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Effect of Ultravoilet Light on Chromium Tolerant Isolate of Aspergillus Niger

Shazia Iram[1], Sumera Abrar[1], Iftihkar Ahmad[2], Ehsan Akhtar[2] and Naila Shuja[2]

[1]Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi.

[2]PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Center Islamabad.

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ABSTRACT

Chromium is the third most toxic heavy which can be removed from the environment by naturally occurring microbes. There is an increasing interest to enhance the bioremediation potential of microorganism; this study is focused on using UV radiation (258 nm) to increase the bioremediating efficiency of chromium tolerant Aperillus niger, isolated from peri-urban agricultural area of Kasur. UV light has a tendency to cause DNA mutations in microbes that may lead to point mutation which in turn can contribute towards tolerance against heavy metals. The conidia of K14 isolate of A.niger were exposed to UV light at the distance of 0.5m from the source and time duration of 1-6 hours. The behavior of A.niger with and without Petri plate cover under different doses of UV light was studied. Control strain was left unexposed to UV light. All the variants after exposure to UV light decreased the growth rate but the variant exposed for 5 hours showed contrasting effect as the growth significantly increased hence point mutations were caused when the Petri plate cover was removed. The mutant variant (irradiated for 5 hours) was taken for further analysis of heavy metal tolerance, biosorption and effect of different media conditions including pH, temperature, batch time and different $Cr(NO_3)_3$ concentrations. The rate of uptake of $Cr(NO_3)_3$ by the mutant variant was faster as compared to the control, whereas there was no significant difference in the biosorption potential of both the variants. Optimum biosorption conditions have been found for both the control and mutant variants at pH 6, temperature 30°C, and 2 hour as optimum retention time for control and 1 hour for the mutant variant. It was concluded that A.niger was differently affected by different time exposure of UV radiation (258nm) with reference to changes in its growth rate, uptake of metal and its tolerance potential and the rate of uptake of chromium was faster for the mutant variant.

Introduction

Heavy metals are present naturally in the earth's crust and are the result of anthropogenic activities (Symon et al., 1986). Thus the elimination of such compounds is essential due to their intense toxicity in environment (Kapoor et al., 1999), which can be done with the help of biomass of fungus, algae and bacteria (Ahalya et al., 2003). Various species of the fungus including genus such as Aspergillus, Penicillium and Rhizopus are known to be very efficient in bioremediation of different heavy metals from contaminated sites in both living and dead form (Kapoor & Viraraghvan, 1995).

The mode of action of UV light explicates its impact on microorganisms. When microbes are exposed to such type of radiations inside cells like bacteria and fungi, UV light can cause DNA mutations. It may lead to point mutation that may contribute to the tolerance to heavy metals (Todar, 2011).

Chromium is available naturally as well as anthropogenic production of chromium is also present in the environment. It is the third most toxic heavy metal that is a threat to the environment globally. The estimated global impact of Cr is on 13-17 million people (Tariq et al., 2005). Due to easy access and low cost many underdeveloped and developing countries increased the production of leather from 35-60% from 1970-95. Tanning services are exceedingly exercised in Nepal, Bangladesh, and India, plus constantly causing unfavorable effects to the environment and human health in Southeast Asia, South America, and Africa (Dhabi et al., 2002). According to a survey conducted by Blacksmith Institute (2010), 75% of the chromium contaminated sites are situated in South Asia. India and Pakistan are on top for the chromium contamination due to the presence of highest number of tannery industries. Moreover, South America was also found to be at a threat of massive number of people being affected by the chromium contamination (Megaraj et al., 2003). An assessment report by the Federal Environmental Protection Agency, stated that the tanneries situated in Sialkot and Kasur are releasing effluents with the concentration of chromium ranging between 182-222 ppm which is in opposition to the standards of 1ppmr by NEQS. The residents of Rawalpindi are also being adversely affected by the discharge of chromium loaded effluents in the local water bodies by a chromium salt producing industry located in the area (Malik et al., 2010). The concentrations of chromium and lead are significantly high in various drinking water supplies in Karachi. The level of lead contamination is extremely high in approximately all the water sources of the city. Furthermore, six areas of Karachi had levels of chromium higher than WHO acceptable limit (50ppb). The occurrence to any one heavy metal in soil or water facilitates the presence of other metals, since vital association was established among both the heavy metals representing the likelihood of similar sources for contamination in the city (Rizwan et al., 2009).

