ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (2): 213-223, 2008
ISSN 1682-296X
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Studies on the Decolorization of Malachite Green by the Local Isolate *Acremonium kiliense*

[1]Amany S. Youssef, [2]Mervat F. El-Sherif and [1]Samy A. El-Assar
[1]Microbiology Division, Department of Botany, Faculty of Science,
[2]Department of Biological Sciences, Faculty of Education,
Alexandria University, Alexandria, Egypt

Abstract: The filamentous fungus *Acremonium kiliense* metabolizes the malachite green, crystal violet, carbol fuchsin and Methylene blue dyes showing different decolorization patterns. Decolorization occurred by biosorption at the initial stage and further biodegradation; about 95.4% malachite green in main medium within 72 h under shacked conditions. It depends on various conditions such as concentration of dye, concentration of cells, composition of medium and agitation. Decolorization rate for malachite green dye in shacked culture were found to be more than the static culture and also depend on biomass concentration. Reusability of *Acremonium kiliense* in malachite green dye removal was examined. Only six cycles (runs) of decolorization could be achieved. UV-VIS and Nuclear Magnetic Resonance (NMR) analysis of samples extracted with ethyl acetate from decolorized culture flask confirmed the biodegradation of malachite green into simpler metabolites.

Key words: *Acremonium kiliense*, triphenylmethane dyes, malachite green, dyeing waste, biotransformation

INTRODUCTION

The dye stuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for color (Mohan *et al*., 2002). Waste water from the textile industry can contain a variety of polluting substances including dyes (McMullan *et al*., 2001). Removal of color from dye bearing wastewater is a complex problem because of difficulty in treating such wastewater by conventional treatment method (Kumar *et al*., 2006). During the dyeing process, 5-10% of the unreacted dyes are lost in effluents (Wong and Yu, 1999). The aesthetic nuisance and toxicity of dyes are of major environmental concern (Banat *et al*., 1996). The treatment of effluents containing dyes has been conventionally carried out to remove color (decolorization) by physical or chemical methods such as adsorption, chemical precipitation, photolysis, chemical oxidation and reduction, electrochemical treatment, etc. These chemical or physical methods are less efficient, costly, of limited applicability and produce wastes, which are difficult to dispose of. The treatment systems based on using microorganisms capable of decolorization/ degrading dyes have received increasing interest owing to their cost, effectiveness, ability to produce less sludge and environmental benignity (Chen *et al*., 2003). Currently, extensive research is being focused on finding an optimal microbial biomass that would be as cheap as possible for the removal of contaminating dyes from large volume of polluted water. Over the past decade many microorganisms capable of decolorizing triphenyl methane dyes at lab scale level have been reported (Sani and Banerjee, 1999; An *et al*., 2002; Gill *et al*., 2002; Sharma *et al*., 2004a; Ren *et al*., 2006).

Malachite Green (MG), an N-methylated diaminotriphenyl methane dye, has been widely used as most efficacious antifungal agent in the fish farming industry (Schnick, 1988). It is also used for coloring purposes amongst all other dyes of each category (Gupta *et al*., 2004). Potential human exposure to malachite green could result from the consumption of treated fish and from working in the dye and aqua culture industries (Alderman and Clifton-Hadley, 1993). MG is highly toxic to human beings, as it affects the immune and reproductive systems and processes carcinogenic properties (Fernandes *et al*., 1991; Rao, 1995). Even though the use of MG dye has been banned in several countries and is not approved by the US Food and Drug Administration, it is still used in aqua culture due to its relatively low cost, ready availability and efficacy (Schnick, 1988). From an environmental stand point, there is concern about the fate of MG and its reduced form

Corresponding Author: Amany S. Youssef, Microbiology Division, Department of Botany, Faculty of Science, Alexandria University, Alexandria, Egypt Tel: 0020122835093

leuco malachite green in aquatic and terrestrial ecosystems, since they occur as contaminants (Burchmore and Wilkinson, 1993) and are potential human health hazards. Biological decolorization of triphenyl methane MG has focused primarily on the decolorization of dye via reduction reaction (Azmi *et al*., 1998; Pointing and Vrijmoed, 2000; Sani and Banerjee, 1999). Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium* was also reported (Bumpus and Brock, 1988; Sani *et al*., 1998; Yuzhu and Viraraghavan, 2001). Biotransformation of MG catalyzed by cytochrom P450 mediated both the reduction and N-demethylation reactions are reported in *Cunninghamella* (Cha *et al*., 2001).

Various fungi such as *Trametes versicolor* (Wong and Yu, 1999), *Aspergillus niger* (Fu and Viraraghavan, 2000), *Phanerochaete chrysosporium* (Fournier *et al*., 2004) and *Pestalotiopsis guepinii* (Saparrat and Hammer, 2006) can also decolorize diverse dyes.

In this study, the ability of the isolated fungus *Acremonium kiliense* to decolorize MG dye and other dyes (crystal violet, carbol fuchsin and Methylene blue) at different conditions is reported.

