, incomplete metal removal can be obtained. Furthermore, these processes have high reagent or energy requirements and generate toxic sludge that requires careful disposal (Wild et al, 1987). The need for cost-effective process and safe method for removing heavy metals from discharging effluents has resulted in search for other unconventional materials such as organic or inorganic sorbents (Allen et al, 1998). The use of microbial biomass of fungi (Kapoor and Viraraghavan, 1995) and bacteria for removal of heavy metals from aqueous solutions is gaining increasing attention. It has been found that both living and death microbial cells adsorb metal ions. Chromium is a transition metal most commonly found in the environment in its trivalent Cr (III) and hexavalent Cr (VI) forms (Anderson, 1997). Naturally occurring Cr is almost exclusively in the trivalent state, as the energy required for its oxidation is high. Hence, the hexavalent form is usually considered to be a man-made product (Bai and Abraham, 2001). The toxicities of the two forms of chromium are vastly different. Trivalent chromium is generally a nontoxic, non-mobile micronutrient (Bai and Abraham, 2002). Hexavalent chromium is water soluble, toxic, and carcinogenic, and is considered a pollutant by the United States Environmental Protection Agency (EPA) (Bai and Abraham, 2003). Chromium is the second most common inorganic contaminant of groundwater at hazardous waste sites (Baral and Engelken, 2002). The solubility and negative charge of it's more common forms, chromate and dichromate lead to limited adsorption in aquifer minerals, and results in high mobility of Cr(VI) in aquifers (Barnhart, 1997). The historical and present day contamination of groundwater and soils by Cr (VI) is a result of its industrial uses, including metal plating (for corrosion resistance), pigment production, and lumber and wood products (for preservation) (Clesceri, 1998).

Metal contaminants are commonly found in soils, sediments, and water. Metal pollutants can be produced through industrial processes such as mining, refining, and electroplating. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability

of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species. Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination. Of the various toxic heavy metals discharged into the environment hrough various industrial wastewaters, constituting one of the major causes of environmental pollution, chromium is one of the most toxic and has become a serious health concern. Extensive use of chromium, e.g., in electroplating, tanning, textile dyeing and as a biocide in power plant cooling water, results in discharge of chromium-containing effluents (Barnhart, 1997). The effluents from these industries contain Cr (VI) and Cr (III) at concentrations ranging from tenths to hundreds of milligrams/litre. While Cr (VI) is known to be toxic to both plants and animals, a strong oxidizing agent and a potential carcinogen, Cr (III) is generally only toxic to plants at very high concentrations and is less toxic or nontoxic to animals (Anderson, 1997).

However, none of these methods are completely satisfactory and all feature due to the following disadvantages: (1) generation of a large amount of secondary waste products due to various reagents used in a series of treatments such as reduction of Cr (VI), neutralization of acidic solution and precipitation, and (2) instability of ion-exchange resins due to serious oxidation by hexavalent chromium. Thus, the development of new, cost-effective, more environmentally friendly methods is needed. Biosorption of heavy metals by biomaterials has been suggested as a potential alternative to the existing physicochemical technologies for detoxification and recovery of toxic and valuable metals from wastewaters. Many biomaterials such as seaweed (Kratochvil et al, 1998; Yun et al, 2001), micro-algae (Gupta et al, 2001), fungi (Kapoor and Viraraghavan, 1995) and various other plant materials (Raji and Anirudhan, 1998; Sharma and Forster, 1993) have been studied for their chromium binding abilities. Particularly, fungal biomass can be cheaply and easily procured in rather substantial quantities, as a by product from established industrial fermentation processes. Furthermore, since such abundant dead fungal biomass is of little use, it has been identified as a potential source of biomaterial for the removal of chromium from wastewaters. The objective of the present investigation is to clarify the mechanism that governs Cr (VI) removal by fungal biomass and to evaluate the effects of various parameters such as contact time, pH, initial Cr (VI) concentration, biomass concentration and temperature. Furthermore, the potential of fungal biomass for the detoxification of Cr (VI) is discussed.

2. Materials and Methods:

2.1 Collection of soil sample:

Soil sample was collected from the garden of P.G Dept of Botany, Utkal University, near the waste dumping site since this soil may contain enormous number of saprophytic fungi.

2.2 Isolation of Organism:

A local isolate of *Mucor* species as used in this study was isolated from the soil using serial dilution technique. It was maintained on Potato Dextrose Agar medium (PDA) (Hi-media, Mumbai). The slants were grown as 30^0C for 7 days and stored at 4^0C for further study.

