Journal of Basic and Applied Research International
7(2): 101-106, 2015
International Knowledge Press
www.ikpress.org

BIODEGRADATION OF MALACHITE GREEN DYE BY *Saccharomyces cerevisiae* AND *Candida albicans* ISOLATED FROM TEXTILE EFFLUENT

ABIOYE OLABISI PETER[1*], PHILEMON GARIS[1], ARANSIOLA SESAN ABIODUN[2] AND OYEWOLE OLUWAFEMI ADEBAYO[1]
[1]Deparment of Microbiology, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.
[2]Bioresources Development Centre, National Biotechnology Development Agency, Onipaanu, Ogbomoso, Oyo State, Nigeria.

AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between all authors. Author AOP designed the study, wrote the protocol and interpreted the data. Author PG anchored the field study and performed preliminary data analysis. Authors ASA and OOA gathered the initial data, managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Received: 27th February 2015
Accepted: 30th March 2015
Published: 13th April 2015

Original Research Article

ABSTRACT

Malachite green (MG) degradation by *Saccharomyces cerevisiae*, *Candida albicans* and their consortium isolated from textile effluent, were investigated under aerobic condition using 20 mg of the dye in 250 ml, 500 ml and 750 ml of mineral salt medium for a periods of 12 days. Biodegradation activities were monitored using UV Spectrophotometer at 617 nm wavelength. The results shows 48%, 68% and 70% degradation by *Saccharomyces cerevisiae* for 250 ml, 500 ml and 750 ml dilutions respectively at the end of 12 days, while 48%, 65% and 68% degradation by *Candida albicans* were observed for 250 ml, 500 ml and 750 ml dilutions respectively at the end of 12 days. 52%, 70% and 77% degradation by their consortium for 250 ml, 500 ml and 750 ml dilutions respectively at the end of 12 days were also observed. The results of this study revealed the ability of *Saccharomyces cerevisiae, Candida albicans* and their consortium for the treatment of effluent containing malachite green dye.

Keywords: Degradation; spectrophotometer; consortium; decolorization.

1. INTRODUCTION

Malachite green (dimethylamino 4 alpha-phenylbenzylidene-4-cyclohexadiene 2,5 ylidenedimethyl ammonium, MG) whose common name is aniline green, is a toxic colored chemical primarily used as a dye, having an affinity to the substrate to which it is being applied. When diluted, it can be used as a topical antiseptic to treat parasites, fungal infections and bacterial infections in fish and fish eggs; it is also used as a bacteriological stain. The compound is not related to the mineral malachite, the name just comes from the similarity of color [1]. The majority of natural dyes are from plant sources–roots, berries, bark, leaves, wood, fungi and lichens. Synthetic dyes quickly replaced the traditional natural dyes, they cost less, they offered a vast range of new colors and they imparted better properties to the dyed

**Corresponding author: Email: bisyem2603@yahoo.com;*

materials, dyes are now classified according to how they are used in the dyeing process [2]. Basic dyes are water-soluble cationic dyes that are mainly applied to acrylic fibers, but find some use for wool and silk. Usually acetic acid is added to the dye bath to help the uptake of the dye onto the fiber. Basic dyes are also used in the coloration of paper [3].

Environmental pollution due to anthropogenic factors has been a serious global problem since the dawn of the industrial revolution. Industrial wastes, some of which are highly toxic are daily discharged into the environment [4,5]. Dyes in water bodies result to rapid depletion of dissolved oxygen due to its high BOD values, as dye effluent with high levels of BOD and COD values are highly toxic to biological life. The high alkalinity and traces of chromium which is employed in these dyes adversely affect aquatic life and also interfere with the biological treatment processes. Therefore, care must be taken with its use, as it is a respiratory poison, teratogen and carcinogen that accumulate in tissues, more toxic at lower pH, phytotoxic and inactivated by light [6].

Indeed, the presence of dye as little as 10 to 20 mg/L in water is visible and affects water transparency, which may have an impact on photosynthesis in aquatic plants and causes a part of aesthetic deterioration. Chemical decolonization of the colored compounds always leads to serious problems, not only due to their color, but because many dyes and their breakdown products may be toxic and mutagenic to living organisms. Therefore from an environmental standpoint, there is concern about the fate of malachite green and its reduced form, leucomalachite green, in aquatic and terrestrial ecosystems, since they occur as contaminants and are potential to human health hazards [7].

