Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 629-643.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

ENHANCEMENT OF THE EFFICIENCY OF *RHODOTORULA MUCILAGINOSA* MH341115 (AUMC13570) IN BIODEGRADATION OF MALACHITE GREEN TOXIC DYE FROM POLLUTED WATER

Somaya Mahmoud Nassar*, Nivien Allam Nafady, Maysa Mohammed Ahmed Ali, Eman Mostafa Mohamed

Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt

Malachite green (MG) dye is a widespread environmental contaminant that endangers human health and the stability of the Earth's biosphere. This research evaluated how environmental and cultural conditions influence the decolorization of malachite green by Rhodotorula mucilaginosa AUMC13570. A single factor was changed for every trial while maintaining the previously optimized variable constant in the one-step optimization method used for process optimization considering six main variables: incubation type (static and submerged), media composition (A, B, and C), temperature (25, 28, 30, and 37 ℃), agitation speed (50, 100, 120, and 150 rpm), inoculum age (one, two, three, four, five, and six) days, and initial dye concentration (50, 100, 200, 300, and 1000 mg/L). The decolorization capability was assessed by observing the reduction in dye absorbance every 2 hours during the 12-hour incubation period. Our findings showed that a dye concentration of 50 mg/L, incubation with agitation, a four-day-inoculum age, an agitation speed of 150 rpm, and a temperature of 37 ℃ produced the highest decolorization rate. An analysis of the data highlighted various factors that influenced the decolorization effectiveness ($p < 0.001$).

Keywords: *Malachite green, Rhodotorula mucilaginosa AUMC13570, biodegradation, polluted, toxic dye*

INTRODUCTION

Access to clean water is necessary due to the rapid population growth and the widespread distribution of pollutants. At the same time, their contamination by various substances, such as heavy metal ions, pathogens, and organic compounds at concentrations higher than a threshold, such as synthetic dyes, pesticides, surfactants, hormones, and endocrine-disrupting chemicals situations where the majority of them are difficult to degrade, including pharmaceuticals and personal care products, attracts much attention[1-3]. Due to their widespread use in the printing, coloring, cosmetics, and leather sectors, water pollution has become a serious environmental problem for humans[3,4]. One of the main sources of industrial water contamination in emerging nations is dye wastewater. Since synthetic dyes are poisonous and noxious, they must be eliminated from aquatic sources to prevent serious harm to human health and maintain a diverse range of marine plants and animals[5]. New solutions for the bioremediation of hazardous substances must be developed to mitigate the increasing environmental harm caused by waste disposal. The fibers do not absorb up to 50% of dyes used in textile dying and remain contaminants, presenting a serious environmental risk. Because of their turbidity and high pollution intensity, dye discharge creates a vibrant hue even at low concentrations. Because dyes degrade toxically, they can majorly affect the aquatic environment[6,7].

For instance, malachite green is the most widely used dye for cotton, silk, paper, leather, paints, and printing inks[5]. Because of its characteristics, it is challenging to extract from aqueous solutions[5].

Received :7/1/2025 & Accepted : 27/3/2025

*Corresponding author: Somaya Mahmoud Nassar, E-mail: s.m.nassarmicro@gmail.com

Malachite green (MG), one of the many synthetic dyes, has been widely used in the textile industry to color cotton, silk, wool, jute, leather, porcelain, and other materials[8]. The Physiochemical property of Malachite Green chloride salt is: IUPAC name ([4-[[4-(dimethylamino)phenyl]-phenylmethylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium; chloride), molecular formula ($C_{23}H_{25}ClN_2$), molecular weight (364.91 gm/mol), and max absorption (615 nm)[7,9,10].

However, MG damages the immune system and causes cancer. Additionally, it was discovered that MG was extremely hazardous to freshwater fish. In animal studies, MG induces procreative defects, promotes internal organ neoplasia, and is pathogenic to cells[9].

Malachite green is acutely harmful to various aquatic and terrestrial animals and persistent in the environment. It has been documented that MG and its derivatives cause mutagenesis and cancer in humans. Additionally, several studies have demonstrated that exposure to this dye lowers fertility, raises the incidence of chromosomal fractures, and may impair respiratory enzymes[10,11]. The toxicity of this dye increases with exposure time, temperature, and concentration. It has been reported to cause[12].

The polluted water with malachite green causes a toxic effect on the germination index (%) of wheat grains (*Triticum aestivum*), maize grains (*Zea mays*), sorghum grains (*Sorghum bicolor*), and radish seeds (*Raphanus sativus*), which reach the significantly lowest GI% (9%, 3%, 11%, 1%)[13].

Malachite green is toxic in *Clarias gariepinus* fish which showed a significant decrease in the levels of antioxidant enzymes (superoxide dismutase and glutathione-S-transferase) and a significant decrease in hematological parameters, including monocyte counts, erythrocyte counts, blood hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular hemoglobin concentration (MCHC), platelet counts PCV, total white blood cell counts (WBCs), large and small lymphocyte counts, and high percentage of damaged in (nucleus and cell) percentage[14].

The field of water treatment uses a variety of physical methods (such as flotation, adsorption, multimodal filtration, reverse osmosis, and screening) as well as chemical procedures (such as ion exchange, oxidation, ozonation, flocculants, coagulants, and neutralization). Nevertheless, despite their distinct benefits, most methods have drawbacks such as high costs, complicated processes, and less-than-ideal results[15-22]. Thus, it is crucial to create low-cost, easy-to-use, eco-friendly techniques to eliminate contaminants in water and wastewater[1]. Yeast cells are a potential remediation method for malachite green dye from wastewater to reuse treated water in various applications properly. They can be obtained from several sources, are reasonably priced, and have a high capacity for dye decolorization under aerobic conditions. Yeasts can decolorize 50 mg/l of malachite green in high percentages after three days: *R. mucilaginosa* AUMC13567, *R. mucilaginosa* AUMC13570, *Saccharomyces cerevisiae* 11688, *R. mucilaginosa* KR264902, *S. cerevisiae* CPak, and *D. rugosa* AUMC13571 decolorize dye with a high proportion of dye degradation of more than 90%. These species provide, 98.41%, 96.65%, 96.49%, 95.59%, 92.80%, and 92.22% dye degradation rates respectively. *R. mucilaginosa* AUMC13567, *R. mucilaginosa* AUMC13570, *Saccharomyces cerevisiae* 11688, *R. mucilaginosa* KR264902, and *D. rugosa* AUMC13571, efficiency in biodegradation of water pollutants with 50 mg/l of malachite green and reducing the toxicity level of this hazardous dye. Using treated effluents for irrigation of wheat grains (*Triticum aestivum*), maize grains (*Zea mays*), sorghum grains (*Sorghum bicolor*), and radish seeds (*Raphanus sativus*) is safe and does not harm the environment significantly the potency of germination increases noticeably[13]. This study aimed to evaluate the probable biodegradation of malachite green dye by the *Rhodotorula mucilaginosa* AUMC13570 yeast strain and the enhancement of this biodegradation ability based on many characteristics, such as environmental and nutritional conditions.