2.3 Inoculum Preparation:

10 ml of sterile distilled water containing 0.01% triton-X 100 was transferred to a sporulated (7 days old) PDA slant culture. The spores were dislodged using the sterile inoculation needle under aseptic condition and the suspension with appropriate dilution ($1X10^7$spores/ml) was used as inoculum throughout the experiment.

2.4 Establishment of medium and growth conditions:

The isolated *Mucor* species was grown in sterilized Czapek Dox broth medium for establishment of the optimum temperature, pH and incubation period that supports the exuberant growth of *Mucor* species. Hence, *Mucor* species was grown in Czapek Dox broth medium in temperature ranging from 20^0C-40^0C, pH ranging from 4 -12 and incubation period from 1day-10 days.

2.5 Determination of biomass:

The biomass formation was determined by cell dry weight measurement of 20 ml culture samples. The samples were filtered through dried and pre- weighed filter paper (Whatman No. 1), followed by washing twice with distilled water and then drying at 80^0C to constant weight. The growth responses ware measured in the form of the biomass produced under certain conditions.

2.6 Determination of soluble protein:

The concentration of soluble protein was estimated by Lowry et al. (1951) using Bovine serum albumin as the reference standard.

2.7 Statistical analysis:

Each experiment was carried out in five replicates. From this, arithmetic means, standard errors of mean

(SEM) were calculated and graphs were plotted using MS-Excel.

3. Results and Discussion:

From the mixed culture, *Mucor* was identified by its specific colony characteristics, colour and microscopic features. The optimum pH for the proper growth of *Mucor* was finally found to be at pH-5.5 where the biomass weight was about 0.33±0.01mg/20ml at a constant temperature of 35^{0}C (Table 1). Maximum biomass (0.35±0.02 mg/20ml) was obtained at 35^{0}C in an incubation of eight days. (Table 2). The fungus Mucor species showed maximum biomass (0.65±0.01mg/20ml) when incubated for 8 days. (Table 3).

pH Range	Biomass in (mg/20ml)
4.0	0.22±0.01
4.5	0.26±0.02
5.0	0.31±0.01
5.5	0.33±0.01
6.0	0.29±0.03
6.5	0.28±0.04
7.0	0.24±0.03
8.0	0.23±0.02
9.0	0.21±0.03
10.0	0.19±0.01
11.0	0.18±0.01
12.0	0.16±0.02

Table 1. Effect of various pH on biomass (mg/20ml) growth of *Mucor* species.

Temperature	pH	Biomass in (mg)
20^{0}C	5.5	0.19±0.01
25^{0}C	5.5	0.24±0.01
30^{0}C	5.5	0.29±0.03
35^{0}C	5.5	0.35±0.02
40^{0}C	5.5	0.25±0.04

Table 2. Effect of various Temperatures on growth of *Mucor* species at pH 5.5.

Incubation period (days)	Biomass in mg./20ml
1	0.04±0.01
2	0.12±0.01
3	0.26±0.02
4	0.37±0.03
5	0.44±0.02
6	0.52±0.01
7	0.58±0.03
8	0.65±0.01
9	0.61±0.01
10	0.59±0.03

Table 3. Effect of various incubation periods on growth of *Mucor sp.* at 35^{0}C and pH 5.5

Further investigation on *Mucor* species was carried out in the presence of chromium in the medium such as when the pure culture of *Mucor* was treated with various concentration of chromium, it was noticed that growth of *Mucor* biomass was obtained up to 0.020 mM concentration of chromium (VI) in the medium and further increase in the concentration reduced the biomass. This may be due to the tolerance of toxicity up to 0.020mM concentration of chromium and further addition of chromium may be acting as toxic for the growth of the organism (Table 4).

Concentration of Chromium (VI) in mM	Duration in days	Biomass in (mg)
Control	8	0.15±0.01
0.005	8	0.23±0.06
0.010	8	0.27±0.04
0.015	8	0.35±0.02
0.020	8	0.42±0.01
0.025	8	0.31±0.01
0.030	8	0.26±0.05
0.035	8	0.19±0.02
0.040	8	0.16±0.04

Table 4. Effect of different Chromium (VI) concentration in the culture media on biomass (mg/20ml) of *Mucor* species in 8 days of incubation.

Analysis of protein content showed 0.78 mg/ml protein at a concentration of 0.005 mM of chromium in the sample and gradually protein content was reduced when organism was grown in higher concentration of chromium as shown in the figure given bellow.

