Global Journal of Microbiology and Biotechnology.
Volume 3, Number 1 (2015), pp. 3-8

http://www.irphouse.com

Optimization of carbon source and incubation conditions for biological treatment of Aniline Blue using *Aspergillus awamori* (MTCC-548)

Sukhvinder Singh Purewal[1]* and Amandeep Singh Thind[2]

[1]Department of Biotechnology, Chaudhary Devi Lal University, Sirsa-125055, Haryana, India
[2]Centre for Biosciences, Central University of Punjab, Bathinda-151001, Punjab, India
Email: purewal.0029@gmail.com

Abstract

The optimized conditions for the removal of aniline blue dye by *Aspergillus awamori* were determined using different factors like: carbon sources (Glucose, Galactose, Lactose, Sucrose, Maltose and Fructose) and incubation conditions [Number of Days, Light vs. Dark and Static vs. Shaking conditions]. The potential of filamentous fungi towards successful dye degradation/adsorption of dye was reported earlier in literature. However, there was no information about biodegradation/adsorption ability of *Aspergillus awamori* towards Aniline blue. Present study focused on adsorption potential of *Aspergillus awamori* for maximum removal of aniline blue. Under the optimized conditions (Fructose, 10 Days, Shaking under light at 28˚C and RPM 130) the approximate removal of dye (78.97%) were obtained.

Keywords: Aniline Blue; Aspergillus awamori; Biodegradation; Carbon Source; Incubation conditions.

Introduction

Water pollution is one of striking problem all over the world. Pollution due to textile industry effluent has increased during recent years. Polluted water may contain mixture of chemical compounds which may either biodegradable or non-biodegradable. The presence of dyes in waste water at a concentration exceeding 100 mg/l can considerably complicate its biodegradation by conventional sludge treatment process. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and the presence of metal ions (Anjali et al., 2007). Numbers of dyes with variable characteristics are produced worldwide (Zollinger,

1987). Dyes attract the attentions of various industries because of their application in printing process, petroleum products and synthesis of coloured fibers (Pierce, 1994; Raffi et al., 1997). Discharge of coloured products in surrounding environments such as lakes and rivers sometimes creates problem in aquatic system leading to non-availability and poor supply of oxygen by acting as a barrier (Kumar and Purewal, 2014; Aksu et al., 2007). Although many physical and chemical parameters are used to degrade or adsorb the coloured pollutant/dye residues from water bodies but these methods are very costly sometimes leads to the production of secondary pollutants. Some chemical pollutants are not degraded easily by physical and chemical methods. They might require some harsher conditions or use of alternate techniques that may boost up the process of degradation/decolorization. Degradation and decolorization by microorganisms are currently in use at industrial level also. Although microorganisms are capable for clearance of pollutants from waste water but they requires some additional cofactors, proper incubation conditions and nutrient sources. Optimized growth of microorganisms results in better treatment of recalcitrant compounds also. There is an urgent need of specific and selective microorganisms that may completely eradicate the coloured compounds from river water and sewage wastes. Use of filamentous fungal strains may be helpful in removal of these compounds completely from the waste water. In our earlier study, *Aspergillus awamori* was used to treat the water containing bromocresol green and eosin yellow (Kumar et al., 2014; Kumar and Purewal, 2014). However, to the best of our knowledge there is no information in the literature about the biodegradation and biosorption of aniline dye by *Aspergillus awamori*.

Material and methods

The present study was carried out by keeping in view the importance of the incubation conditions, carbon source type and incubation time.

Microorganisms

Certified filamentous fungal strain *Aspergillus awamori* (MTCC-548) was purchased from Microbial Type Culture Collection, IMTECH, Chandigarh. For maintenance and further growth of *Aspergillus awamori* Czapek agar medium was used.

Chemicals

Aniline blue was purchased from Himedia. Others chemicals such as Sodium nitrate ($NaNO_3$), Potassium Chloride (KCl), Magnesium Sulphate ($MgSO_4.7H_2O$), Ferrous Sulphate ($FeSO_4.7H_2O$), di-potassium hydrogen orthophosphate (K_2HPO_4), Zinc Sulphate ($ZnSO_4.7H_2O$), Copper Sulphate (II) ($CuSO_4.5H_2O$) and sucrose were also purchased from HiMedia.. All reagents used were of analytical grade. Before using, glassware's were washed with tap water and sterilized in an oven at 180°C for 3 h.