MATERIALS AND METHODS

Organism and culture conditions: *Acremonium kiliense* strain used in the present study was obtained from extensive isolating program from soil and sludge samples. *A. kiliense* was routinely maintained on glucose peptone medium (g L^{-1}) containing glucose (20), peptone (5), KH_2PO_4 (1) and $MgSO_4.7H_2O$ (0.5). The cells used in dye degradation studies were always grown in main medium (g L^{-1}) containing $(NH_4)_2SO_4$ (1.5), KH_2PO_4 (0.5), K_2HPO_4 (0.5), $MgSO_4$. $7H_2O$ (0.1), $CaCl_2$ (0.1), $FeSO_4$ (0.07) and yeast extract (0.5). The final pH of the medium was adjusted to 7.0.

Dyes and chemicals: Malachite Green dye (MG) was obtained from Koch-Light Laboratories LTD (England), where as Crystal violet and other dyes and fine chemicals were obtained from BDH chemicals Ltd. Poole England. The chemical structure of MG dye is shown in Fig. 1.

Enrichment isolation of dye decolorizing microorganisms: The soil and sludge samples collected from the vicinity of textile dye manufacturing/textile processing industries and soil of Faculty of Science at El-Shatby and Moharam Bey at April, 2006 were enriched on main medium containing MG dye. The plating of serially diluted enriched populations was carried on main medium agar plates containing 5 mg L^{-1} of MG dye and incubated at 37±2°C. The morphologically distinct colonies showing clear zone around their colonies were further purified and stored on main medium or glucose-peptone dye agar slants. The isolates were given specific codes on the basis of the samples used.

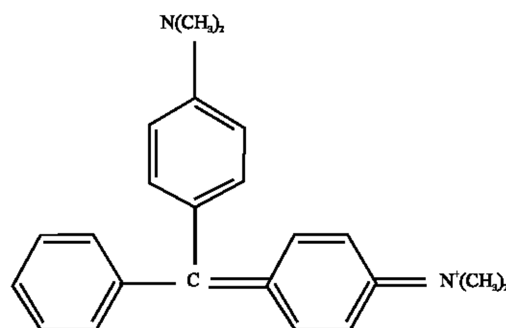

Fig. 1: Chemical structure of malachite green

Screening of isolated microorganisms for dye decolorization: Four morphologically distinct bacterial and fungal isolates capable of decolorizing the dye on main medium dye agar plates were grown in 250 mL Erlenmeyer flasks containing 50 mL main medium supplemented with MG dye (5 mg L^{-1}). The flasks were inoculated with a loopful of bacterial suspension or 2 discs of fungal mycelium of 9 old agar plate and incubated on rotary shaker (150 rpm) at 37±2°C for 96 h. After every 12 h, 5 mL sample was taken out aseptically and centrifuged at 10,000 rpm for 15 min. The cell free supernatant was used to determine the percentage decolorization of MG dye. One morphologically distinct fungal isolate showing more than 90% decolorization of the added dye within 72 h was selected for further studies.

Identification of selected strain microscopy and photography: Scanning electron micrographs were made for cells at the late-exponential-phase in electron microscope unit, Faculty of Science, Alexandria University. *Acremonium kiliense* was identified by Al Azhar University, Fermentation Biotechnology and Applied Microbiology (Domsch *et al*., 1980).

Decolorization assay: The decolorizing activity was expressed in term of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima (max) of MG dye (615.3 nm). The uninoculated medium supplemented with MG dye was used as a reference. Decolorization activity (%) was calculated according to the formula:

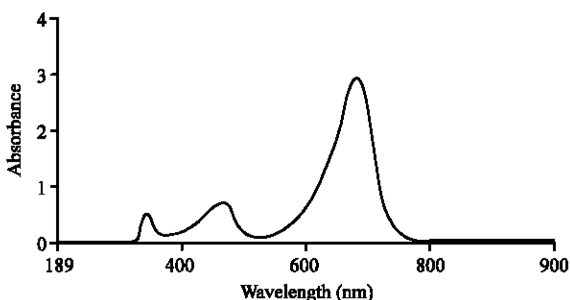

Fig. 2: UV-Vis spectra of MG

$$\text{Decolorization activity (\%)} = \frac{\text{Initial absorbance} - \text{Observed abservance}}{\text{Initial absorbance}} \times 100$$

Degradation of MG dye was monitored by measuring the UV spectra (Beckman DU 60 spectrophotometer). Absorbance of the culture broth was measured at 615.3 nm. Figure 2 showed the absorption spectrum of MG dye.

Media composition: MG concentration (5 mg L^{-1}) was kept constant and 2 discs of fungal growth were used for inoculation. The previously described medium (main medium) was used and called medium A. five other media B, C, D, E and F were used as follows:

Medium B: Medium A+$NaNO_3$ (2 g L^{-1}) instead of $(NH_4)_2SO_4$.

Medium C: Medium B+sucrose (2 g L^{-1}) + KCl (0.1 g L^{-1}) instead of $CaCl_2$.