MATERIALS AND METHODS

Selection of Yeast Strain

Rhodotorula mucilaginosa AUMC13570 was selected to enhance its ability in bioremediation of the wastewater polluted with malachite green because of the following reasons: High capacity to break down malachite green dye as the degradation percentage reached 96.65% after three days[13], its high effectiveness in lowering the phytotoxic effect of MG, and the fact that the metabolites of the biodegraded dye were

safe for use in the growth of radish (*Raphanus sativus*) grains, maize (*Zea mays*) seeds, sorghum (*Sorghum bicolor*) seeds, and wheat (*T. aestivum*) seeds[13], and the detoxification effect of biodegradation of malachite green which confirmed by Fourier Transform Infrared Spectroscopy Analysis (FTIR) results**[13]**. It is easy to obtain this type of yeast by isolating it from a source available in nature. It was isolated from Carrot pickled and genetically identified using internal transcribed spacer (ITS) sequences in nuclear ribosomal DNA[23]. BLAST from the NCBI website was used to assess this strain.

Cultivation of *Rhodotorula mucilaginosa* AUMC13570

The yeast strain was re-cultivated on MYE solid media that included 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% agar. According to**[23]**, the cultured yeast cells were inoculated into 50 mL of sterilized malt yeast extract (MYE) broth medium that contained 1% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract. The broth (MYE) medium was sterilized and its pH was adjusted to seven. A loop of yeast inoculum was cultured in 50 mL of the sterilized medium. The flask was shaken in an Environ-Shaker 3597-1 shaker at 100 rpm for three days at 28 °C to create an aerobic state[13, 14].

Environmental and Nutritional Factors

An experiment was designed using a single factor that was changed for every trial while maintaining the previously optimized variable constant in the one-step optimization method used for process optimization considering six main variables: incubation type (static and submerged), media composition (A, B, and C), temperature (25, 28, 30, and 37ºC), agitation speed (50, 100, 120, and 150 rpm.), inoculum age (one, two, three, four, five, and six) days of propagation, and initial dye concentration (50, 100, 200, 300, and 1000 mg/L). According to[5, 25-29], these parameters were identified as the primary significant factors in malachite green degradation**.**

Effect of Type of Incubation

The modified medium consisted of distilled water supplies with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract and (50 mg/l) of malachite green, the pH was adjusted to 5.8[14, 24], and the medium was sterilized by autoclaving at 121ºC for (20) minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum were added to 50 milliliters of modified media containing malachite green dye after three days of incubation. The mixture was incubated for twelve hours, under one of the tested incubation types: static (surface culture), or submerged (rotating culture) fermentation was conducted in an Environ-Shaker 3597-1 shaker at 100 rpm, and the flasks were incubated at 28ºC. As explained by [13, 14, 24].

Effect of Media Composition

Three types of media were tested as a decolorized medium for the enhancement of the *R. mucilaginosa* AUMC13570, medium A consisted of distilled water (plain), medium B distilled water supplies with 5% glucose, and medium C distilled water supplies with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract. Each one had a consistent concentration of malachite green (50 mg/l), the pH was adjusted to 5.8[13, 24], and the medium was sterilized by autoclaving at 121ºC for 20 minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum after three days of cultivation were added to 50 milliliters of media containing malachite green dye, and the mixture was incubated at 28ºC in the aerobic state in an Environ-Shaker 3597-1 at 100 rpm. As explained by [13, 14, 24, 25].

Impact of Varying Agitation Speeds

Modified MYE media was composed of distilled water with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract supplied with 50 mg/l of MG, and the pH was adjusted to 5.8[13, 24]. The medium was sterilized by autoclaving at 121ºC for 20 minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum after being cultivated in three days were added to 50 milliliters of decolorizing media containing malachite green dye, and the mixture was incubated for twelve hours, the flasks were sealed with sterile cotton wool and incubated at 28ºC in aerobic conditions under shaking with one of tested agitation rates were (50, 80, 100, 120, and 150 rpm). As explained by[5, 25-29].

Different inoculum age

Modified MYE media was composed of distilled water with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract supplied with 50 mg/l of MG, and the pH was adjusted to 5.8. The medium was sterilized by autoclaving at 121ºC for 20 minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum after one of each tested period of cultivation (1, 2, 3, 4, 5, and 6 days) were added to 50 milliliters of modified MYE media containing malachite green dye, the mixture was incubated for twelve hours, the flasks were sealed with sterile cotton wool and incubated at 28ºC under shaking (120 rpm) in aerobic conditions [5, 24-29].

Different Incubation Temperatures' Effects

Modified MYE media was composed of distilled water with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract supplied with 50mg/l of malachite green, and the pH was adjusted to 5.8. The medium was sterilized by autoclaving at 121ºC for 20 minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum after four days of cultivation were added to 50 milliliters of modified MYE media and the mixture was incubated for twelve hours, the flasks were sealed with sterile cotton wool and incubated at one of the tested temperatures (25ºC, 28ºC, 30ºC, and 37ºC) under shaking (120 rpm) in aerobic conditions[5, 24-29].

Impact of varying initial Malachite Green concentration

Modified MYE media was composed of distilled water with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract supplies with one of the tested malachite green concentrations (50, 100, 200, 300, 500, and 1000 mg/l), and the pH was adjusted to 5.8. The medium was sterilized by autoclaving at 121ºC for 20 minutes. It was allowed to stay until ambient temperature was achieved before inoculating with test organisms. Ten milliliters of inoculum after four days of cultivation were added to 50 milliliters of modified MYE media and the mixture was incubated for twelve hours, the flasks were sealed with sterile cotton wool and incubated at one of the tested temperatures of 37ºC under shaking (120 rpm.) in aerobic conditions[5, 24-29].

Spectrophotometric Analysis of degradation malachite green dye

Over the twelve hours, the biodegradation of MG by yeast cells was observed every two hours. After centrifuging a five ml sample at 5000 x g for 7 minutes in a cold environment (using a CRU-5000 Centrifuge IEC), 3 ml of the medium was removed, and the supernatant was measured at 620 nm. The analysis was conducted in triplicate at Assiut University's Botany and Microbiology Department, Faculty of Science, using the Mnicam/UV-vis spectrophotometry Helios Gamma[12-14, 24, 30, 31].

The following formula was used to determine the percentage of decolorization:

$$Decolorization\ (\%) = \frac{Ab-Ob}{Ab} * 100$$

Where **Ab** is the Initial absorbance and **Ob** is the observed absorbance**.**

Investigation of Statistics

The average of three studies ± standard error was used to obtain the basic statistics, the mean and standard errors. The IBM-SPSS package version 2147[32]. was used for all statistical analyses. One-way ANOVA (Analysis Of Variance) and the Duncan multiple comparison test were used to determine whether there were significant differences between means; Significance of difference was defined at the 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **) and 5% level ($p < 0.05$; *).

RESULTS AND DISCUSSION

R. mucilaginosa AUMC13570 is a yeast strain that possesses a high ability to degrade malachite green dye, it can grow in solid media supplied with 50 mg/l malachite green dye, and the decolorization index was 2.39 after three days of incubation. *R. mucilaginosa* AUMC13570 possesses a high ability to degrade malachite green dye in broth media supplies with 50 mg/l malachite green during three days of incubation, the first day the degradation percentage on the first day reached 72.7 %, the second day reached 83.6% on the third day reach 96.65% and *R. mucilaginosa* AUMC13570 can also absorb 0.6% of 50 mg/l malachite green in its dead cell[13]**.**

Effect of incubation type

The result of the investigation of the impact of incubation type on the degradation efficiency of *R. mucilaginosa* AUMC13570 recorded that following a 12 hrs incubation period with agitation at 100 rpm, the decolorization of MG accomplished a rate of 89 ± 0.4 %. In contrast, MG removal without agitation was considerably lower ($p < 0.001$) at 46.1 ± 0.6 %, as shown in **Table 1 and Fig. 1.** The complete removal of malachite green dye with 100% decolorization percentage was noted after a (24 hrs.) incubation period with agitation at 100 rpm, while without agitation the complete removal of malachite green dye with 100% decolorization percentage was noted after a (50 hrs.) incubation period. The increased decolorization noted under agitated conditions may be attributed to improved nutrient and oxygen distribution[33]. Agitation results in higher cell proliferation than static conditions, which could contribute to decolorization by promoting enzyme release[26]. Similar results were reported by[34], who observed a 133% increase in MG decolorization with agitation at 120 rpm versus static conditions.

