

COMPARATIVE ASSESSMENT OF BIOSORPTION OF MALACHITE GREEN DYE FROM ITS AQUEOUS SOLUTION BY LIVING AND DEAD HYPHOMYCETOUS FUNGI

Shyam Singh Mehra, Harish Pal Bhati, Permod Kumar and M.U. Charaya

Microbiology Laboratory, Department of Botany, CCS University, Meerut-250004

Email: ssingh.dungar@gmail.com

Abstract: The dead biomass of *Aspergillus nidulans* Eidan and *Humicola grisea* Traaen was found to be quite effective in adsorbing the dye malachite green from its aqueous solutions. In most of the case, the dead (autoclaved) biomass proved to be more effective than the living biomass. Changes in surface properties, modification of binding sites and increase in surface area due to autoclaving may be the possible reasons for increase efficiency of dead biomass.

Keyword: Dye pollution, Biosorption, Malachite green, Dead fungal biomass

INTRODUCTION

The industrial wastes including the effluents from dye, paper and pulp industries as also distilleries are the major contributors to water pollution. Approximately 700,000 tones and 10,000 different types of dyes and pigments are produced annually across the world, and are extensively used in many industries including textile, leather, pulp, pharmaceuticals, cosmetic, tannery, paper, food and plastics—the textile industries ranking first in the usage of dyes (Azhar *et al.*, 2005; Jain *et al.*, 2003; Sadettin and Donmez, 2006; Kiran *et al.*, 2009; Aksakal and Uzun, 2010). The production of dyes in India alone is estimated to be around 60,000 tons (Banate *et al.*, 1996). The coloured effluents containing dyes are toxic, mutagenic, carcinogenic as well as allergenic (Bakshi *et al.*, 1999; O'Mahony *et al.*, 2002; Aksu and Cagatay, 2006; Kumar *et al.*, 2006). The removal of colour from waste effluents becomes environmentally important because even a small quantity of dye in water can be toxic and is highly visible (Chou *et al.*, 2001).

In view of the various shortcomings of conventional dye removal technologies, environment-friendly alternatives for the removal of dyes by fungi through three principal mechanisms: biosorption, bioaccumulation and biodegradation have attracted the attention of scientists (Kaushik and Malik, 2009). The biological materials mainly yeast and fungi, are major candidates for the development of such devices which may be called biotrap (Crusberg, 2004).

Malachite Green ($C_{52}H_{54}N_4O_{12}$), a basic cationic triarylmethane dye, has been widely used not only for the dyeing of cotton, silk, paper, leather wool and jute but also in the manufacture of paints and printing inks (Gupta *et al.*, 2004). Mouthri and Singra Charya (2009) reported that malachite green exhibited toxic effects on the growth of *Polyporus elegans*, *Trametes versicolor*, *Lenzites betulina* and *Mucor mucedo*. Khataee *et al.* (2010) investigated decolourisation of malachite green by *Chlorella*, *Cosmarium* and *Euglena* sps. and found

that all of these species possess high decolourization efficiency.

The fungi may be classified into two categories according to their life state: (i) living cells that biodegrade and biosorb dyes; and (ii) dead cells (fungal biomass) which adsorb dye (Fu and Virraghavan, 2001). The use of dead biomass is preferred against the living by many workers since (a) dead organisms are not subject to toxicity limitations; (b) these do not require continuous supply of nutrients; and (c) these can be regenerated simply and may be reused for many cycles (Akar and Tunali, 2005; Padmes *et al.* 2005; Kumar, 2012 and Kumar and Charaya, 2013). The present study was carried out to assess the efficiency of living and dead biomass of *Aspergillus nidulans* and *Humicola grisea* to adsorb malachite green from its aqueous solution of different concentrations.