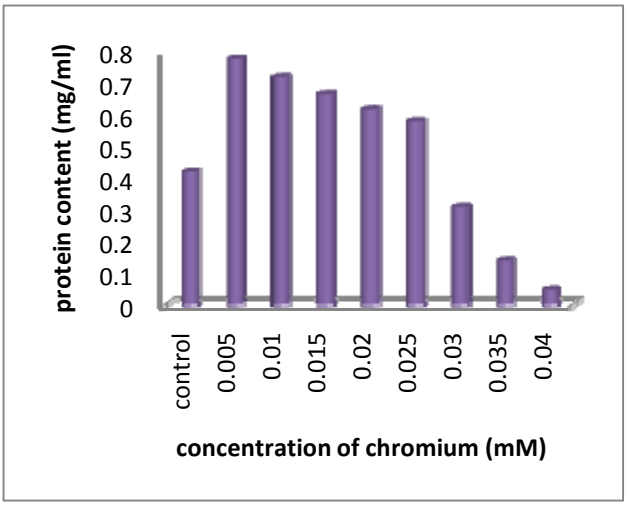

Figure 1. Effect of chromium on protein content of *Mucor* species on 8th day of incubation.

Chromium contamination of the environment has

become an important issue due to the potential health threat it poses. Conventional technologies to clean up heavy metal ions from contaminated waters have been utilized, but they remain cost-ineffective. Therefore, the use of dead fungal biomass for the detoxification of Chromium (VI) from contaminated waters may be a novel and cost-effective alternative. The use of dead fungal biomass has the following advantages: it is abundant and very cheap, the process does not require a continuous nutrient supply for maintaining the cells in good physiological conditions, and dead cells are not subjected to physiological constraints such as metals toxicity. Mucor was able to bioremediate the chromium present in the medium at lower concentration. Hence it can be used to treat the effluents containing chromium at a lower cost and it is also eco-friendly so it will not hamper to the environment.

Acknowledgement:

This work was financially supported by the UGC-RGNF, New Delhi. The authors are also grateful to the Head of the Department, P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar for providing the necessary laboratory facilities.

References:

[1] Allen HE, Garrison AW, Luther GW. Metals in Surface Waters. Chelsea, Michigan, Ann Arbor Press: 1998: 262-267.

[2] Anderson RA. Chromium as an essential nutrient for humans. Regul. Toxicol. Pharmacol. 1997: 26 (1): S35–S41.

[3] Bai RS, Abraham TE. Biosorption of Cr (VI) from aqueous solution by Rhizopus nigricans. Bioresour. Technol. 2001:79 (1): 73–81.

[4] Bai RS, Abraham TE. Studies on chromium (VI) adsorption-desorption using immobilized fungal biomass. Bioresour. Technol. 2003: 87 (1): 17–26.

[5] Bai RS, Abraham TE. Studies on enhancement of Cr (VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Res. 2002: 36 (5): 1224–1236.

[6] Baral A, Engelken RD. Chromium-based regulations and greening in metal finishing industries in the USA. Environ. Sci. Policy 2002: 5 (2): 121–133.

[7] Barnhart J. Occurrences, uses, and properties of chromium. Regul. Toxicol. Pharmacol. 1997:26 (1): S3–S7.

[8] Clesceri LS, Greenberg AE and Eaton AD. *Standard methods for the examination of water and wastewater.* 20th ed. American Public Health Association, Washington, 1998; 1325 p.

[9] Clesceri LS, Greenberg AE, Eaton, AD. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, 1998: pp 366–368.

[10] Gupta VK, Shrivastava AK, Jain N. Biosorption of chromium (VI) from aqueous solutions by green algae Spirogyra species. Water Res. 2001: 35 (17): 4079–4085.

[11] Kapoor A, Viraraghavan T. Fungal biosorption-an alternative treatment option for heavy metal bearing wastewaters: a review. Bioresour. Technol. 1995: 53 (3): 195–206.

[12] Kratochvil D, Pimentel P, Volesky B. Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environ. Sci. Technol. 1998: 32 (18): 2693–2698.

[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951:193:265-75.

[14] Raji C, Anirudhan TS. Batch Cr (VI) removal by polyacrylamide-grafted sawdust: kinetics and thermodynamics. Water Res. 1998: 32 (12): 3772–3780.

[15] Sharma DC, Forster CF. Removal of hexavalent chromium usingsphag num moss peat. Water Res. 1993: 27 (7): 1201–1208.

[16] Wild J, Barnes D, Forster CF, Hrudey SE. Liquid Wastes from the Metal Finishing Industry. John Wiley and Sons, New York 1987: pp. 21–62.

[17] Yun, YS, Park D, Park JM, Volesky, B. Biosorption of trivalent chromium on the brown seaweed biomass. Environ. Sci. Technol. 2001: 35 (21): 4353–4358.

10/1/2010