Moreover, agitation significantly boosts decolorization, as shown by[35], who achieved 93% MG removal under agitated conditions compared to 44% under static conditions following 120 hours of incubation. This is in agreement with the work of[36], which reported that the level of decolorization of malachite green by *Bacillus cereus* and *Pseudomonas earoginosa* under the shaking condition was the highest at 92.4 ± 0.9, 95.7 ± 0.4 %, respectively. Also, the results agreed with the result of[26] who found that *Flavobacterium* sp. decolorized 150 mg/L Malachite Green under agitated conditions at 160 rpm reached 91 ± 1 % which was significantly higher ($p < 0.001$) than MG removal without agitation 39 ± 1 %, after 3 days of the incubation period.

Table 1: Impact of incubation type on the degradation of malachite green dye by *Rhodotorula mucilaginosa* AUMC13570.

Incubation (hrs.)	Static	Submerged
2hrs	5.9 ± 0.1^{a}	14.5 ± 0.2^{a}
4hrs	6.7 ± 0.4^{a}	26.4 ± 0.6^{b}
6hrs	7.7 ± 0.3^{a}	51.5 ± 0.3^{c}
8hrs	10.5 ± 0.1^{b}	65.3 ± 0.6^{d}
10hrs	12.9 ± 0.5^{c}	73.3 ± 0.3^{e}
12hrs	46.1 ± 0.6^{d}	89.0 ± 0.4^{f}
F-value	674^{***}	4490^{***}

The mean percentage of MG removal values is presented as triplicate samples ± standard errors. Values with different superscript letters in the same column for each parameter were significant differences at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$; *).

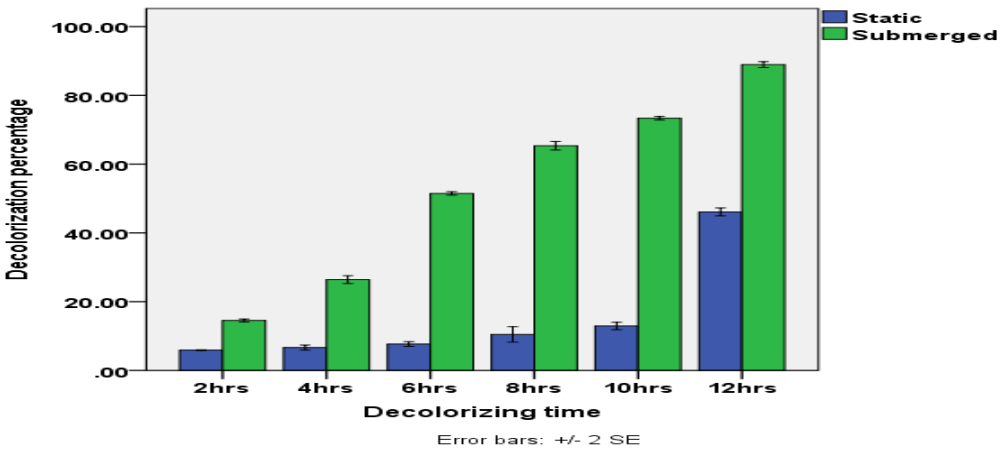

Fig. 1: Impact of incubation type on the degradation of malachite green dye by *Rhodotorula mucilaginosa* AUMC13570.

Effect of media composition

Three media were investigated for their impact on the degradation efficiency of *R. mucilaginosa* AUMC13570. The results illustrate that media (C) which consists of distilled water supplies 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract significantly enhanced the degradation efficiency of *R. mucilaginosa* AUMC13570 ($p < 0.001$) reached 89.0±0.3 % after (12 hrs.) comparing to media (B) and (A) in which the degradation efficiency of *R. mucilaginosa* AUMC13570 reached 65.1±0.9% and 47.6 ± 1.5%, respectively, after the same incubation period. The complete degradation was achieved after 30 hrs of incubation period in the case of using the media (C). On the contrary, in the case of using media (B) and (A), the complete degradation was noted after 48 and 70 hrs, respectively, as shown in **Table 2**.

Fig. (2) presented the significant enhancement in degradation efficiency of *R. mucilaginosa* AUMC13570 when using media (C) and, on the contrary, when using media (B) and (A)**.**

The significant enhancement in degradation efficiency of *R. mucilaginosa* AUMC13570 when using media (C) may be due to the media's components, such as glucose (carbon source), and yeast extract, which are recorded as important in growing microorganisms and enhancing the microbial efficiency in producing enzymes that degrade the malachite green dye.

Media (C) contain yeast extract, which contains (total nitrogen, phosphate, ash, salt, and vitamins) and peptone, which can be used as a nitrogen source as it consists of a sufficient quantity of amino acids (tryptophan, lysine, arginine, histidine, methionine, and phenylalanine. Peptone is important for the growth of the microorganisms involved in the fermentation process. **Al-Tohamy et al.**[35] found it crucial to add a carbon and nitrogen source to the dye for biodegradation experiments using bacterial strains. Our result agrees with the result of[26] who recorded the effectiveness of carbon sources for the decolorization of malachite green by *Flavobacterium* sp.

Our result also agrees **with[36],** who records that the active utilization of carbon sources promotes the production of ligninolytic enzymes, such as laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase. These enzymes were primarily responsible for dye decolorization.

According to[37] the polysaccharides and phenolic compounds may promote microbial growth, increasing laccase production. A phenolic extract enhanced MG decolorization[39].

Despite media (A) having the lowest significant impact on the degradation efficiency of *R. mucilaginosa* AUMC13570 ($p < 0.001$) among other tested media the degradation reaching 47.6 ± 0.6% and complete degradation after 70 hrs, this result represented an alternative new low-cost bioremediation without using any carbon source differ than the result of[40], which recorded no decolorization in the medium lacking a carbon source.

Table 2: Different medium compositions' effects on decolorizing the malachite green dye by *Rhodotorula mucilaginosa* AUMC13570.

Incubation (hours)	Media (A)	Media (B)	Media (C)	F-value
2hrs	7.3±0.4^{a}	8.2±0.7^{a}	18.41±0.2^{b}	211.9***
4hrs	9.4±0.1^{a}	13.1±0.7^{a}	29.8±0.5^{b}	442.1***
6hrs	15.4±0.4^{a}	38.51±0.9^{b}	53.7±0.2^{c}	1106.1***
8hrs	19.3±0.8^{a}	53.5±0.5^{b}	66.9±0.6^{c}	1567***
10hrs	36.9±0.6^{a}	61.9±1.8^{b}	74.6±0.3^{c}	311.4***
12hrs	47.6±1.5^{a}	65.1±0.9^{b}	89.0±0.3^{c}	422.5***

The percentage of MG removal values is the mean of triplicate samples ± standard errors. Values with different superscript letters in the same raw for each parameter, and significance of difference were defined at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$; *). **Media A** consisted of distilled water (plain)**, media B** distilled water supplied with 5% glucose**, and media C** distilled water provided with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract. Each one had a consistent concentration of malachite green (50 mg/l).