Fungus can survive in intense stressful environments like, lack of nutrients, acute pH and temperature, exposure of UV light in addition to occurrence of high concentrations of heavy metals. The presence of cell wall escorts to enhancement of the

Tele:

E-mail addresses: sumeraabrar75@gmail.com

metal binding sites in fungus (Gupta et al., 2000), where as the presence of the melanin content in the filamentous fungus leads to the resistant towards UV light (Anand et al., 2006). Typically the exposure of the ultraviolet (UV) light to the living organisms result in damaging consequences. On the contrary, the amount of production of yeast increased after the exposure to UV light. The increase in the yeast production was due to the release of nitrogenous material (amino-N substance) from the medium on which yeast was cultured, which helped the growth and reproduction of yeast cells (Highlands, 2011). The rate of fermentation brewer's yeast was reported to be faster after the exposure to ultra violet light (Taneer et al., 1923). The aim of the present study was to enhance the bioremediating efficiency of chromium tolerant strain of Aspergillus niger isolated from the heavily contaminated soil of Kasur by UV light.

Materials and Methods

Source of fugal culture and effect of UV light on Growth of Aspergillus niger

Isolate of Aspergillus niger (K14) was isolated from the contaminated soils of Kasure. The conidia of the 24 hour grown fungi were placed as the material for irradiation. The source of the UV light was from the tube light present in the laminar flow of 258nm of the wavelength. The distance between the exposed Petri plates and UV light was 0.5m. The fungus was exposed in the presence and absence of the cover of the Petri plate. The exposure time duration was 1, 2, 3, 4, 5 and 6 hours as (a, b, c, d, e and f variants). The irradiates were labeled as K14a, K14b, K14c, K14d, K14e and K14f respectively and the non-irradiated (control) K14. Petri plates were incubated for 7 days and radial growth (cm) was recorded on daily basis and death curve was established. Each irradiated A.niger variant was tested in 3 replications (Petri dishes) in order to study the changes in the behaviour and growth after the exposition to different time exposures to UV light. The variants showing enhanced growth after the exposition to UV (K14e) was selected for the tolerance analysis and biosorption potential in comparison to the non-irradiated control (K14).

Heavy metal tolerance analysis of mutant variant of A. niger Fungal variant, K14e of Aspergillus niger was tested for its tolerance potential against different concentrations of $Cr(NO_3)_3$. Potato Dextrose Agar media was amended with different concentrations of $Cr(NO_3)_3$ (500, 1000, 1500 and 2000 ppm) and was used for the comparison of the tolerance efficiency by irradiated and non irradiated variant of A.niger. For tolerance analysis of wild and mutant variants, inoculums of test fungi were stippled in centre of metal containing plates. Then the plates were incubated at 29ºC for 7 days. The growth of fungi was monitored from the point of inoculation. Tolerances of fungi were studied by the comparison the level of metal uptake by control and irradiated variants of A.niger. Different parameters of tolerance analysis were measured i.e., analysis of death curve, radial growth of test fungi, mean radial growth of fungi and tolerance index.

Biosorption analysis of mutant variant of A. niger

The biosorption potential of K14e was evaluated. The adsorbent was prepared by culturing discs of A.niger of 0.5cm size in Potato Dextrose broth at 29˚C and 111 rpm speed of the rotator shaker for 7 days. Thick biomass was obtained on the 7th day which was then filtered, dried and crushed, dead biomass was used as adsorbent. Initial concentrations were prepared by dissolving $Cr(NO_3)_3$ in deionized water separately to give different metal concentrations. All glass wares were washed with 0.1 M HCl before and after each experiment to avoid binding of the metal to it. Different concentrations of (500, 1000, 1500 and 2000 ppm) metal solution were added in the same medium. After the preparation of the metal solution, 0.5 gm of dead fungal biomass was suspended into 50 ml of metal solution in 250 ml conical flask. The flask was then agitated on the rotatory shaker for four hours at 111rpm at room temperature and samples were collected after each hour. The initial pH of the metal solutions was adjusted to 5.6 (Kapoor & Viraraghavan, 1995). After each experiment, the mixture was filtered through Wattman No. 1 filter paper and evaluated for the amount of metal concentration present in the filtrate by atomic absorption spectrophotometer (Javaid & Bajwa, 2008).