Medium D: Medium A+peptone (2 g L^{-1}) and KCl (0.1 g L^{-1}) instead of $(NH_4)_2SO_4$ and $CaCl_2$, respectively.

Medium E: Medium D+glucose (5 g L^{-1}).

Medium F: Medium A+glucose (5 g L^{-1}).

Different MG concentrations: Effect of different MG concentrations on biodegradation by *A. kiliense* cells was studied by taking six different MG concentrations (1.0, 2.5, 5.0, 6.25, 7.5, 10.0 and 20.0 mg L^{-1}) in flasks containing the main medium inoculated with 2 fungal discs in each flask.

Different cell concentrations (size of inoculum): MG concentration (5 mg L^{-1}) was kept constant in 50 mL main medium flasks. Different numbers of equal fungal discs (of diameter 0.5 cm) were used (1, 2, 4, 6, 8 and 10) in two sets. First set was maintained on the main medium and the other set was maintained on glucose peptone medium.

Tap water and saline degradation activity: The main medium was replaced by tap water and saline.

Different pH of phosphate and acetate buffer: The main medium was replaced by acetate buffer 0.02 M (pH 3.6, 4.4 and 5.2) or phosphate buffer 0.02 M (pH 7.0, 7.5 and 8.0).

Reusing *A. kiliense* cells: Four and eight equal fungal discs obtained from both glucose peptone medium and main medium reinoculated into main medium flasks. This was repeated several times till the organism loss its ability to decolorize the dye (complete decolorization).

Different heavy metals: Nickel chloride, zinc chloride, cadmium chloride, sodium arsenate and sodium selenite were separately added to the main medium with three different concentrations for each, 0.01, 0.05 and 0.1 mg mL^{-1}, respectively.

Decolorization of other dyes by the isolated *A. kiliense*: Decolorization of other dyes (Methylene blue, Crystal violet, Carbol fuchsin) at a concentration of 2.5 mg L^{-1} were investigated.

Analytical procedure

NMR and UV spectral analysis: The metabolites produced during the biodegradation of MG at 0 h (control) and after decolorization of medium (72 h) were extracted with equal volume of ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary evaporator. The crystals obtained were dissolved in a small volume of methanol and the same sample was used for NMR and UV spectral analysis. NMR analysis was carried on an instrument 500 ECA Jeol. UV- VIS spectral analysis was carried out using PERKIN ELMER LAMBADA 4/VIS spectrophotometer.

RESULTS AND DISCUSSION

Acremonium sp. are filamentous, cosmopolitan fungi commonly isolated from plant debris and soil. The decolorization activity of the fungus *A. kiliense* indicates its natural adaptation to survive in the presence of toxic dye (MG). Similar studies were conducted by Abd-El-Rahim *et al.* (2002) for the isolation of microorganisms adapted to high dye concentrations from sites near textile industry complex.

Data obtained by using Environmental Scanning Electron Microscope (ESEM) FEI Quanta 200 showed colonies of *A. kiliense* reaching 1.8-3.3 cm in diameter in ten days on malt extract agar medium, stained brown chlamydospores 4-8 m. simple phialides arising from submerged hyphae. Conidia cylindrical (Fig. 3).