MATERIAL AND METHOD

One strain each of *Aspergillus nidulans* and *Humicola grisea*, isolated from soils from the dye-polluted sites (at Partapur Industrial Area, Meerut, were used in the present study. The fungal cultures were maintained on Potato Dextrose Agar (PDA) plates. The spore suspension of *Aspergillus nidulans* and *Humicola grisea* were separately inoculated in MGY (Malt Glucose Yeast Peptone) broth medium and were allowed to incubate for 15-20 days at 30°C. After sufficient growth of the fungus had developed, the biomass was separated from the broth medium and the wet biomass washed thrice with tap water. One half of the washed biomass was used as living biomass while the other half of the biomass was autoclaved at 15 psi for 20 minutes to obtain dead biomass. Thirty six packets each containing 10 mg biomass of *Aspergillus nidulans* (9 of living; 9 of dead biomass) and *Humicola grisea* (9 of living; 9 of dead biomass) were prepared and used for biosorption experiments. Malachite green was used to assess the ability of the fungal biomass to adsorb dyes. Stock solution of malachite green was prepared in a manner so as to obtain different concentrations

(i.e. 100ppm, 200ppm and 300ppm) of malachite green in respective solution.

100 ml of 100 ppm malachite green solution were taken in each of a set of nine 250 ml flasks (Set A). Similarly, two sets of nine flasks each were prepared for (i) 200 ppm dye solution (Set B) and (ii) 300 ppm dye solution (Set C). To three flasks of set A were added 10 mg of living *A. nidulans* biomass (subset A1); to three flasks were added 10 mg of dead *A. nidulans* biomass (subset A2); while three flasks were kept as control (subset A3). The flasks of sets B and C were also treated similarly, thus yielding subsets B1, B2, B3 and C1, C2, C3. The flasks were shaken simultaneously on an orbital shaker at 150 rpm for about 10 minutes. After 10 minutes of continuous shaking, the solution of each flask was filtered through a plastic sieve to remove the fungal biomass and unadsorbed dye in supernatant was estimated using a *uv-vis* spectrophotometer (Model SL- 159) at 620 nm wave length. Similar procedure was repeated for *Humicola grisea*. The adsorption capacity and Q-values were calculated using the formula: $Q = V (C_i - C_f) / m$ where, Q = specific dye uptake (mg/g) of biomass, C_i and C_f are the initial and final dye concentrations (mg/ l), m = adsorbent dosage (g); and V is the volume of dye solution.

RESULT AND DISCUSSION

Both the fungal strains under test i.e. *Aspergillus nidulans* and *Humicola grisea* were found to be quite efficient at malachite green adsorption from the solutions of different concentrations of malachite green dye the percentage dye adsorption ranging from 83.07% to 94.83% (Tables 1.1, 1.2). Dead fungal biomass showed greater biosorption of malachite green dye, where the biosorption was

94.83%, 91.73% and 92.50% for 100, 200 and 300 ppm in case of *Aspergillus nidulans*; and 90.34%, 91.03% and 90.57% for 100, 200 and 300 ppm in case of *Humicola grisea*. Maximum biosorption upto 91.29% and 94.83% for *Aspergillus nidulans*; and 91.31% and 91.03% for *Humicola grisea* were recorded using living and dead biomass, respectively. The minimum dye removal by living biomass of *Aspergillus nidulans* (86.21% with 200 ppm dye conc.) and *Humicola grisea* (83.07% with 300 ppm conc.) were obtained.

In a present study, it is found that the performance of dead biomass was almost always more than dead by living biomass. A number of workers including Akar and Tunali (2005), Padmesh *et al.* (2005), Kumar and Charaya (2012) have suggested that living biomass may be subjected to toxic effects of dyes (and other pollutants) at elevated concentrations; therefore, nonviable or dead biomass may be preferred to overcome this disadvantage. A number of workers in the past have also found to dead biomass to be more dependable and efficient for biosorption (Abedin, 2008; Nanthakumar, 2009; Kumar, 2011). Fu and Viraraghavan (2001) believed that the better performance of dead biomass in contrast to living biomass is due to greater adsorption strength, change in surface property and increase in surface area due to cell rupture after death. Baranaglu and Arica (2007) proposed that heat treatment can modify surface binding sites *via* denaturation of proteins on the cell wall structures.