The effect of pH on biosorption capacity of control and irradiated isolates (A.niger) was studied at different pH i.e. 4, 6, & 9, with time interval of 2 hours on rotary shaker at the concentration on which maximum adsorption was recorded. To analyse the effect of temperature and find the optimum temperature for biosorption capacity of A.niger, 1gm (control and irradiated isolates each) of biomass was studied at different temperatures i.e., at 22˚C, 30˚C and 37˚C with the time interval of 2 hours on rotary shaker. Analysis was done by Langmuir and Freundlich isotherm.

Results

Effect of UV light on growth of Aspergillus niger

The effect of different doses of UV light on the growth of fungus was analyzed with and without the Petri plate cover. The fungi were exposed with the time interval of hours i.e., 1, 2, 3, 4, 5 and 6 hours with Petri plate cover. The recorded data for the 7 days showed that with the increasing exposure to UV light the growth of the fungus simultaneously decreased. Control culture showed the best growth (Fig 1).

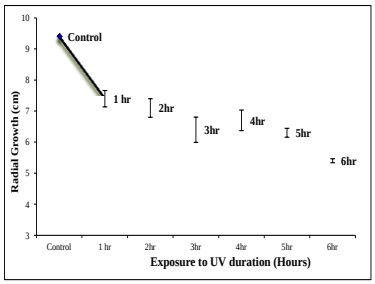

Fig 1. Growth response of A. niger (K14) after the exposure to the UV light for 1-6 hours with Petri plate cover

Exposure of the isolate K14 to different doses of the UV light caused the fungal growth efficiency decrease significantly in subcultures K14a, K14b, K14c, K14d and K14f (UV exposure after 1, 2, 3, 4 and 6 hours respectively), but it increased in K14e (at 5 hours) hence point mutation was caused. The growth of all UV light variants lag behind control variant K14 except K14e. The premier considerable variation was recorded between the control K14 and K14d and K14f. As the radial growth of the strain K14e was found to be in contrast to the depleting growth by increasing the exposure to UV light, so this culture was selected for further analysis of metal tolerance and biomass production (Fig 2).

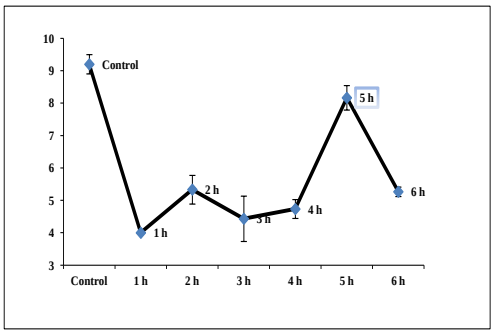

Fig 2. Growth response of A.niger (K14e) after the exposition to the UV light for 1-6 hours without Petri plate cover

Tolerance Analysis

The exposed K14e UV variant (irradiated for 5 hours) was further selected for the comparison of tolerance analysis with control (non-irradiated) variant at different concentrations of Cr $(NO_3)_3$ i.e., 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. These results indicated that the radial growth of the irradiated variant was higher as compared to the control. The incubation period for Aspergillus niger was 168 hours (7 days). Both the variants showed maximum growth at 1000 ppm, the density was observed to be very thick and dark black in color (Fig 3).