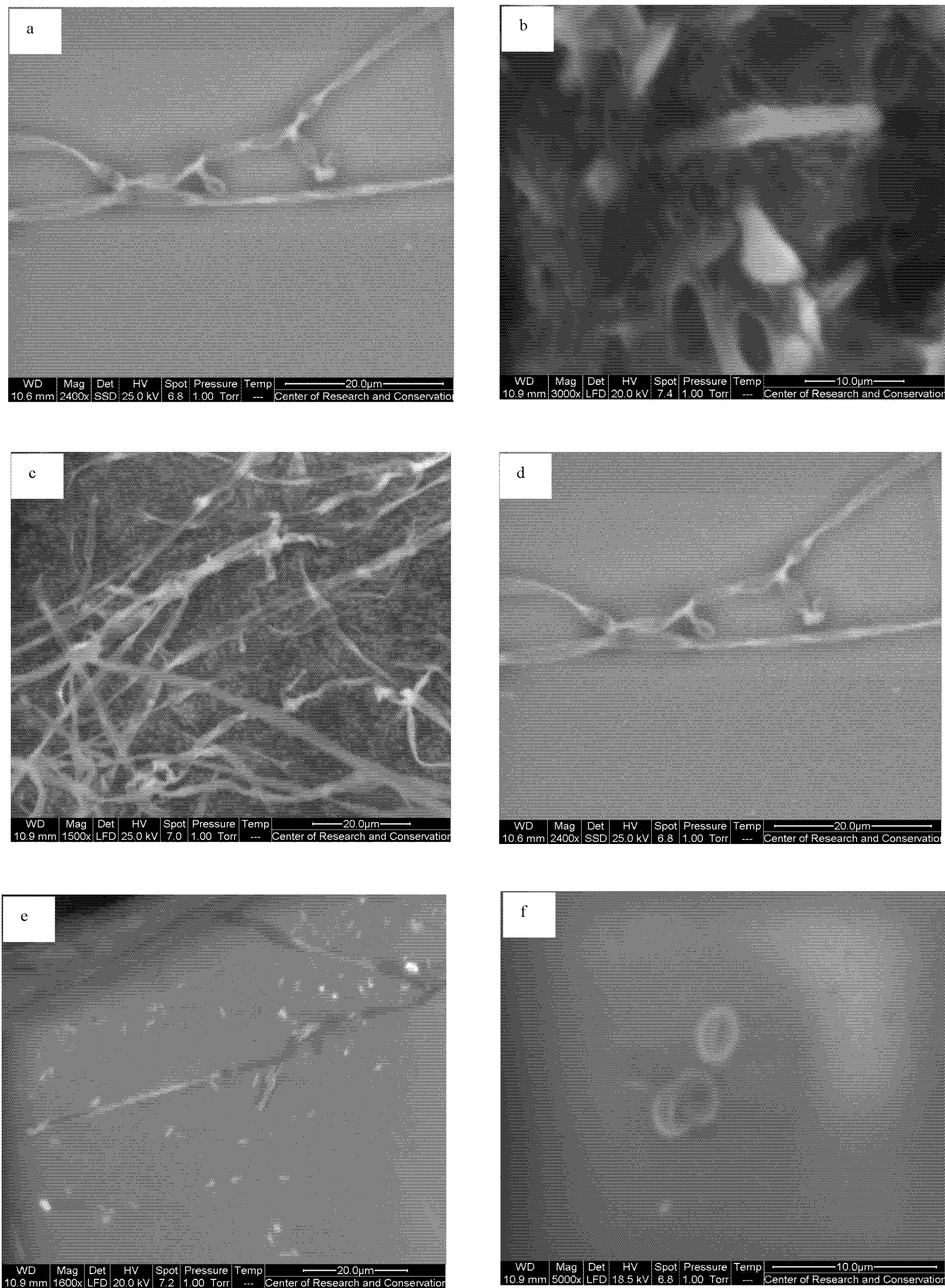

Fig. 3: Electron microscopy of *A. kiliense*. (a and b) Chlamydospores, (c) Orthotropic phialides, (d) Reduced phialides (simple phialides), (e) Orthotropic phialides and (f) Cylindrical conidia

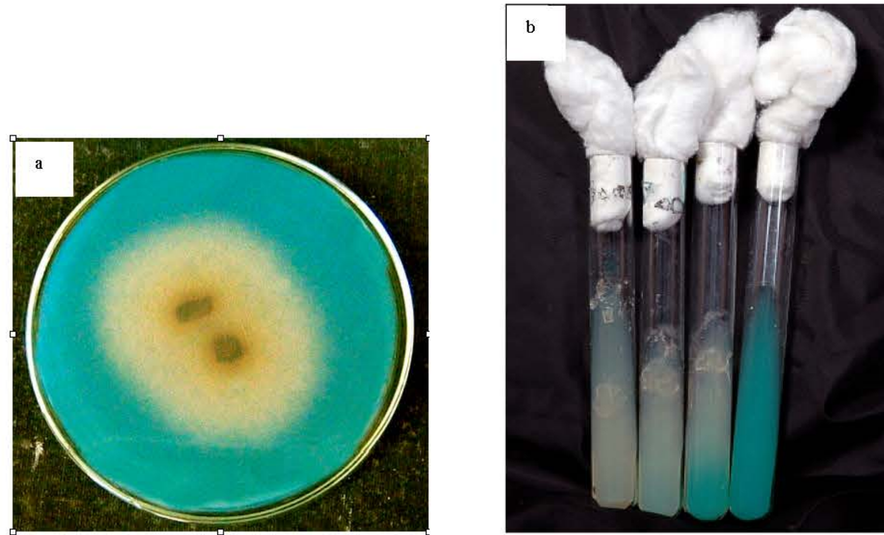

Fig. 4: Decolorization of M.G. on solid medium (a) clear zone plate after 5 days and (b) slants at different time intervals 2, 4, 6 and 8 days (from right to left)

Table 1: Percentage decolorization of Malachite green in static and shacked conditions using various medium compositions A, B, C, D, E and F

Types of media	Decolorization % after (h)							
	12	24	36	48	60	72	84	96
A*	52.3	76.3	81.9	90.6	94.30	96.7	98.1	96.3
A**	47.8	57.9	62.9	70.0	83.00	73.7	86.3	94.0
B*	51.4	63.5	65.5	77.4	85.60	97.9	97.1	96.7
B**	42.8	55.4	63.3	67.8	77.90	84.9	88.8	95.1
C*	25.8	57.9	74.1	78.5	83.80	91.5	95.9	97.8
C**	16.4	32.3	47.2	53.1	62.60	74.0	81.3	94.7
D*	23.8	37.3	49.4	61.3	67.55	84.3	92.5	99.0
D**	20.4	28.0	34.3	35.0	48.00	62.4	73.0	92.4
E*	23.6	34.7	38.2	42.8	41.30	46.7	53.1	82.2
E**	19.8	27.1	33.4	45.4	45.50	51.1	66.9	84.3
F*	38.8	39.5	56.5	59.1	59.60	82.4	93.4	98.9
F**	45.8	46.0	46.1	59.3	68.10	79.1	81.5	95.4

*: Shacked, **: Static

Plating studies of the fungus carried on main medium agar plates containing 5 mg L^{-1} of MG dye and incubated at 37±2°C for 8 days showed clear zone around the fungal colonies. Also, agar slant of the fungus showed increased degree of color removal within the same incubation time (Fig. 4).