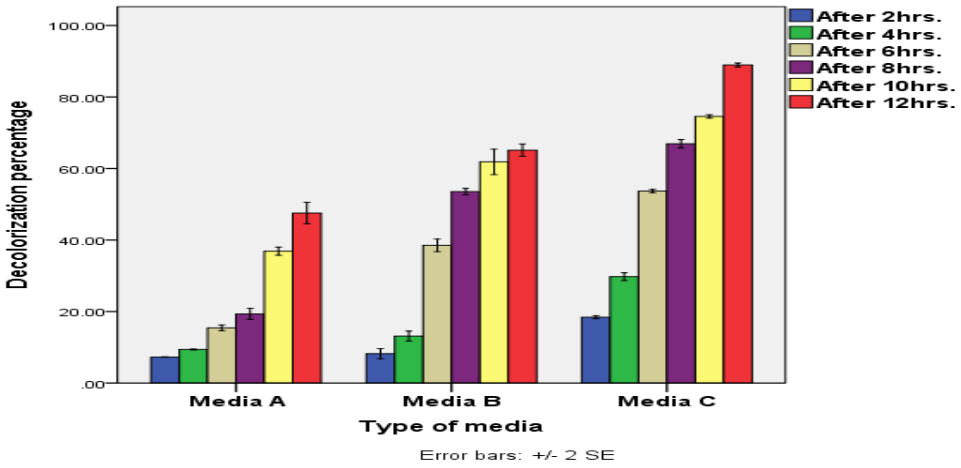

Fig. 2: Different medium compositions' effects on decolorizing the malachite green dye by *Rhodotorula mucilaginosa* AUMC13570. Media A consisted of distilled water, media B distilled water supplied with 5% glucose, and media C distilled water provided with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract. Each one had a consistent concentration of malachite green (50 mg/l).

Effect of different agitation speeds

Among the tested five agitation speeds the agitation speed of 120 rpm possesses the significant highest impact on the enhancement of the degradation efficiency as 89.6 ± 0.7 % was achieved after (12 hrs.) compared to 86.7 ± 0.5 % degradation at 100 rpm agitation speed, 72.3 ± 0.5% degradation at 80 rpm agitation speed, 66.9 ± 0.6 % degradation at 150 rpm agitation speed and the lowest degradation percentage 35.7 ± 0.5 % was achieved at agitation speed (50 rpm.) as illustrated in **Fig. 3** and **Table 3.** The increase in decolorization percentage in high agitation speed may be due to enhanced nutrition and oxygen dispersion.

It was noted that decolorization was completely 100% achieved after (14, 24, 28, 77, and 80 hrs.) at agitation speeds (120, 100, 80, 150, and 50 rpm.) respectively. In the current study, in all agitation speeds except agitation speed 50 rpm, *R. mucilaginosa* AUMC13570 dramatically decreased the color of MG from dye aqueous solutions by more than 66.7% after 12 hrs incubation period.

Our result is different than[33] who found that the shaking did not significantly affect discoloration at 100 rpm or at 150 rpm as the decolorization of 100 mg L^{-1} Malachite green dye by *Stenotrophomonas maltophilia* reached 98.33 ± 0.5% at 100 rpm and reached 98.66 ± 0.6% at 150 rpm after 48 hours.

Barapatre et al.[34] recorded a result similar to our result that *Aspergillus flavus* (F10) achieved enhancement in decolorization of 150 mg L^{-1} MG with agitation at (120 rpm.) and reached 70% after 8 days but *R. mucilaginosa* AUMC13570 decolorization 100% of 50 mg of MG after only14 hours with agitation at (120 rpm).

Table 3: Effect of different agitation speeds on decolorizing the malachite green dye by *Rhodotorula mucilaginosa* AUMC13570.

Incubation (hrs.)	Agitation speed					
	50 rpm	**80 rpm**	**100 rpm**	**120 rpm**	**150 rpm**	**F-value**
2hrs	6.6 ± 0.5^{a}	7.6 ± 0.1^{a}	19.0 ± 0.1^{c}	49.6 ± 0.4^{d}	16.0 ± 0.0^{b}	1198.9^{***}
4hrs	10.0 ± 0.2^{a}	14.6 ± 5.9^{b}	30.3 ± 0.2^{c}	55.6 ± 0.7^{e}	33.1 ± 0.1^{c}	602.9^{***}
6hrs	12.6 ± 0.8^{a}	35.1 ± 1.3^{b}	54.0 ± 0.1^{d}	58.6 ± 0.2^{e}	47.5 ± 0.1^{c}	765.7^{***}
38hrs	18.3 ± 0.2^{a}	55.0 ± 0.3^{b}	67.2 ± 1.0^{d}	76.7 ± 0.8^{e}	57.5 ± 0.9^{c}	1188.1^{***}
10hrs	27.2 ± 0.2^{a}	56.2 ± 1.1^{b}	74.7 ± 0.2^{d}	82.0 ± 0.7^{e}	60.0 ± 0.2^{c}	1202.5^{***}
12hrs	35.7 ± 0.5^{a}	72.3 ± 0.5^{c}	86.7 ± 0.5^{d}	89.6 ± 0.7^{e}	66.9 ± 0.6^{b}	1554.0^{***}

The mean values of the percentage of MG removal are presented as triplicate samples ± standard errors. Values with different superscript letters in the same raw for each parameter were significant differences at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$; *).

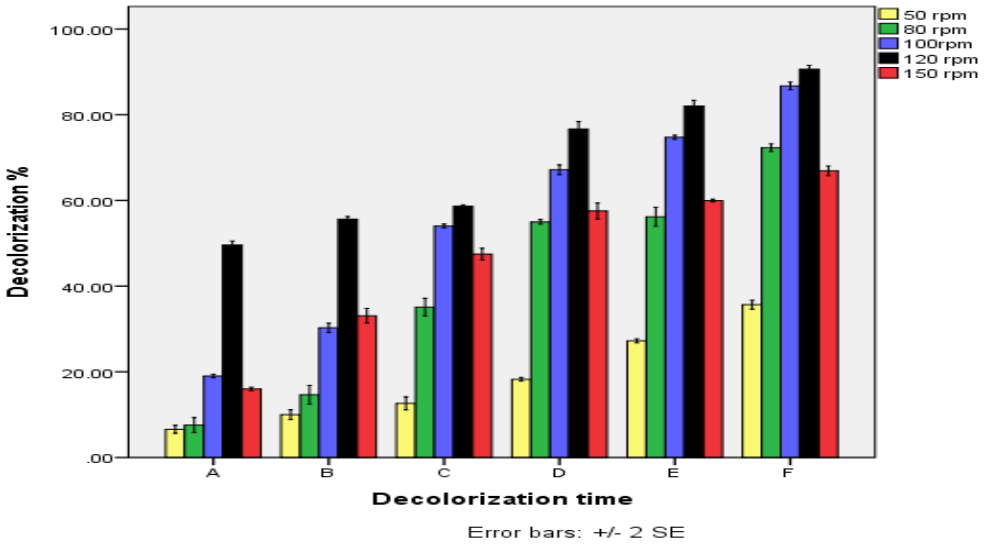

Fig. 3: Effect of different agitation speeds on decolorizing the malachite green dye by *Rhodotorula mucilaginosa* AUMC13570. A: 2hrs, B: 4hrs, C: 6hrs, D: 8hrs, E: 10hrs, and F: 12hrs.