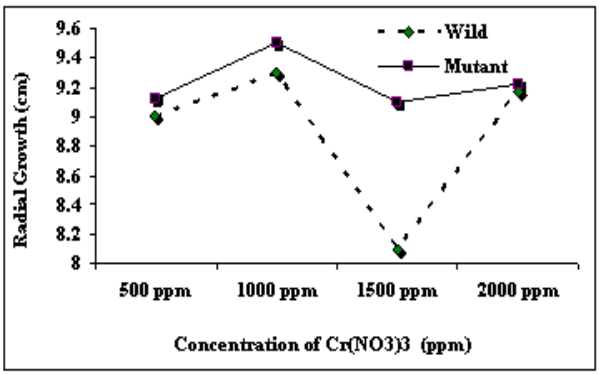

Fig 3. Radial growth (cm) of A.niger wild and mutant variants against Cr $(NO_3)_3$

The spore size also increased at higher concentration. Growth rate was declined at 1500 ppm of the metal. The irradiated variant was found to be more tolerant towards chromium nitrate as compared to the non-irradiated variant. Tolerance index of non-irradiated variant of A.niger was found to be most tolerant at 2000 ppm and least tolerant was shown at 1500 ppm, 1000 ppm and 500 ppm (Table 1). The mutant variant is found to be most tolerant at 500 ppm, followed by 2000 ppm>1000ppm>1500ppm respectively (Table 2).

Biosorption potential of control and mutant variants of A.niger

Effect of different chromium concentrations (500, 1000, 1500 and 2000 ppm) on non-irradiated variant of A.niger batch was conducted for 4 hours at 29°C and sample was collected at the interval of one hour for chromium estimation. Maximum adsorption was achieved at 2 hour by all concentrations of metal. At 500 ppm the equilibrium maintains after 3 hour and the metal uptake is to about 81% and the maximum adsorption was achieved after 2 hours i.e., 98%. At 1000 ppm equilibrium maintains after the time period of 2 hour and metal uptake was 100% and then gradually decreased with increase in the time and similar adsorption pattern was observed at 2000 ppm and 1500ppm, maximum adsorption (94%) was achieved at 2 and 4 hour (fig 4).

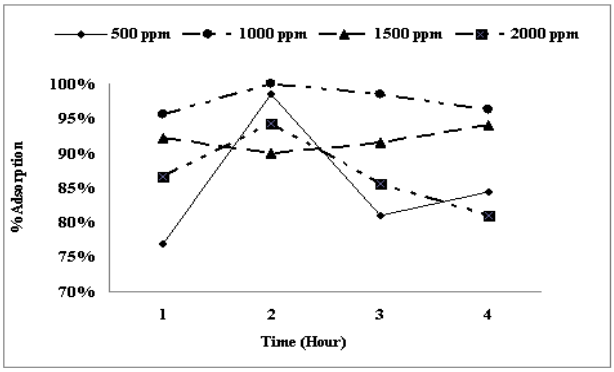

Fig 4. The plot of residual $Cr(NO_3)_3$ concentration with time by dead biomass of Control variant of A.niger

Similar concentrations, temperature and time period was tested for the uptake of metal for the irradiated variant of A.niger and maximum adsorption was achieved for almost all the concentrations at 1 hour (fig 5). Maximum amount of chromium uptake was recorded at 1500 ppm in the time period of 1 hour which was about 98.8%, and then gradually the uptake rate reduced with the increasing time period. Similarly the equilibrium was maintained at the time period of 3 hour and the metal uptake was about 94%, for the 1000 ppm and then decreased at 4 hour. However, the maximum level of metal uptake by irradiated variant at 500 ppm and 2000 ppm, increased with increase in the time. Maximum level of metal uptake (83% and 87%) was recorded for 500 ppm and 2000 ppm at 4 hour respectively.