Biological degradation, the chemical dissolution of materials by microorganism or by other biological means, seems to be promising, given that the process is environmentally friendly to chemical decomposition. The term biodegradation is often used in relation to ecology, waste management, biomedicine and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic materials can be degraded aerobically with oxygen or anaerobically, without oxygen [8].

Fungi are well known organisms with high potential for degradation of different pollutants, using different metabolic pathways. It is well documented that the effectiveness of degradation depends on the dye structure, concentration, adaptation of microorganisms, their activity and biomass concentration. The well-known fungi strains highly capable of colour removing are as follows: *Pseudozyma rugulosa, Candida krusi, Rhodotorulla* sp., *Phanerochaete chrysosporium, Funaliatrogi, Coriolus versicolor* and *Cyathus* species [7], which can decolorize effluents as well. Studies on the biodegradation of malachite green have focused primarily on the decolourization of these dyes via reduction reactions. Although malachite green and the leuco-metabolites of malachite green are toxic, this research therefore provides initial steps for malachite green biodegradation, with further detoxification by different microflora present in the environment. This investigation was aimed at isolation and characterization of yeasts with ability to utilize malachite green as sole source of carbon.

2. MATERIALS AND METHODS

2.1 Samples Collection and Preservation

Malachite green dye was collected from the Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria. The dye was of commercial grade and the specifications are presented in Table 1 below. The organisms that were used for biodegradation activities were isolated from textile effluent collected from Funtua Textile Industry, Katsina State, Nigeria.

Table 1. Malachite green dye specifications

CAS number	2437-29-8
Molecular formula	$C_{52}H_{54}N_4O_{12}$
Molecular weight	927.02
Absorbance	617nm

Source: Deng et al. [9].

2.2 Isolation of Organisms

Serial dilution of effluent sample was made using sterile normal saline as the diluent. Aliquots (0.1 ml) of the dilution sample was aseptically transferred onto freshly prepared Sabouraud dextrose agar using pour plate method and incubated at 25ºC for 48 hours. The colonies were counted and expressed as colonies forming units per ml (cfu/ml). The colonies were sub-cultured repeatedly on Sabouraud dextrose agar to get a pure culture and was later stored in an agar slants in their pure forms for further characterization and biodegradation experiment.

The pure cultures of yeast isolates were identified on the basis of their cultural, morphological, colony

shape, mycelium formation Gram staining, germ tube test, nitrate reduction test, carbohydrate fermentation test and physiological characteristics in accordance with methods described by Cruickshank et al. [10] and with reference to Holt [11]

2.3 Standardization of Inoculum for Degradation of Malachite Green Dye

Cells from the stock were inoculated into 20 ml nutrient broth (oxoid) contained in 100 ml Erlenmeyer flask. It was incubated at 25ºC for 24 hours [12].

2.4 Preparation of Decolorizing Medium

The decolorizing medium (M9 synthetic medium) was prepared using 1 litre of distilled water according to Deng et al. [9] with little modification. It is composed of KH_2PO_4 0.5g L^{-1}, NaCl 0.5g L^{-1}, $CaCl_2.6H_2O$ 1.0g L^{-1}, $NaB_4O_7.H_2O$ 0.2g L^{-1}, $MnCl_2.H_2O$ 0.1g L^{-1}, $ZnSO_4.7H_2O$ 0.1g L^{-1}, $CuSO_4.5H_2O$ 0.1g L^{-1}, NH_4Cl_3.0g L^{-1}, $MgSO_4$ 0.3g L^{-1}, $(NH_4)_2\ SO_4$ 1.0g L^{-1}, $NaHCO_3$ 0.2g L^{-1}, 20 mg of malachite green dye was added to 250 ml, 500 ml and 750 ml of the M9 synthetic medium respectively. The pH was adjusted 7.0 with 0.1N HCl and 0.1N NaOH using OHAUS STATER, model 300 pH meter. The medium containing malachite green dye was autoclaved at 121ºC for 15 minutes.

2.5 Sterilization of Decolorizing Medium

After the preparation of the mineral salt medium in which malachite green dye was added and the pH adjusted to neutral, the medium was sterilized by autoclaving at 121ºC for fifteen (15) minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms.