Impact of Age of Inoculum

In the investigation of the impact of Age of inoculum on the degradation efficiency of *R. mucilaginosa* AUMC13570 on 50 mgL^{-1} of malachite green following 12 hrs, the result showed that there is a significant difference in the degradation efficiency of *R. mucilaginosa* AUMC13570 between different inoculum ages at $p < 0.001$, **Fig. 4**. The highest degradation of efficiency of *R. mucilaginosa* AUMC13570 is achieved when the inoculum age was four days reached 98.0±0.3%, followed by the degradation percentage when the inoculum age was three days reached 96.3±10%, followed by the degradation percentage when the inoculum age was five days reached 89.0±0.3%, and followed by the degradation percentage when the inoculum age was six days reached 87.1±0.7%. In contrast, the degradation significantly decreased when the inoculum age was one day and two days reached (48.8±0.0% and 65.5±0.2%) respectively, **Table 4**. *Rhodotorula mucilaginosa* AUMC13570 completely decolorized 50 mg L^{-1} malachite green dye after (14, 18, 20, 24, 30, and 48 hours) when the inoculum age was (4, 3, 5, 6, 2, and 1) respectively.

Table 4: Different *Rhodotorula mucilaginosa* AUMC13570 inoculum Age Effect on decolorizing Malachite green dye.

	Age of inoculum						
Time	**One day**	**Two days**	**Three days**	**Four days**	**Five days**	**Six days**	**F-value**
2hrs	11.6 ± 0.1^{a}	15.1 ± 0.0^{b}	25.9 ± 0.7^{e}	60.7 ± 2.1^{f}	18.8 ± 0.5^{d}	18.7 ± 0.1^{c}	25856^{***}
4hrs	20.1 ± 0.5^{a}	21.5 ± 0.1^{b}	27.4 ± 1.5^{c}	62.9 ± 0.6^{e}	30.1 ± 1.6^{d}	27.7 ± 0.1^{c}	2439^{***}
6hrs	23.6 ± 0.1^{f}	27.3 ± 0.5^{e}	46.5 ± 0.2^{d}	95.2 ± 0.3^{c}	53.9 ± 1.3^{b}	50.1 ± 0.5^{a}	5693^{***}
8hrs	38.0 ± 0.4^{f}	53.6 ± 0.2^{e}	53.7 ± 0.6^{d}	96.7 ± 0.2^{c}	67.1 ± 1.3^{b}	64.6 ± 0.3^{e}	3517^{***}
10hrs	40.9 ± 0.4^{f}	63.9 ± 0.1^{e}	79.9 ± 1.1^{d}	97.7 ± 0.2^{c}	74.7 ± 1.3^{b}	71.5 ± 0.3^{a}	4839^{***}
12hrs	48.8 ± 0.0^{f}	65.5 ± 0.2^{e}	96.3 ± 10^{d}	98.0 ± 0.3^{c}	89.0 ± 0.3^{b}	87.1 ± 0.7^{a}	3726^{***}

The mean values of the percentage of MG removal are presented as triplicate samples ± standard errors. Values with different superscript letters in the same raw for each parameter were significantly different at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$; *).

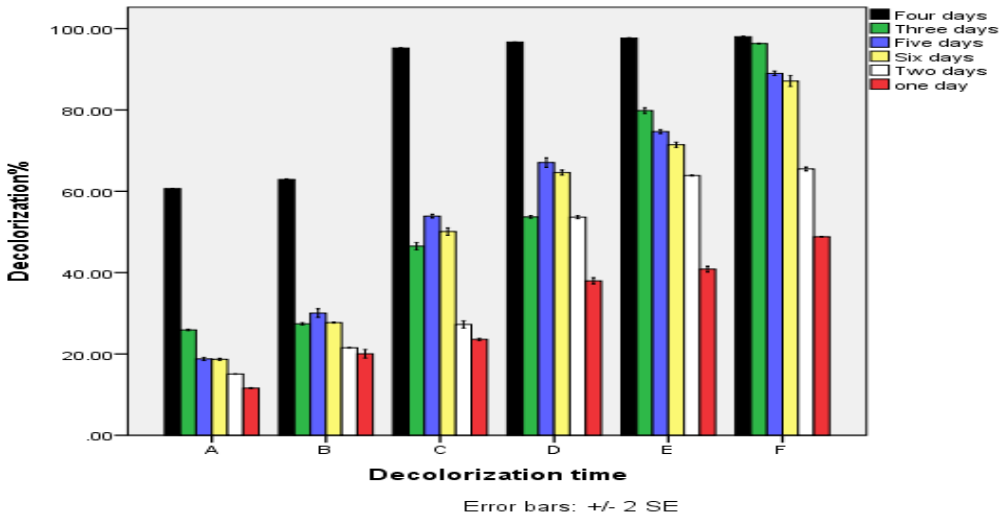

Fig. 4: Different *Rhodotorula mucilaginosa* AUMC13570 inoculum Age Effect on decolorizing Malachite green dye. A: 2hrs, B: 4hrs, C: 6hrs, D: 8hrs, E: 10hrs, and F: 12hrs.

Impact of incubation temperature degrees

The result of the investigation of the impact of different incubation temperature degrees on the degradation efficiency of *R. mucilaginosa* AUMC13570 on 50 mgL^{-1} of malachite green, following a 12 hrs, recorded that the degradation efficiency of *R. mucilaginosa* AUMC13570 is a significant difference at temperature degrees 25, 28, 30, and 37℃ **Fig. 5**. The highest degradation of efficiency of *R. mucilaginosa* AUMC13570 is achieved at 37℃, reached 98.8 ± 0.3 %, followed by degradation percentage at 30℃, reached 70.2±0.9 %. In contrast, the degradation significantly decreased at 28℃ reached 63.5±1.2 %, and significantly reduced at 25℃ reached 60.1±0.6% **Table 5**. *Rhodotorula mucilaginosa* AUMC13570 completely decolorized 50 mg L^{-1} malachite green dye after 14 hours at 37℃, after 26 hours at 30℃, after 40 hours at 28℃, and after 25 hours at 80℃.

Alaya et al.[33] recorded different results where the decolorization percentage of 100 mgL^{-1} Malachite green dye by *Stenotrophomonas maltophilia* is reduced at 30℃ and 35℃ reached (91.14 ± 0.42% and 89.86 ± 0.67%) respectively compared to the high decolorization percentage reached 96.77 ± 0.62% at 28℃ after two days of incubation.

Kalpana et al.[27] recorded a similar result to ours, noting that the rate of dye decolorization is influenced by the incubation temperature, which affects enzyme activity and microbial development.

El-Bendary et al.[41] found a similar temperature-dependent trend, with *Pseudomonas plecoglossicide* MG2 achieving 92% decolorization of 50 mg/L MG after 96 hours of incubation at 35 °C.

Similarly[40], the incubation temperature influenced decolorization efficacy, where *Klebsiella pneumoniae* WA-1 demonstrated a maximum biodegradation rate of 100% for MG at 37 °C, with an initial concentration of 5 mg/L and a 12-hour incubation period.

Also[26], similar to our result, the rate of dye decolorization is influenced by the incubation temperature, which affects enzyme activity and microbial development. The highest decolorization activity occurs between 30 °C and 40 °C, with maximum decolorization of 150 mg/L of MG 91 ± 1 % observed at 37 °C after 3 days.

However, our result shows that *Rhodotorula mucilaginosa* AUMC13570 completely decolorized 50 mg L^{-1} malachite green dye after 14 hours at 37℃, which is higher in degradation efficiency than the following results[40] recorded that *Klebsiella pneumoniae* WA-1 degraded only 5 mg/L, [41]recorded that *Pseudomonas plecoglossicide* MG2 achieving 92% decolorization of 50 mg/L MG after 96 hours of incubation, and[33] who recorded the high decolorization percentage of 100 mgL^{-1} Malachite green dye by *Stenotrophomonas maltophilia* is 96.77 ± 0.62% at 28℃ after two days of incubation.