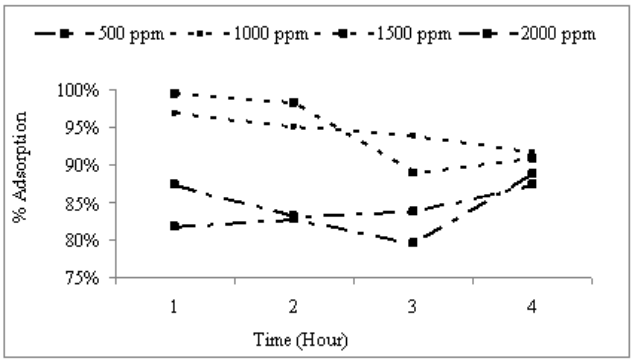

Fig 5. The plot of residual $Cr(NO_3)_3$ concentration with time on irradiated variant of A.niger

The removal of chromium by non-irradiated and irradiated variant at three different temperatures i.e., 22°C, 30 °C and 37 °C and maximum initial adsorption rate of Cr by the non-irradiated and irradiated variant was 83% and 79% at 30°C (fig 6).

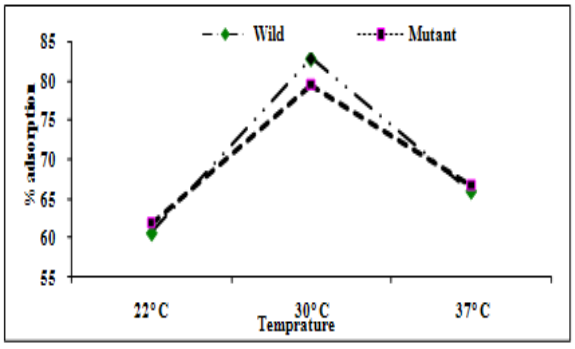

Fig 6. Effect of initial temperature on the equilibrium Cr $(NO_3)_3$ uptake by non-irradiated and irradiated variant of A.niger

Table 1. The tolerance index of non-irradiated variant against different concentrations of Cr(N03)3

$Cr(N0_3)_3$ Conc.	Tolerance index
500 ppm	1.068
1000 ppm	1.051
1500 ppm	0.987
2000 ppm	1.645

Table 2. The tolerance index of mutant variant against different concentrations of $Cr(N0_3)_3$

$Cr(N0_3)_3$ Conc.	Tolerance index
500 ppm	1.021
1000 ppm	1.103
1500 ppm	0.969
2000 ppm	1.048

pH is one of the most vital factors that persuade the sorption potential of the adsorbent. The best pH for the biosorption was 6. The equilibrium uptake of Cr(NO3)3 was 98% for the non-irradiated variant and 99.3% for the irradiated variant (fig.7). Langmuir and the Freundlich models are statically significant isotherm models for the analysis of the metal uptake. The Freundlich model (at 95% confidence level) was best fitted isotherms to experimental data than Langmuir model and 100% adsorption was achieved at 1000 ppm by wild strain after 2 hour (Fig 8,9,10,11).

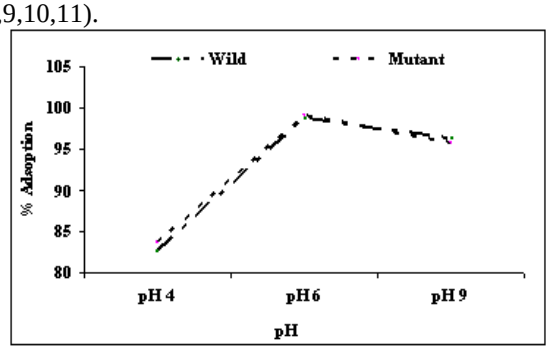

Fig 7. Effect of pH on non- irradiated and irradiated variants of A.niger

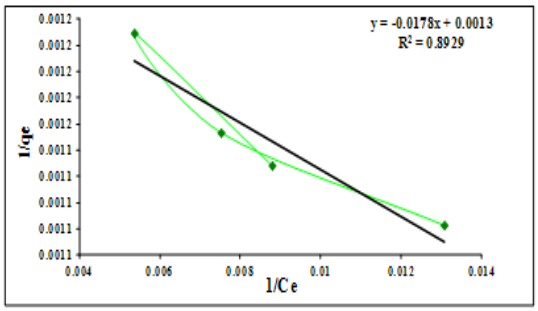

Fig 8. Langmiur model for biosorption of Cr on dead variant of wild A.niger at 1000ppm