Culture media composition

Four stock solutions were prepared for media preparation. Stock solutions were: **Stock-A** containing $NaNO_3$ 40 g/L, KCl 10 g/L, $MgSO_4.7H_2O$ 10 g/L, $FeSO_4.7H_2O$ 0.2 g/L, $FeSO_4.7H_2O$ 0.2 g/L; **Stock-B** containing K_2HPO_4 20 g/L; **Stock-C** containing $ZnSO_4.7H_2O$ 1g/100ml; **Stock-D** containing $CuSO_4.5H_2O$ 0.5g/100 ml. Added equal volume of **Stock-A** and **Stock-B** (50 ml), 1 ml of **Stock-C** and **Stock-D**, Sucrose was used as a carbon source (30g) in flask and prepared final volume 1 L distilled water. In addition to above minerals Aniline Blue dye was dissolved in media (100mg/L). Culture media was sterilized in an autoclave at 121°C for 20-25 minutes. Controlled media was prepared in two parts: Czapek broth and Czapek broth + Aniline blue. The remaining part of media containing dye was divided into ten parts for determination of biodegradation efficacy of *Aspergillus awamori* (MTCC-548) for period of 10 days at temperature 30±2°C.

Selected parameters

Adsorption potential of certified culture *Aspergillus awamori* was optimized with respect to the effect of incubation time (0-10 Days), carbon source (Glucose, Galactose, Lactose, Sucrose, Maltose and Fructose), incubation conditions (Shaking vs. Static under light and dark both). Adsorption potential was observed under different culture conditions by changing the one factor at a time. Duration of experiment was for 10 days. Solution without inoculum was served as control. After a regular interval of time the biomass was removed from the solution using Whatmann no. 1 filter paper.

Decolorization/Adsorption assay

The adsorption potential of filamentous fungal strain *Aspergillus awamori* was observed in C-limited Czapek broth amended with aniline blue dye (100 mg/l). Each flask containing 150 ml of media with aniline blue dye was inoculated with fungal disc separately. Flasks were incubated at 28±2° C for 10 days. Filtrate of each flask was observed for the decrease in dye concentration using Elico S-159 spectrophotometer at λmax (494 nm) of aniline blue. Adsorption percentage was calculated using the formula:

$$\text{Adsorption potential (\%)} = \frac{\text{Absorbance}_C - \text{Absorbance}_S}{\text{Absorbance}_C} \times 100$$

Where:

Absorbance_C is the absorbance of Control solution without fungal strain

Absorbance_S is the absorbance of sample treated with fungal strain

Results and Discussion

Effect of carbon source on adsorption

Different carbon sources such as glucose, galactose, lactose, sucrose, maltose and fructose were used at 30 g/L to investigate their effect on adsorption potential of *Aspergillus awamori* towards aniline blue. Maximum adsorption of aniline blue dye (78.97 %) was observed with fructose whereas the minimum adsorption (53.22 %) occurs in the presence of galactose (Figure 1). The results of the present research work clearly demonstrate that during the process of biological treatment of waste containing aniline blue, fructose may boost up the degradation/adsorption potential of filamentous fungi applied. The change in dye concentration in waste by adsorption/degradation by mycelium of fungi with reduction of dye intensity in solution was reported (Knapp et al., 1995; Fu and Viraraghavan, 2002).