Table 5: Effect of different incubation temperature degrees on malachite green dye decolorizing by *Rhodotorula mucilaginosa* AUMC13570.

Incubation by hours	Incubation temperature degree				
	25℃	28℃	30℃	37℃	F-value
2hrs	4.8±0.5^{a}	10.5±1.4^{b}	19.5±0.8^{c}	60.7±0.3^{d}	910.6***
4hrs	9.5±0.3^{a}	18.8±0.6^{b}	23.9±1.1^{c}	75.2±0.3^{d}	1893***
6hrs	16.2±0.7^{a}	26.7±0.8^{b}	34.1±0.6^{c}	81.9±0.7^{d}	1706.5***
8hrs	57.1±0.2^{a}	54.0±1.0^{b}	41.1±0.7^{c}	91.5±0.6^{d}	1004.8***
10hrs	57.3±0.3^{a}	59.1±0.1^{a}	58.1±1.2^{a}	97.0±0.2^{b}	957.2***
12hrs	60.1±0.6^{a}	63.5±1.2^{b}	70.2±0.9^{c}	98.8±0.3^{d}	454.6***

The mean values of the percentage of MG removal are presented as triplicate samples ± standard errors. Values with different superscript letters in the same raw for each parameter were significantly different at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$;*).

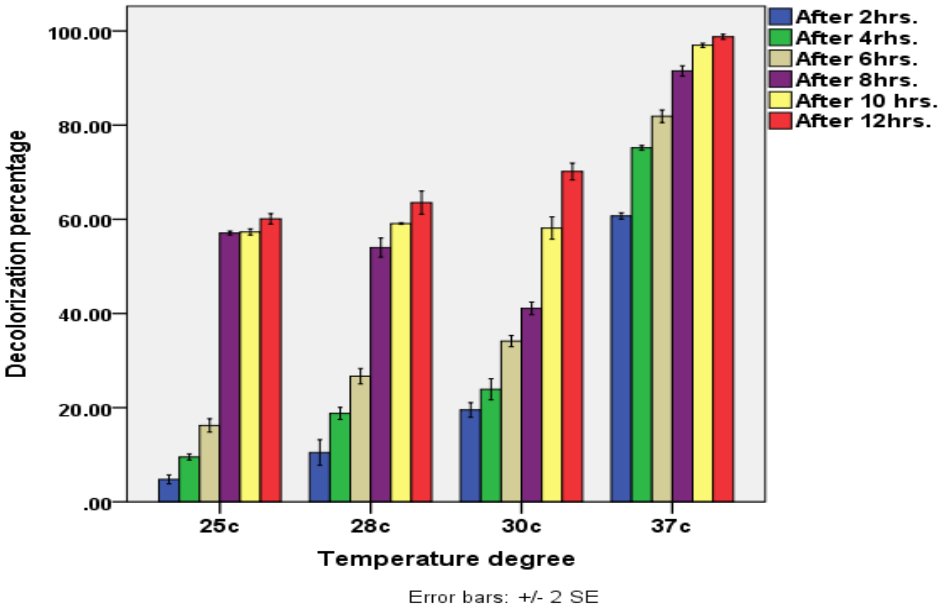

Fig. 5: Effect of different incubation temperature degrees on malachite green dye decolorizing by *Rhodotorula mucilaginosa* AUMC13570.

Effect of malachite green dye concentrations

Studied the decolorization ability of *Rhodotorula mucilaginosa* AUMC13570 and its tolerance at a different dye concentration of 50, 100, 200, 300, 500, and 1000 mg L^{-1}, recorded that the decolorization rate of malachite green dye significantly decreased with the increase in malachite green concentration (mg.) **Fig. 6.** *Rhodotorula mucilaginosa* AUMC13570 decolorize 99.3±0.1% of 50 mg L^{-1} malachite green dye, decolorize 89.1±1.3% of 100 mg L^{-1} malachite green dye, decolorize 46.6±1.0% 200 mg L^{-1} malachite green dye, decolorize 25.6±0.3 of 300 mg L^{-1} malachite green dye, 6.4±1.3 of 500 mg L^{-1} malachite green dye after 12 hours **Table 6.**

Rhodotorula mucilaginosa AUMC13570 completely decolorized 50 mg L^{-1} malachite green dye after 13 hours, 100 mg L^{-1} malachite green dye completely after 14 hours, and 200 mg L^{-1} malachite green dye completely after two days. The malachite green concentration of 300 mg was not completely decolorized; the highest decolorization percent was 55% after 48 hours. The malachite green concentration of 500 mg was not completely decolorized, but the highest decolorization percent was 50% after three days.

The malachite green concentration of 1000 mg was very toxic to *Rhodotorula mucilaginosa* AUMC13570 and inhibited its growth of yeast and enzymes so no decolorization percentage was observed even after four days.

Similar results were recorded by[33] who found a significant decrease with increasing the dye concentration. *Stenotrophomonas maltophilia*, a compost bacterium, decolorized $99.46 \pm 0.50\%$ of 100 mgL^{-1} Malachite green dye, $98.24 \pm 0.57\%$ of 200 mg Malachite green dye, $85.9 \pm 0.53\%$ of 300 Malachite green dye, $67.4 \pm 0.51\%$ of 500 mg Malachite green dye after two days.

Also, **Alshehrei**[38] discovered that as dye concentration increased, the percentage of dye decolorization for malachite green declined; *B. cereus* recorded the maximum percentage at (100 mg/L), with 91.2% for MG. Malachite green could be decolored by *Pseudomonas aeruginosa* up to 90% at a dosage of 100 mg/L. Reduced dye decolorization activities were seen at 500 mg/L to 1500 mg/L concentrations.

After all kinds of analysis (Degradation process), the chemical changes (before and after degradation experiment) were recorded using Fourier Transform Infrared Spectroscopy Analysis (FTIR). These results were published in previous research[13]. The FTIR spectrum results of Malachite Green (MG) show distinct peaks in the fingerprint region, indicating amines and ketones. The peaks in the range of 500-3500 cm^{-1} indicate the presence of –OH, -NH-, and -C-H (amides and amines), while the peaks in the range of 1614.8 cm^{-1} and 1219.3 cm^{-1} suggest the presence of amines. The peaks in the range of 1445.4 - 511.1 cm^{-1} correspond to metabolites like alkane, alkene, and alkyl. The 1500-600 cm-1 peaks are associated with specific peaks for the aromatic metabolite mono-substituted and para-disubstituted benzene rings. After degradation by *R. mucilaginosa* AUMC13570 new peaks were observed, indicating the degradation of malachite green and the formation of new compounds. The new peaks at 2956 cm^{-1} for O-H stretching vibrations, 1712 cm^{-1} for C-H stretching, 1652 cm^{-1} and 1579 cm^{-1} for alkenes C=C stretching of benzene rings, and 1464 cm^{-1} for C-H were identified. The peaks at 1377 cm^{-1}, 1283 cm^{-1}, and 1197 cm^{-1} indicate alcohol, while 722 cm^{-1}, 700 cm^{-1} indicate halo compound. The reduction of peaks along the fingerprint region, such as 941, 904, 832, 725, 658, and 511 cm-1, can be attributed to the loss of the metabolites' aromaticity, indicating the complete degradation of MG.