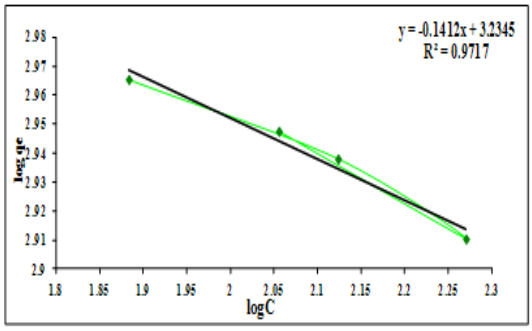

Fig 9. Freundlich model for biosorption of Cr on dead variant of wild A.niger biomass at 1000ppm

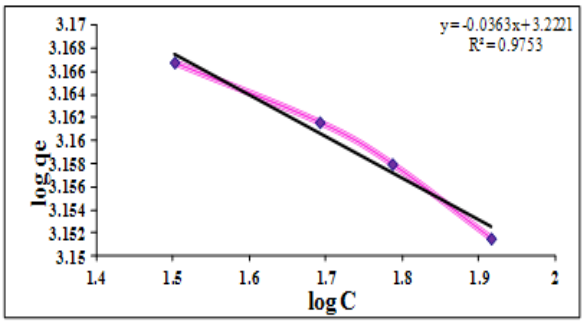

Fig 10. Langmiur model for Biosorption Cr on dead Mutant A.niger Biomass at 1500ppm

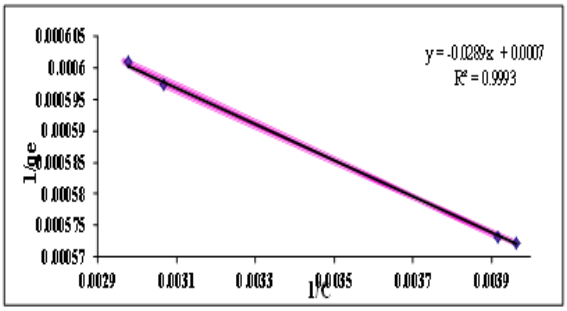

Fig 11. Freundlich modelfor Biosorption Cr on dead, Mutant A.niger Biomass at 1500 ppm

Discussion

The present study is the first detailed experimental demonstration on effect of the exposure to the UV light on the growing conidia of Aspergillus niger and it's on metal tolerance and biosorption potential with the mounting time exposure of ultraviolet light the growth of fungus decreased till the restricted level (1-4 hr). Other than that the fungus achieved the highest level of growth after 5 hour exposure to the UV light, and the growth eventually reduced after the longer durations. These results are contradictory to many authors. A study was conducted by Farques et al. (2002) in order to study the effect of UV-B radiation on the condia of 65 different isolates of B. bassiana, 23 of Metarhizium anisopliae, 14 of Metarhizium flavoviride and 33 isolates of Paecilomyces fumosoroseus. These isolates were irradiated by artificial sunlight (295 to 1100 nm) at an UV-B radiation, for one, two, four and eight hours. The time duration of two hours of exposure to the UV light or more was found to be damaging to all isolates tested. However on the other hand, Müller-Kögler (1965) concluded that the fungal conidia are not affected subsequent to longer exposure to the ultraviolet light. Whereas prolonged exposure to UV of about 1.5 hours lead to faster growth of the fungus and the spore production was also found to be enhanced. But the rate of