From the result of the present study, it may be safely concluded that dead biomass of *A.nidulans* and *H. grisea* may serve as efficient components of biosorption-based effluent treatment system for the removal of malachite green.

Table 1.1 Biosorption of malachite green by living and dead biomass of *Aspergillus nidulans* from aqueous solution of the dye.

| Initial concentration of malachite green in the solution | Type of biomass | Dye remaining in the solution | Dye adsorbed by <i>Aspergillus nidulans</i> | % Biosorption | Q- Value |
|--|-----------------|-------------------------------|---|---------------|----------|
| 100 | L | 8.71 | 91.29 | 91.29 | 912.9 |
| | D | 5.17 | 94.83 | 94.83 | 948.3 |
| 200 | L | 27.76 | 172.24 | 86.12 | 1722.4 |
| | D | 16.33 | 183.47 | 91.73 | 1834.7 |
| 300 | L | 33.08 | 266.92 | 88.97 | 2669.2 |
| | D | 22.5 | 277.5 | 92.5 | 2775 |

Table 1.2 Biosorption of malachite green by living and dead biomass of *Humicola grisea* from aqueous solution of the dye.

| Initial concentration of malachite green in the solution | Type of biomass | Dye remaining in the solution | Dye adsorbed by <i>Humicola grisea</i> | % Biosorption | Q- Value |
|--|-----------------|-------------------------------|--|---------------|----------|
| 100 | L | 8.69 | 91.31 | 91.31 | 913.1 |
| | D | 9.66 | 90.34 | 90.34 | 903.4 |

| | | | | | |
|-----|----------|-------|--------|-------|--------|
| 200 | L | 28.69 | 171.31 | 85.65 | 1713.1 |
| | D | 17.94 | 182.06 | 91.03 | 1820.6 |
| 300 | L | 50.77 | 249.23 | 83.07 | 2492.3 |
| | D | 28.29 | 271.71 | 90.57 | 2717.1 |