Also, the phytotoxicity tests before and after (Degradation experiments), have shown that the degradation by-products produced from the original dye are less toxic[13]. The impact of Malachite Green dye and its degradation metabolites by *R. mucilaginosa* AUMC13570 on the germination index of seeds and grains of wheat, maize, sorghum, and radish reach 43%, 35%, 48%, 47% respectively comparing to their germination index in MG dye which was 9%, 3%, 11%, 1% respectively and improvement rate degradation of MG metabolites produced by *R. mucilaginosa* AUMC13570 significantly increased reach 3, 10, 3, 40 respectively.

The results showed that the germination index of seeds and grains in untreated Malachite Green solution was low compared to the metabolites produced by *R. mucilaginosa* AUMC13570 after degradation indicating the safety of using yeast in bioremediation of dye-polluted water and potential positive environmental impact[13].

Table 6: Different Malachite green dye concentrations affect *Rhodotorula mucilaginosa* AUMC13570 decolorizing efficiency.

	Malachite green dye concentration (mg.)						
Time	**50**	**100**	**200**	**300**	**500**	**1000**	**F-value**
2hrs	49.8 ± 0.4^{f}	34.7 ± 0.3^{d}	9.1 ± 0.7^{b}	14.2 ± 2.1^{c}	3.7 ± 0.5^{a}	0 ± 0^{a}	435.6^{***}
4hrs	55.8 ± 0.3^{f}	46.5 ± 0.4^{e}	15.3 ± 1.5^{c}	19.2 ± 0.6^{c}	6.3 ± 1.6^{b}	0 ± 0^{a}	552.3^{***}
6hrs	58.8 ± 0.2^{f}	52.4 ± 0.9^{e}	19.6 ± 0.2^{d}	23.0 ± 0.3^{c}	6.4 ± 1.3^{b}	0 ± 0^{a}	1229.7^{***}
8hrs	80.8 ± 0.7^{f}	61.1 ± 0.2^{e}	21.9 ± 0.6^{d}	23.9 ± 0.2^{c}	6.4 ± 1.3^{b}	0 ± 0^{a}	2199.1^{***}
10hrs	94.2 ± 0.9^{f}	67.9 ± 0.5^{e}	38.1 ± 1.1^{d}	25.9 ± 0.2^{c}	6.4 ± 1.3^{b}	0 ± 0^{a}	1980.5^{***}
12hrs	99.3 ± 0.1^{f}	89.1 ± 1.3^{e}	46.6 ± 1.0^{d}	25.6 ± 0.3^{c}	6.4 ± 1.3^{b}	0 ± 0^{a}	2750.8^{***}

The mean values of the percentage of MG removal are presented as triplicate samples ± standard errors. Values with different superscript letters in the same raw for each parameter were significantly different at 0.01 ($p < 0.001$; ***), 0.1 ($p < 0.01$; **), and 5% level ($p < 0.05$; *).

Conclusion

In summary, *Rhodotorula mucilaginosa* AUMC13570 has the potential to degrade Malachite Green as a target pollutant in an aqueous solution in a high percentage. The parameters that influence the degradation of Malachite Green by *R. mucilaginosa* AUMC13570 were represented and found to be more tolerant degrading the dye in a wide range of incubation type (static and submerged), media composition (A, B, and C), temperature (25, 28, 30, and 37℃), agitation speed (50, 100, 120, and 150 rpm), inoculum age (one, two, three, four, five, and six) days, and initial dye concentration (50, 100, 200, 300, and 1000 mg/L). The degradation efficiency of *R. mucilaginosa* AUMC13570 is enhanced from 96.65% decolorizing percentage of 50 mgL^{-1} after three days of the incubation period to completely decolorized 50 mg L^{-1} malachite green dye with 100% decolorization percentage after 13 hours, 100 mg L^{-1} malachite green dye completely after 14 hours, and 200 mg L^{-1} malachite green dye completely after two days incubation periods using the ideal conditions: a shaking type of incubation, media supplies with 3% glucose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract, 37℃ temperature degree, 120 rpm agitation speed, four days inoculum age, and 50 mg initial dye concentration.

REFERENCE

1. B. Abdollahi, A. Najafidoust, E. A. Asl and M. Sillanpaa, "Fabrication of ZiF-8 metal-organic framework (MOFs)-based CuO-ZnO photocatalyst with enhanced solar-light-driven property for degradation of organic dyes", ***Arab J Chem***, 14(12), 103-444 (2021).
2. D. Akgün and M. Dükkancı, "G-C3N4 supported Ag/AgCl@ MIL-88A MOF based triple composites for highly efficient diuron photodegradation under visible LED light irradiation", ***JWPE***, 51, 103-469 (2023).
3. M. Khosravi-Far, R. M. Mohebat, Ghaedi, and M. Tabatabaee, "Efficient photocatalytic degradation of malachite green dye from wastewater using facilely synthesized ternary ZIF-8/Ag/Ag2S nanocomposite: Optimization by RSM (CCD)", ***Process Saf Environ Prot***, 190, 46-62 (2024).
4. X. Zhang, N. Yuan, T. Chen, B. Li and Q. Wang, "Fabrication of hydrangea-shaped Bi2WO6/ZIF-8 visible-light responsive photocatalysts for degradation of methylene blue", ***Chemosphere***, 307, 135-949 (2022).
5. A. Elhalil, H. Tounsadi, R. Elmoubarki, F. Z. Mahjoubi, M. Farnane, M. Sadiq and N. Barka, "Factorial experimental design for the optimization of catalytic degradation of malachite green dye in aqueous solution by Fenton process", ***Water Resour Ind***, 15, 41-48 (2016).
6. D. A. Yaseen and M. Scholz, "Textile dye wastewater characteristics and constituents of synthetic effluents: a critical review", ***Int J Environ Sci Technol***, *16*, 1193-1226 (2019).
7. A. Vignesh, K. Manigundan, J. Santhoshkumar, T. Shanmugasundaram, V. Gopikrishnan, M. Radhakrishnan and R. Balagurunathan, "Microbial degradation, spectral analysis, and toxicological assessment of malachite green by *Streptomyces chrestomyceticus* S20", ***Bioprocess Biosyst Eng***, 43, 1457-1468 (2020).
8. H. S. Rai, S. Singh, P. P. S. Cheema, T. K. Bansal and U. C. Banerjee, "Decolorization of triphenylmethane dye-bath effluent in an integrated two-stage anaerobic reactor", ***J Environ Manag,*** 83(3), 290-297 (2007).
9. X. Zhou, J. Zhang, Z. Pan and D. Li, "Review of methods for the detection and determination of malachite green and leuco-malachite green in aquaculture", ***Crit Rev Anal Chem***, 49(1), 1-20 (2019).
10. S. J. Culp, P. W. Mellick, R. W. Trotter, K. J. Greenlees, R. L. Kodell and F. A. Beland, "Carcinogenicity of malachite green chloride and leucomalachite green in B6C3F1 mice and F344 rats", ***Food Chem Toxicol***, 44(8), 1204-1212 (2006).
11. A. Stammati, C. Nebbia, I. De Angelis, A. G. Albo, M. Carletti, C. Rebecchi and M. Dacasto, "Effects of malachite green (MG) and its major metabolite, leucomalachite green (LMG), in two human cell