fructification was found to be deteriorating after 4.5 hours exposure to the ultraviolet light. Moreover, the fructification was entirely stopped after 30-48 hours respectively. These findings are the outmost parallel to present investigation. In the present study in order to get the correct time to cause mutation in the fungus, a comprehensive preliminary study was conducted, so different trials with different time exposures were monitored and the effects were evaluated. It is complicated to evaluate the findings which are related to duration of ultraviolet light essential for initiation of mutations. Sharma et al., (1992) stated that when B. bassiana was exposed to ultraviolet light for four hours the toxin production was found to be higher possibly due to some cell mutation. Furthermore, Claudia et al. (2011) concluded that low intensity of laser radiation can help in the control of pathogenicity in the seeds of maize caused by fungus. Fusarium specie was exposed to UV light at five different time durations i.e., 30, 60, 180, 300 and 600 seconds and at two different intensities (I1 = 16.3 e and I2 = 4.6 mW/cm2). The blend of I1 and I2, at 5 min of irradiation period, reduced the number of contaminated seeds with Fusarium up to 61.11% when evaluated with reference to control seed which was non-irradiated. So, the effect of radiation on microbes merely depends on the type of strain, the time duration and the intensity of the radiation. In this study, different time durations were monitored starting form seconds to minutes and ultimately to hours, with the lid cover of the Petri plate on, when no such significant difference on the growth of A.niger on PDA media was observed then the, lid of the Petri plate was removed. This led to the mutation in the culture of A.niger (K14e) which was exposed to the 5 hours of UV light (258nm). The growth rate of this culture was increased significantly. So, it is concluded that, the intensity of the UV light has evident positive effects on the growth of the fungus, depending on the strain and exposure duration. Ultra Violet (UV) light exposure can bring many changes in the structure of DNA of the microbes; it can have both positive and negative effects on them. A lot of work has been done and declared the use of UV light for disinfection of many types of microbes whereas, on the other hand researchers are also working on studying the positive effect of UV light on growth of microbes holding bioremediating potential and trying to increase the remediating efficiency. An experiment was conducted by Ailian et al. (2006), in order to study the effect of laser radiations on the rate of degradation of lignin by white rot fungus. This specific strain already had the tendency to biodegrade lignin and this experiment was conducted in order to increase its degradation potential. Helium and neon laser radiations were used with the wave length of 632.5nm. After the exposure for 20 min of laser radiation the degradation rate of lignin were increased from 39% to 88%. Furthermore, when the exposure time was 10min the degradation rate increased from 43% to 50%. Esterase isozyme analysis was also conducted, and the irradiated strains were found to stable. Similar results were obtained by this study, the mutant culture (irradiate for 5 hour) of chromium tolerant A.niger increased its metal tolerance potential, as compared to the control (non-irradiated). It showed faster growth in the chromium amended concentrations (500, 1000, 1500 and 2000 ppm) on the Petri plates, Hence leading to faster rate of bioremediation. Han et al. (2006) stated that the optimal pH for lead and chromium uptake was 6.0 for pre-treated biomass of fungus. Cereal chaff was used as a biosorbent for Pb and Cr adsorption and results showed that there was an increase in the Pb uptake with the increase in pH the best biosorption was achieved at pH 5-6 in addition the optimum pH for chromium and lead was found to be 9. Selatnia et al. (2006) reported that the pre-treated biomass of Streptomyces rimosus can be used efficiently as a biosorbents due to its enhanced efficiency of adsorption for the deduction of lead ions from solution. Furthermore, Kapoor and Viraraghavan, (1995) testified the high potential of A.niger for different heavy metals like, lead, cadmium and copper. In another research, they recommended that for the better biosorption of metals by Aspergillius niger, presence of carboxylate and amine group plays a very significant role. Kapoor et al. (1999) concluded that eight hour is the correct equilibrium time for the maximum biosorption for cadmium, copper nickel and lead. Tsezos et al. (1997) investigated then biosorption potential of Rhizopus arrhizus against uranium and according to his findings the equilibrium was attained after 10 hr. In present study, the maximum adsorption was achieved at 2 hour by the control isolate at all the chromium concentrations (500, 1000, 1500, and 2000 ppm) and 100% adsorption was recorded at 1000 ppm. On the other hand, maximum adsorption of Cr(NO3)3 was achieved after 1 hour by the irradiated variants and 99.3% adsorption was recorded at 1500 ppm. The results indicate that the irradiated variants apart from the faster growth also attained special characteristics in which it is capable of faster rate of sorption as well.

Conclusion

It was concluded that A.niger was differently affected by different time exposure of UV radiation (258nm) with reference to changes in its growth rate, uptake of metal and its tolerance potential. Moreover, the rate of uptake of chromium was faster for the mutant variant, in the biosorption experiment.

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