L= Living fungal biomass; D= Dead fungal biomass

REFERENCES

- Abedin, R.M.A.** (2008). Decolorization and biodegradation of crystal violet and malachite green by *Fusarium solani* (martius) saccardo. A comparative study on biosorption of dyes by the dead fungal biomass. *Am. Euras. J. Bot.* **1**:17–31.
- Akar, T. and Tunali, S.** (2005). Biosorption performance of *Botrytis cinerea* fungal by-products for removal of Cd(II) and Cu(II) ions from aqueous solutions. *Miner. Eng.* **18**: 1099–1109.
- Aksakal, O. and Uzun, H.** (2010). Equilibrium, kinetic and thermodynamic studies of the biosorption of textile dye (Reactive Red 195) onto *Pinus sylvestris* L. *J. Hazard. Mater.* **181**: 666–672.
- Aksu, Z. and Cagatay, S.S.** (2006). Investigation of biosorption of Gemazol Turquoise Blue–G reactive dye by dried *Rhizopus arrhizus* in batch and continuous systems. *Sep. Purif. Technol.* **48**: 24–35.
- Azhar, S.S., Liew, A.G., Suhardy, D., Hafiz, K.F. and Hatim, M.D.I.** (2005). Dye removal from aqueous solution by using adsorption on treated sugarcane bagasse. *Ameri. J. App. Sci.* **2**: 1499–1503.
- Bakshi, D.K., Gupta, K.G. and Sharma, P.** (1999). Enhanced biodecolorization of synthetic textile dye effluent by *Phanerochaete chrysosporium* under improved culture conditions. *World J. Microbiol. Biotechnol.* **15**: 507–509.
- Banat, I.M., Nigam, P., Singh, D. and Marchant, R.** (1996). Microbial decolorization of textile dyes containing effluents: a review. *Biores. Technol.* **58**: 217–227.
- Bhole, B.D., Ganguly, B., Madhuran, A., Deshpande, D. and Joshi, J.** (2004). Biosorption of methyl violet, basic fuchsin and their mixture using dead fungal biomass. *Curr. Sci.* **86**: 1641–1644.
- Chou, K.S., Tsai, J.C. and Lo, C.T.** (2001). The adsorption of Congo red and vacuum pump oil by rice hull ash. *Biores. Technol.* **78**: 217–219.
- Crusberg, T.C., Mark, S.S. and Dilorio, A.** (2004). Biomineralisation of heavy metals. In “*Fungal Biotechnology in Agricultural, Food and Environmental Application*” (ed., Arora, D.K.) Marcel–Dekker, U.S.A. pp: 409–417.
- Fu, Y. Z. and Viraraghavan, T.** (2001a). Fungal decolorization of dye wastewaters: a review. *Biores. Technol.* **79**: 251–262.
- Gupta, M.A., Krishnan, L. and Gajbe, V.** (2004). Adsorption kinetics and column operation for the removal and recovery of malachite green from wastewater using bottom ash. *Sep. and Puri. Technol.* **40**: 87–96.
- Jain, R., Bhargava, M. and Sharma, N.** (2003). Treatment and decolorisation of an azo dye in industrial effluent. *J. Sci. Ind. Res.* **62**: 813–819.
- Kaushik, P. and Malik, A.** (2009). Fungal dye decolourisation: recent advances and future potential. *Environmental International* **35**, 127–141.
- Khataee, A.R., Zarei, M. and Pourhassan, M.** (2010). Bioremediation of Malachite Green from contaminated water by three microalgae. *Neural Network Modeling Clean* **38**: 96–103.
- Kiran, I., Ilhan, S., Caner, N., Iscen, C.F. and Yildiz, Z.** (2009). Biosorption properties of dried *Neurospora crassa* for the removal of Burazol Blue ED dye. *Desalination.* **249**: 273–278.
- Kumar, K.V., Ramamurthi, V., and Sivanesan, S.** (2006). Biosorption of malachite green, a cationic dye onto *Pithophora* sp. a fresh water algae. *Dyes Pigments* **69**: 102–107.
- Kumar, P.** (2011). *Studies on Certain Biotechnological Aspects of Microbe–Metal Interactions*. Ph.D. thesis, C.C.S. University, Meerut (INDIA).
- Kumar, P. and Charaya, M.U.** (2012). Effect of treatment with lead sulphate on soil mycobiota. *J. of Plant Development Sci.* **4**: 89–94.
- Kumar, P. and Charaya, M.U.** (2013). A comparative assessment of the efficiencies of living vs. dead biomass of *Aspergillus niger* Link to adsorb basic fuchsin from its aqueous solutions. *Plant Archives* **13**: 485–488.
- Mouthri, B. and Singara Charya, M.A.** (2009). Decolourisation of crystal violet and malachite green by fungi. *Sci. World J.* **4**: 28–33.
- Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy, P.** (2009). Investigation on biosorption of reactive blue 140 by dead biomass of *Aspergillus niger* HM11: Kinetics and isotherm studies. *Global J. Biotechnol. Biochem.* **4**: 169–178.
- O’Mahony, T., Guibal, E. and Tobin, J.M.** (2002). Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzy. Microb. Technol.* **31**: 456–463.
- Padmesh, T.V.N., Vijayaraghavan, K., Sekaran, G. and Velan, M.** (2005). Batch and column studies on biosorption of acid dyes on fresh water macro algae *Azolla filiculoides*. *J. Hazard. Mater.* **125**: 121–129.
- Sadettin S. and Donmez, G.** (2007). Simultaneous bioaccumulation of reactive dye and chromium (VI) by using *Phormidium thermophil* sp. *Enz. Microb. Technol.* **41**: 175–80.