2.6 Decolorization of Dye by Yeasts Cells

20 ml of decolorizing medium containing malachite green dye in 50 ml Erlenmeyer flask was inoculated with 2 ml of standardized inoculum that was incubated for 24 hours. All experiments were performed in duplicates. The flasks were plugged with sterilized cotton wool and incubated at 25ºC, an aerobic condition was provided by shaking the flask as described by Ren et al. [13] throughout the duration of the experiment. Initial absorbance of malachite green dye was taken at 617 nm using UV-VIS spectrophotometer (UV-VIS spectrophotometer Model 752) after centrifugation at 1200 rpm for fifteen (15) minutes. The experiment lasted for twelve (12) days and absorbance was taken at three (3) days interval alongside the control. In order to monitor decolorization activities, the percentage decolorization was calculated from the following equation according to Saranja et al. [14] as shown below:

$$\%\ decolorization = \frac{Ao - At}{Ao} \times 100$$

Where Ao and At represents initial and final dye concentrations in mg/L respectively.

3. RESULTS AND DISCUSSION

Fig. 1 shows the percentage degradation of 20 mg of MG in 250 ml dilutions. At the end of twelve days, 48.81% degradation by *Saccharomyces cerevisiae*, 48.34% degradation by *Candida albicans* and 52.70% degradation by their consortium were recorded respectively.

At the end of twelve days, the percentage degradation of 20 mg of MG in 500 ml dilutions as shown in Fig. 2 is 68.77% by *Saccharomyces cerevisiae*, 65.14% by *Candida albicans* and 70.44% by their consortium were recorded respectively.

Percentage degradation of 20 mg of MG in 750 ml dilutions is shown in Fig. 3, at the end of twelve days, 70.82% degradation by *Saccharomyces cerevisiae*, 68.89% degradation by *Candida albicans* and 77.41% degradation by their consortium respectively.

The consortium was observed to have the highest percentage decolorization of 52%, 70% and 77% at 250ml, 500ml and 750ml dilutions respectively at the end of 12 days. These could be due to synergistic relationship between the microorganisms [15].

The time effect on decolorization rate was significant, as degradation activities considerably increases with increase in time, thus showing the color removal efficiency of *Saccharomyces cerevisiae, Candida albicans* and their consortium as a function of time. This is in agreement with the work of Parshetti et al. [16] on biodegradation of malachite green by *Kocuria rosea* MTCC 1532, according to them 50 mg l^{-1}MG was completely decolorized under aerobic culture condition within 5 hours, whereas under anaerobic culture condition, only 80% was decolorize within three (3) hours.

The result shows high potential of *Saccharomyces cerevisiae, Candida albicans* and their consortium to decolorize MG dye in conjunction with dye concentrations and time, this is in agreement with the work of Youssef et al. [17] who studied the decolorization of malachite green by *Acremonium*

kiliense, according to them 95.4% MG was decolorized within 72 hours when the concentration of the dye was 5 mg L^{-1} but decolorization was only 35.48% when the dye concentration was doubled. They have attributed this trend to be due to inhibition of fungal growth at high dye concentration.

Indeed several studies [18,19] attributed decolorization to biodegradation when the major visible light absorbance peaks of the dye disappeared, these decolorization is most probably due to biodegradation of dye in which aromatic ring of dye are taken as sole carbon and energy source [20].