It was found that *A. kiliense* has the highest decolorization rate for MG dye as compared with the other tested isolates. The results obtained showed that *A. kiliense* cells decolorized 95.4% MG (5 mg L^{-1}) within 72 h by simultaneous biosorption (initially up to 12 h) and further biodegradation. It was observed that cells within 12 h were dark green while after 72 h were almost white. This indicates that MG decolorization occurs in the first 7 h is due to biosorption of dye inside the cells and the cells start biodegradation of MG after 10:12 h. Similar results were observed with *Saccharomyces cerevisae* cells (Jadhav and Govindwar, 2006) which clearly show that high percentage of decolorization of MG is mainly due to microbial biotransformation and not to biosorption.

In present study we tried to find out the effect of various conditions on the decolorization of MG dye by *A. kiliense* cells. Effect of various medium composition on the decolorization of MG (5 mg L^{-1}) was tested under static and shacked conditions (Table 1). All types of media used showed decolorization of MG in the range of 82.2-99% after 96 h. The best results 98.9 and 99% were obtained with media F and D, respectively under shacked conditions. This may be due to presence of peptone in medium D and glucose in medium F. Glucose as carbon

source starts cells growth, the cell mass increase and causes higher decolorization of MG. The results showed also that shaking generally favored higher decolorization and faster biodegradation of MG than that obtained with static cultures. This may be due to an increase in mass and oxygen transfer between cells and the medium, factors that optimize the action of oxidative enzymes. This result is on line with Sani *et al.* (1998) and Machado *et al.* (2006) who compare static and shake culture in decolorization of dyes by *Phanerochaete chrysosporium* and basidiomycetes fungi, respectively. Also, Ren *et al.* (2006) studies revealed that color removal of triphenylmethane dyes was due to soluble cytosolic enzymes and the enzyme was an NADH/NADPH-dependant oxygenase.

In decolorization studies using different MG concentrations (Fig. 5). *A. kiliense* was able to decolorize MG up to a concentration of 10 mg L^{-1}, however the rate of decolorization at this concentration was very low. This may due to inhibition of fungal growth and thus biotransformation did not occur (Sani and Banerjee, 1999; Cha *et al.*, 2001). 95.4% MG dye was decolorized within 72 h when the concentration of the dye was 5 mg L^{-1} but decolorization was only 35.48% when the dye concentration was doubled. On the other hand when MG dye concentration was 1 and 2.5 mg L^{-1} decolorization occurred faster within 36 and 48 h, respectively.

Effect of cell concentration (by using different numbers of equal fungal discs) on decolorization of MG was tested within 72 h (Table 2). Fungal discs (inocula) were obtained from both glucose peptone medium and main medium. The best results were obtained with glucose peptone medium discs and by increasing their numbers. This may attributed to the presence of glucose. Rai *et al.* (2007) reported that a minimum level of glucose as a supplementary carbon source is required to maintain maximum color removal efficiency. Table 2 showed that four fungal discs (as inocula) show 95%, six discs 96.3%, eight discs 973% and ten discs 98.1% decolorization within 12 h.

When decolorization pattern of MG dye was studied by inoculating *A. kiliense* cells in tap water without adding any organic or inorganic compounds, the cells showed less ability to decolorize MG (32.8%) within 16 h compared with main medium, unlike reported in other similar studies (Jadhav and Govindwar, 2006) done on biotransformation of MG by *Saccharomyces cerevisiae*. On the other hand, using saline water showed 80.2% decolorization within the same time (Table 3). Using phosphate and acetate buffers (0.02 M) with different pH

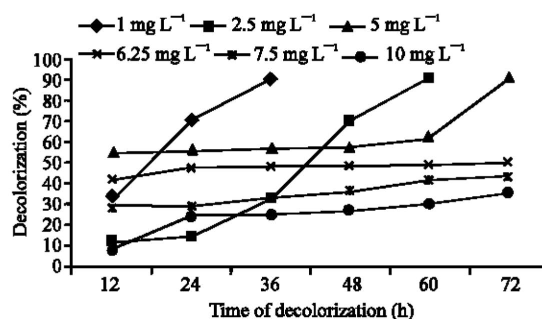

Fig. 5: Percentage decolorization of different concentrations of Malachite green

Table 2: Percentage decolorization of MG using different numbers of equal fungal discs (Inocula) obtained from glucose peptone medium (G.p.) or Main Medium (M.m.)