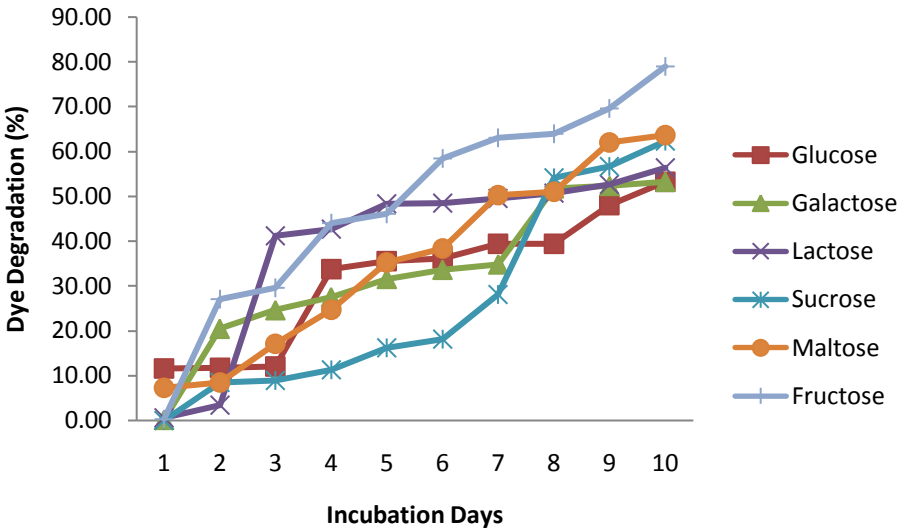

Fig.1. Effect of incubation days and carbon sources on adsorption potential of *Aspergillus awamori*

Effect of incubation conditions

Optimization of incubation time (Days) is one of the necessary factor in preliminary study of any waste water treatment plan. Keeping in view the importance, the change in dye concentration was observed after every 24 hrs during the experiment period (10 Days). Dye concentration was decreased with increased incubation time demonstrating the correlation with incubation period (Figure 1). Maximum adsorption of dye (78.97%) by fungal strain was found on 10^{th} day of the experiment.

Biological treatment of waste is one of the alternative techniques that can attract the attention of environmentalists and other research concerned with environment management. The success rate of treatment process also depends on the condition provide during the treatment by microorganisms. Microorganism are very sensitive to the condition in which they are living and further optimized growth

results in better removal of waste from the surrounding environment. Present research work was carried out in both dark and light conditions to investigate the effect of these parameters on fungal efficiency towards dye removal. Although in the presence of dark conditions the removal of dye was observed 98.94% but light conditions significantly enhance the adsorption potential of fungal strain by 99.07% (Figure 2). Further, maximum adsorption of dye was found with shaking the solution containing dye at 28˚C with rpm 130 in comparison with static conditions that result in adsorption percentage only up to 78.97 % (Figure 3).

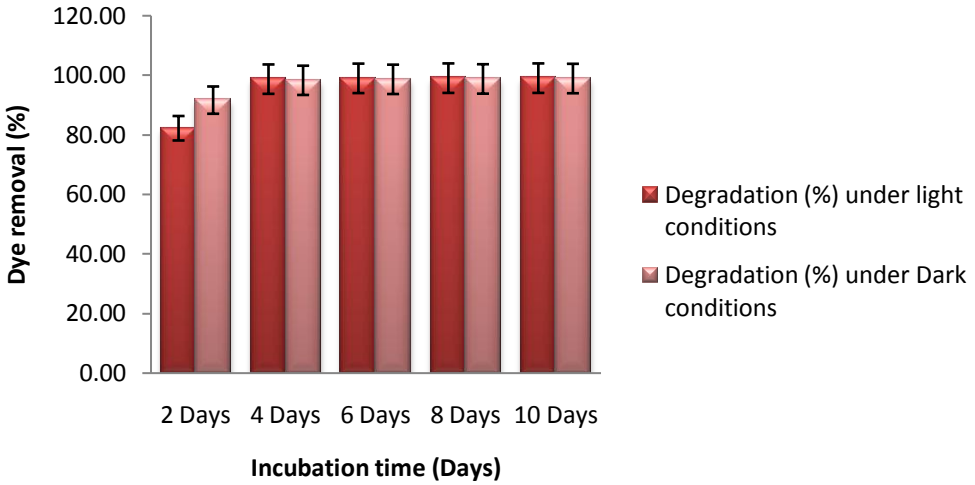

Fig.2. Effect of dark and light conditions on adsorption of dye by *Aspergillus awamori*