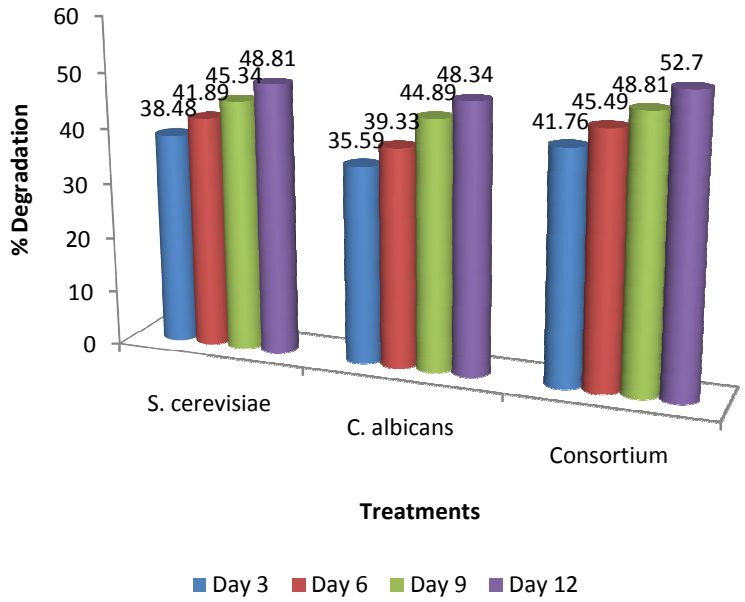

Fig. 1. Percentage degradation of 20 mg of MG in 250 ml dilutions by *Saccharomyces cerevisiae, Candida albicans and* their consortium

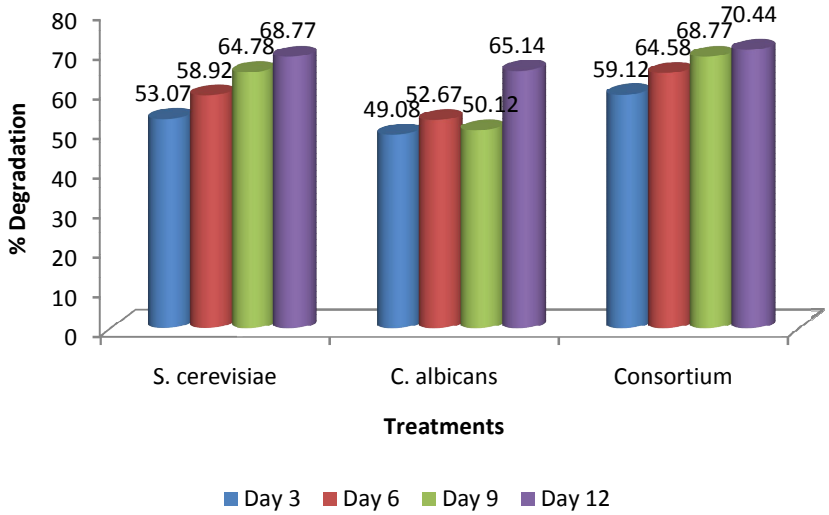

Fig. 2. Percentage degradation of 20 mg of MG in 500 ml dilutions by *Saccharomyces cerevisiae, Candida albicans and* their consortium

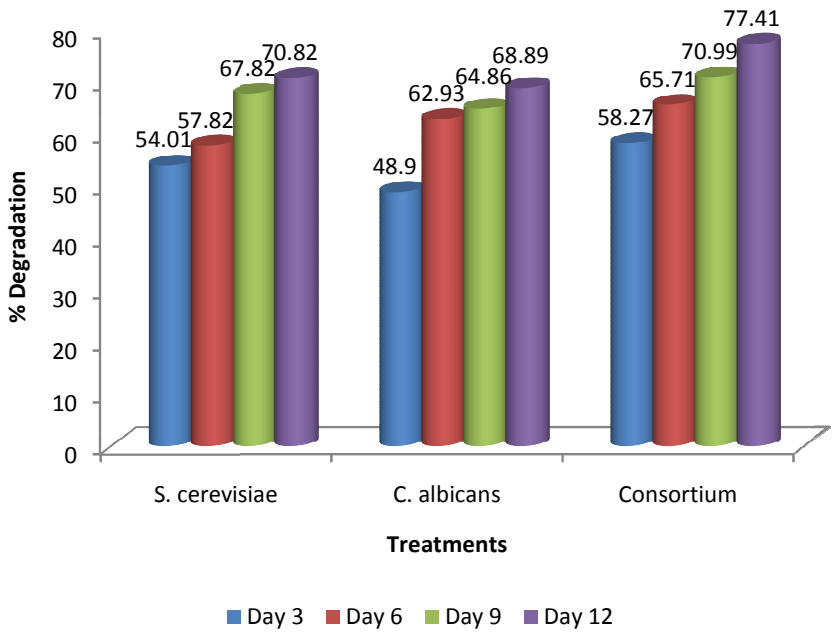

Fig. 3. Percentage degradation of 20 mg of MG in 750 ml dilutions by *Saccharomyces cerevisiae*, *Candida albicans* and their consortium