Medium	No. of fungal discs	Decolorization % after (h) 12	24	36	48	60	72
Glucose peptone medium	1	57.1	63.8	71.5	86.0	97.8	
	2	74.7	84.1	98.1			
	4	95.0					
	6	96.3					
	8	97.3					
	10	98.1					
Main medium	1	31.8	32.8	32.3	40.6	48.9	50.0
	2	33.3	34.0	52.3	64.0	82.0	93.2
	4	39.8	43.6	46.3	89.7	90.8	94.3
	6	44.3	47.6	47.8	53.4	63.0	92.0
	8	54.7	55.6	61.6	73.9	80.9	92.1
	10	60.8	62.3	68.4	82.3	84.8	92.4

Table 3: Percentage decolorization of Malachite green using main medium, tap water and saline

Media	Decolorization % after (h) 6	12	16
Main medium	77.5	97.3	
Tap water	9.1	25.2	32.8
Saline	54.8	73.3	80.2

Table 4: Percentage decolorization of MG using acetate and phosphate buffers

Buffers	pH	Decolorization % after (h) 8	12	16
Acetate	3.6	27.2	52.0	74.2
	4.4	40.6	47.0	78.4
	5.2	49.6	75.4	96.8
Phosphate	7.0	48.6	52.0	82.5
	7.5	7.1	29.1	35.5
	8.0	8.3	24.4	33.5

values as media showed that 96.8% MG dye was decolorized by using acetate buffer (pH 5.2), while 82.5% by using phosphate buffer (pH 7) within 16 h (Table 4).

A. kiliense cells showed also an ability to decolorize all other dyes tested vis, methylene blue, crystal violet

and carbol fuchsin. The results obtained showed that *A. kiliense* cells decolorize 90.7% methylene blue, 92.4% crystal violet and 73.1 carbol fuchsin within 48 h by simultaneous biosorption then further biodegradation (Table 5). The results are on line with those reported by Ren *et al*. (2006) where by they reported the decolorization of more than 90% crystal violet, basic fuchsin and MG by a newly isolated *Aeromonas hydrophila* strain. Similarly, An *et al*. (2002) isolated a *Citrobacter* sp. capable of decolorizing 80% malachite green and 90% crystal violet and methyl red dye.

Repeated use of the fungal discs were performed to examine the reusability of *A. kiliense* sp in MG dye removal (Table 6). The best results were obtained when glucose peptone medium discs used. Decolorization occurred by using 8 fungal discs (as inocula) need 12 h in each run. On the other hand, using 4 fungal discs need 24 h for maximum decolorization. During the 6 repeated run by using 8 fungal discs *A. kiliense* sp. showed approximately higher biological decolorization rate (97.8%) than obtained from the first run. This might be attributed to an adaptation effect, since *A. kiliense* was repeatedly exposed to the dye. The result indicated that it hold excellent reusability in repetitive biological operations. Similar results were obtained by Daneshvar *et al*. (2007) for the reusability of microalga *Cosmarium* sp. in MG removal.

In the next part of our study we tried to find out the effect of adding heavy metals with different concentrations on the decolorization of MG dye (5 mg L^{-1}) within 72 h. the best results were obtained with 0.1 mg mL^{-1} of both zinc chloride, nickel chloride, sodium selenite, cadmium and 0.05 mg mL^{-1} sodium arsenate (Table 7). For zinc chloride 95.9%, sod arsenate 94.8%, nickel chloride 88.9%, cadmium 87.8% and sod. Selenite 87.2% decolorization. Similar studies are conducted by Pointing and Vrijmoed (2000) where by they reported that two subtropical strains of basidiomycetes were capable of dye decolorization in the presence of up to 0.25 mM Cd^2, Cu^2 and Zn^ where as decolorization by *P. chrysosporium* was completely inhibited by all metals at concentration as low as 0.1 mM.

Figure 6 shows a typical time dependent UV-VIS spectrum of MG solution during biodegradation. The absorbance peaks, corresponding to dye, diminished which indicated that the dye had been removed. The spectrum of MG in visible region exhibits a main peak with a maximum at 615.3 nm. The decrease of absorbance peak

Table 5: Percentage decolorization of different dyes by *A. kiliense* sp.

Dye	Decolorization % after 12 (h)	24 (h)	48 (h)
Malachite green	91.7	95.2	96.0
Methylene blue (1 ml L^{-1} medium)	56.8	81.4	90.7
Crystal violet (1 ml L^{-1} medium)	76.8	82.0	92.4
Carbol fuchsin (1 ml L^{-1} medium)	73.6	73.2	73.1

Table 6: Percentage decolorization of MG by reusing different number of fungal discs (Inocula)