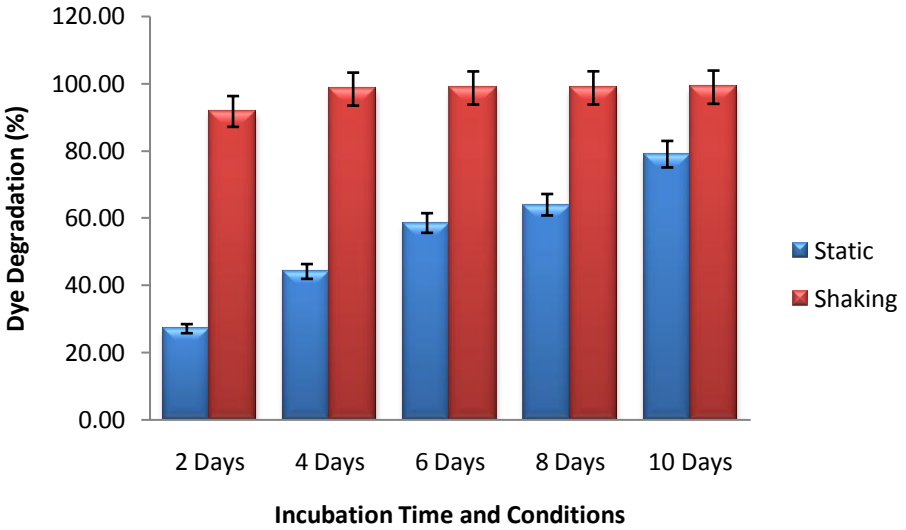

Fig.3. Effect of incubation conditions on percent dye removal.

Conclusion

Present study clearly demonstrates the dye clearance potential of *Aspergillus awamori*. Thus keeping in view the importance of filamentous fungal strains towards solving various environment related issues, they may be used at industrial scale for the purpose of waste water treatment. It was also observed that carbon source significantly affects the degradation/adsorption potential of microorganisms used. Incubation conditions might be another important factor that has to be optimized before using any waste water treatment.

Acknowledgements

Authors want to thank **Chairperson, Department of Biotechnology, Chaudhary Devi Lal University, Sirsa** for providing necessary facilities for the present research work.

References

1. Aksu, Z., Sen Cagatay, S., and Gonen, F., 2007, "Continuous fixed bed biosorption of reactive dyes by dried *Rhizopus arrhizus*: determination of column capacity", J. Hazard Mater., 143(1-2), pp. 362-371.
2. Anjali, P., Poonam, S. and Leela, I., 2007, "Bacterial decolorization and degradation of azo dyes" Int. Biodet. Biodegr., 59, pp. 73–84.
3. Fu, Y., and Viraraghavan, T., 2002, "Dye biosorption sites in *Aspergillus niger*" Bioresource Technol., 82, pp. 139-145.
4. Knapp, J. S., Newby, P. S., and Reece, L. P., 1995, "Decolorization of dye by wood rotting basidiomycete fungi", Enzyme Microb. Tech., 17, pp. 664–668.
5. Kumar, N., and Purewal, S. S., 2014, "Decolorization potential of *Aspergillus awamori* (MTCC-548) against skin irritating Eosin yellow dye" J. Microbiol. Biotech. Res., 4(4), pp. 62-65.
6. Kumar, N., Purewal, S. S., and Thind, A. S., 2014, "Decolorization Potential of *Aspergillus awamori* (MTCC-548) Against Brilliant Green Dye: A Toxic Dye for Digestive System" J. Env. Sci. Comput. Sci. Eng. Technol., 3(3), pp. 1188-1192.
7. Pierce, J., 1994, "Colour in textile effluents-the origins of the problem" J. Society Dyers Colourists, 110, pp. 131-134.
8. Raffi, F., Hall, J. D., and Cerniglia, C. E., 1997, "Mutagenicity of azo dyes used in foods, drugs and cosmetics before and after reduction by *Clostridium* species from the human intestinal tract", Food Chem. Toxicol., 35, pp. 897-901.
9. Zollinger, H., 1987, "Color Chemistry Synthesis, Properties and Applications of Organic Dyes and Pigments" New York: VCH Publishers. pp. 92–102.