4. CONCLUSION

From the result obtained, it can be concluded that *Saccharomyces cerevisiae*, *Candida albicans* and their consortium isolated from textile effluent have the ability to degrade malachite green dye under aerobic condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhilin VK, Mittal A, Krishnan L, Gajbe V. Adsorption kinetics and column operations for the removal and recovery of malachite green from wastewater. Technology Journal. 2010;40:87-96.
2. Alhassani HA, Rauf MA, Ashrafs S. Efficient microbial degradation of Toluidine Blue dye by *Brevibacillus* sp. Dyes and Pigments Journal. 2007;3:395–400.
3. Kvavadze E, Bar YO, Belfer CA, Boaretto EJN, Matskevich Z, Meshveliani T. 30,000-Year-Old Wild Flax Fibers, Journal of Science. 2009;325:1340-1359.
4. Oghenejoboh KM, Ohimor EO. Contamination of Soil and Rivers by Lead from Used Engine Oil: A Case Study of Choba Community in Port-Harcourt, Nigeria, Journal of Pollution Research. 2012;32**:**1–6.
5. Dwivedi AH, Pande UC. Photochemical Degradation of Halogenated Compounds: AReview, Sci. Revs. Chem. Commn. 2012;241-452.
6. Schwinge J, Neal PR, Wiley DE, Fletcher DF, Fane AG. Spiral wound modules and spacers review analysis, Journal of science. 2011;242**:**129 –153.
7. Daiyong C, Manjunath K, Sullia SB. Biological treatment of combined textile dye effluent in ecology of Fung, Biological Journal. 2011;65:63–67.
8. Ogunlaja O, Aemere O. Evaluating the efficiency of a textile wastewater treatment plant located in Oshodi, Lagos. African Journal of Pure and Applied Chemistry. 2009;3**:**13-30.
9. Deng D, Guo J. Zeng, Sun G. Decolorization of Anthraquinone, triphenylmethane and Azo dye by a new isolated bacillus cereus strain DC 11q. Biodegradation and Biodeterioration Journal. 2008;62:263-269.
10. Cruickshank W, Min Q, Kangbi W, Junmei D, Zhongzhong L. Decolorizing Activity of Malachite Green and Its Mechanisms Involved

in Dye Biodegradation by Achromobacter xylosoxidans MG1, Journal of Molecular Micro Biotechnology. 1975;20**:**220–227.

11. Holt O. Dye degradation by microbes, Microbiology Journal. 1977;231:205–220.
12. Oranusi NA, Ogugbue CJ. Effect of pH and nutrient starvation on biodegradation of triphenylmethane by *pseudomonas* sp. Journal of Applied Science and Environmental Management. 2005;3:39-43.
13. Ren S, Guo J, Zeng G, Sun G. Decolorization of triphenylmethane, azo and anthraquinone dyes by a newly isolated Aeromonas hydrophila strain. Applied Microbiology and Biotechnology Journal. . 2006;72**:**1316-1321.
14. Saranraj p, Sumonthi V, Reetha D, Stella D. Decolorization and Degradation of Direct Azo dyes and Biodegradation of textile dye Effluent by using bacteria isolated from textile dye effluent. Journal of Ecobiotechnology. 2010;2:7-11.
15. Manjinder SK, Harvinder SS, Deepak KS, Bhupinder SC, Swapandeep SC. Decolorization of various azo dyes by bacterial consortium, Dyes and Pigments Journal. 2005;67:55–61.
16. Parshetti G, Kalme S, Saratale G, Govindwar. Biodegradation of malachite green by Kocuria rosea MTCC 1532. Journal of Solvent. 2006;53**:**492-498.
17. Youssef AS, Sherif MF, Assar SA. Studies on the decolorization of malachite green by the local isolate Acremonium kiliense. Biotechnology Journal. 2008;7**:**213-223.
18. Sun YA, Sang K, Yong LC, Young S, Cherol H, Young CL. Decolorization of triphenylmethane and azo dyes by *Citrobacter* sp. Biotechnology Journal. 2002;24**:**1037–1040.
19. Chen KC, Wu JY, Liou DJ, Hwang SCJ. Decolorization of textile dyes by newly isolated bacterial strains. Bioresources technology. 2003;101:57–68.
20. Pourrezaei P, Afzal A, Ding N. Physico-chemical processes review. Environment Research Journal. 2010;76:997–1072.