Medium	No. of fungal discs	Decolorization % after																	
		1st cycle			2nd cycle		3rd cycle		4th cycle		5th cycle		6th cycle		7th cycle				
		3 h	8 h	12 h	12 h	16 h	8 h	24 h	12 h	24 h	12 h	24 h	24 h	48 h	12 h	24 h	36 h	48 h	60 h
Glucose peptone medium	4 discs	44.9	62.1	86.7	84.3	86.0	74.8	96.9	83.3	92.6	82.6	97.5	67.5	92.9	58.5	66.7	73.3	81.6	86.7
	8 discs	1st cycle			2nd cycle		3rd cycle		4th cycle		5th cycle		6th cycle		7th cycle				
		3 h	8 h	12 h	12 h	16 h	8 h		12 h		12 h		12 h		24 h		48 h		60 h
		64.8	80.9	92.3	90.5	91.8	93.3		97.8		94.4		97.8		40.8		72.7		72.7
Main medium	4 discs	1st cycle			2nd cycle														
		3 h	8 h	12 h	24 h		36 h		48 h		60 h		72 h		96 h				133
		19.4	21.8	29.3	36.6		40.3		43.7		47.0		60.9		74.7				82.3
	8 discs	1st cycle							2nd cycle			3rd cycle							
		3 h	8 h	12 h	24 h	36 h	48 h	60 h	12 h		24 h	24 h			48 h				60 h
		29.3	36.6	40.0	48.3	63.3	64.8	90.8	66.0		91.8	44.4			74.8				74.8

Table 7: Percentage decolorization of Malachite green using different heavy metals

	Decolorization % after			
	12	24	48	72
Heavy metals ($mg\ mL^{-1}$)	(h)			
Cadmium				
0.01	61.50	86.10	70.80	69.10
0.05	76.70	87.20	73.90	59.60
0.10	57.00	61.20	66.40	87.80
Sodium selenite				
0.01	15.40	57.00	79.40	82.10
0.05	74.30	84.60	87.50	79.70
0.10	82.20	85.00	86.40	87.20
Nickel chloride				
0.01	78.70	81.70	83.60	87.30
0.05	85.40	88.40	79.50	78.10
0.10	75.20	78.70	85.50	88.90
Zinc chloride				
0.01	72.60	76.80	77.10	80.20
0.05	78.70	81.70	89.00	92.50
0.10	85.40	88.40	90.80	95.90
Sodium arsenate				
0.01	50.30	78.10	87.00	88.90
0.05	86.20	90.10	90.80	94.80

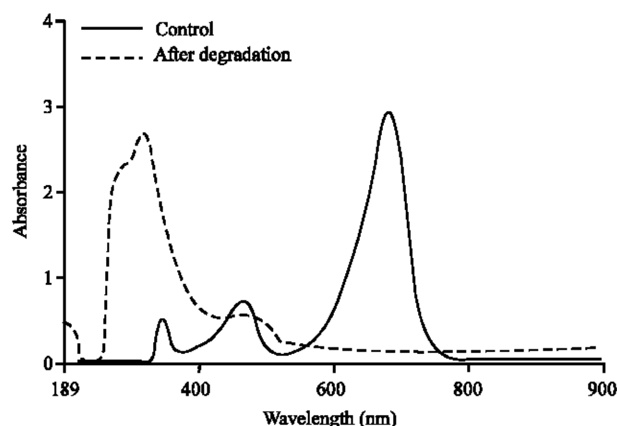

Fig. 6: UV-VIS spectra of extracted MG Dye at control and after degradation with *A. kiliense*

of MG at λ = 615.3 nm in Fig. 6 showed a rapid degradation of the dye. According to the literature (Chen *et al.*, 2003) biodecolorization of dyes can be due to adsorption to biomass or biodegradation. If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will disappear or new peak will appear. As shown in Fig. 6 the main absorbance peak approximately disappeared within 72 h. In addition extra absorbance peaks appeared in decolorized solution, probably resulting from the absorbance metabolites or degraded fragments of dye molecules (Daneshvar *et al.*, 2007). These results indicated that color removal by *A. kiliense* may be largely attributed to biodegradation. To confirm this observation MG dye and the metabolites from ethyl acetate extracts of *A. kiliense* cultures incubated with MG were analyzed by NMR spectra (Fig. 7A, B). It indicate that the four methyl groups of MG appear at 3.4 ppm as a singlet while the product show singlet at 4.3 ppm corresponding to only two methyl group. Demethylation of 2 methyl group takes place. So, MG is transformed to Didesmethyl Malachite green (Fig. 8). This study confirmed that the decolorization of MG by *A. kiliense* could be attributed to demethylation and the demethylated metabolites of MG did not exhibit absorption at 615.3 nm. Similar NMR studies was obtained by Sharma *et al.* (2004b) showed biodegradation of the aromatic rings of the MG into simpler metabolic intermediates.

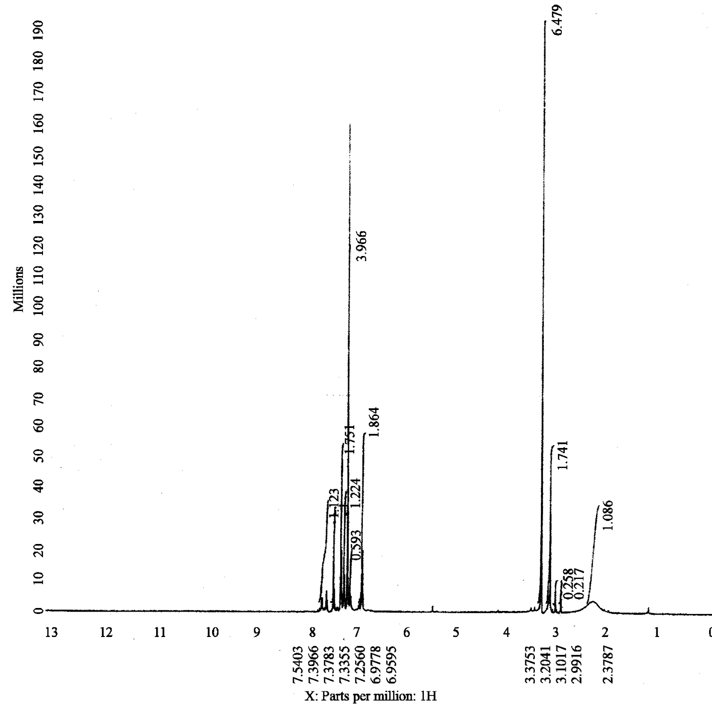

Fig. 7A: NMR analysis of MG dye extracted with ethyl acetate (control)

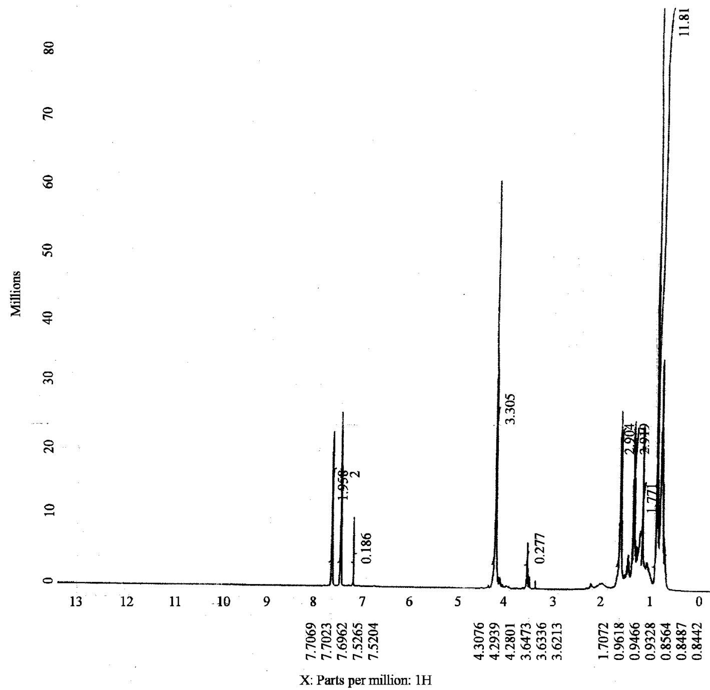

Fig. 7B: NMR analysis of products extracted with ethyl acetate formed by degradation of MG dye by *A. kiliense* cells. The final product formed after degradation was Didesmethyl-MG

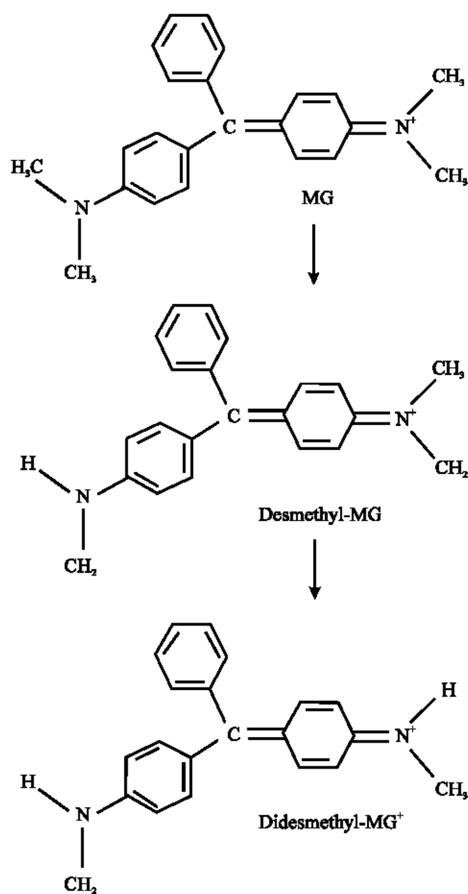

Fig. 8: Proposed mechanism for metabolism of MG

CONCLUSION

The present study revealed the ability of *A. kiliense* sp. to decolorize MG and other dyes. Results obtained from this work showed that this *Fungal* sp. possessed high decolorization efficiency and reusability. Selection of optimal conditions can induce the decolorization performance of cells for MG. UV and NMR studies confirmed that color removal of MG by *A. kiliense* cells may be largely due to biodegradation.

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