lines", ***Toxicol In Vitro***, 19(7), 853-858 (2005).
12. J.P. Jadhav and S. P Govindwar, "Biotransformation of malachite green by *Saccharomyces cerevisiae* MTCC 463", ***Yeast***, 23(4), 315-323 (2006).
13. N. Allam, E. M. Mohamed, S. M. Nassar and M. A. Ali, "Screening of Yeast Ability To Decolorization And Complete Biodegradation of Malachite Green Textile Dye and Investigation of Their Phytotoxicity", ***Bull Pharm Sci Assiut Univ***, 47(1), 179-195 (2024).
14. S. Nassar, A. E. D. H. Sayed, N. A. Nafady, M. M. Ali, and E. M. Mohamed, "Bioremediation of the toxic effects induced by the malachite green dye in *Clarias gariepinus* using *Rhodotorula mucilaginosa* MH298827", ***Sci Afr***, 26, e02496 (2024).
15. A. Ahmad, S. B. Kurniawan, J. Ahmad, J. Alias, N. Marsidi, N. S. M., Said and H. A. Hasan, "Dosage-based application versus ratio-based approach for metal-and plant-based coagulants in wastewater treatment: Merits, limitations, and applicability", ***J Clean Prod***, 334, 130-245 (2022).
16. Y. Gao, X. Fang, D. Chen, N. Ma and W. Dai, "Ternary photocatalyst of ZIF-8 nanofilms coupled with AgI nanoparticles seamlessly on ZnO microrods for enhanced visible-light photocatalysis degradation", ***J Taiwan Inst Chem Eng***, 131, 104-146 (2022).
17. V. R. Moreira, Y. A. R. Lebron, L. V. de Souza Santos and M. C. S. Amaral, "Low-cost recycled end-of-life reverse osmosis membranes for water treatment at the point-of-use", ***J Clean Prod***, 362, 132-495 (2022).
18. S. Thanigaivel, S. Vickram, S. Manikandan, S. R. Deena, R. Subbaiya, N. Karmegam and W. Kim, "Sustainability and carbon neutralization trends in microalgae bioenergy production from wastewater treatment: A review", ***Bioresour Technol***, 364, 128057 (2022).
19. B. Swanckaert, J. Geltmeyer, K. Rabaey, K. De Buysser, L. Bonin and K. De Clerck, "A review on ion-exchange nanofiber membranes: properties, structure and application in electrochemical (waste) water treatment", ***Sep Purif Technol***, 287, 120-529 (2022).
20. W. Shen, D. Mukherjee, N. Koirala, G. Hu, K. Lee, M. Zhao and J. Li, "Microbubble and nanobubble-based gas flotation for oily wastewater treatment: a review", ***Environ Rev***, 30(3), 359-379 (2022).
21. Y. Q. Wang, L. X. Hu, J. H. Zhao, Y. Han, Y. S. Liu, J. L. Zhao and G. G. Ying, "Suspect, non-target and target screening of pharmaceuticals and personal care products (PPCPs) in a drinking water system", ***Sci Total Environ***, 808, 151-866 (2022).
22. A. Azari, R. Nabizadeh, A. H. Mahvi and S. Nasseri, "Magnetic multi-walled carbon nanotubes-loaded alginate for treatment of industrial dye manufacturing effluent: adsorption modelling and process optimisation by central composite face-central design", ***J Environ Anal Chem***, 103(7), 1509-1529 (2023).
23. M. Mohamed, M. Ahmed and S. Nassar, "Isolation and molecular identification of yeasts in different food stuff and determine their abilities for phenols and flavonoids productivity by HPLC analysis", ***Yeast***, 4(3), 71-79 (2019).
24. S. Ren, J. Guo, G. Zeng and G. Sun, "Decolorization of triphenylmethane, azo, and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain", ***Appl Microbiol Biotechnol***, 72, 1316-1321 (2006).
25. Y. Yang, G. Wang, B. Wang, L. Du, X. Jia and Y. Zhao, "Decolorization of malachite green by a newly isolated *Penicillium* sp. YW 01 and optimization of decolorization parameters", ***Environ Eng Sci***, 28(8), 555-562 (2011).
26. M. N. Maniyam, P. Gunalan, H. H. Azman, H. Abdullah and N. S. Yaacob, "Enhancing biodecolorization of Malachite Green by *Flavobacterium* sp. through monothetic analysis", ***J Indian Chem Soc***, 101(12), 101-452 (2024).
27. R. Kalpana, M. Maheshwaran, E. Vimali, M. R. Soosai, C. S. Shivamathi, I. G.

Moorthy and P. Varalakshmi, "Decolorization of textile dye by halophilic *Exiguobacterium* sp. VK1: biomass and exopolysaccharide (EPS) enhancement for bioremediation of Malachite Green", ***ChemistrySelect***, 5(28), 8787-8797 (2020).

28. S. Abu-Hussien, H. Hemdan, B. A. Alzahrani, O. M. A. S. Alswat, F. A. Alatawi, M. A. Alenezi and S. M. El-Sayed, "Microbial degradation, spectral analysis and toxicological assessment of malachite green dye by *Streptomyces exfoliatus"*, ***Molecules***, 27(19), 6456 (2022).
29. M. Abewaa, A. Mengistu, T. Takele, J. Fito and T. Nkambule, "Adsorptive removal of malachite green dye from aqueous solution using Rumex abyssinicus derived activated carbon", ***Sci Rep***, 13(1), 14701 (2023).
30. N. A. Oranusi and C. J. Ogugbue, "Effect of pH and nutrient starvation on biodegradation of azo dyes by *Pseudomonas* sp", ***J Appl Sci Envir Manag***, 9(1), 39-43 (2005).
31. F. M. Yılmaz, M. Karaaslan and H. Vardin, "Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (*Prunus cerasus* L.) pomace", ***J Food Sci Technol***, 52, 2851-2859 (2015).
32. J. P. Stevens, "*Intermediate statistics: A modern approach"*, ***Routledge*** (2013).
33. V. Alaya, R. K. Kodi, E. Ninganna, B. Gowda and M. B. Shivanna, "Decolorization of Malachite green dye by *Stenotrophomonas maltophilia* a compost bacterium", ***Bull Natl Res Cent,*** 45, 1-13 (2021).
34. A. Barapatre, K. R. Aadil and H. Jha "Biodegradation of malachite green by the ligninolytic fungus *Aspergillus flavus*", ***CLEAN–Soil, Air, Water,*** 45(4), 1600045 (2017).
35. R. Al-Tohamy, J. Sun, M. F. Fareed, E. R. Kenawy and S. S. Ali, "Ecofriendly biodegradation of Reactive Black 5 by newly isolated *Sterigmatomyces halophilus* SSA1575, valued for textile azo dye wastewater processing and detoxification", ***Sci Rep***, 10(1), 12370 (2020).
36. C. E. Edoamodu and U. U. Nwodo, "Decolourization of synthetic dyes by laccase produced from *Bacillus* sp. NU2", ***Biotechnol Biotechnol Equip***, 36(1), 95-106 (2022).
37. Z. Vipotnik, M. Michelin and T. Tavares, "Biodegradation of chrysene and benzo [a] pyrene and removal of metals from naturally contaminated soil by isolated *Trametes versicolor* strain and laccase produced thereof", ***Environ Technol Innov***, 28, 102737 (2022).
38. F. Alshehrei, "Optimization of malachite green and crystal violet decolorization by *Bacillus cereus* and *Pseudomonas earoginosa"*, ***Ann Biol Res***, 9(1), 1-8 (2018).
39. K. Murugesan, I. H. Yang, Y. M. Kim, J. R. Jeon and Y. S. Chang, "Enhanced transformation of malachite green by laccase of *Ganoderma lucidum* in the presence of natural phenolic compounds", ***Appl Microbiol Biotechnol***, 82, 341-350 (2009).
40. C. Li, D. Liu, X. Yang, S. Chen, J. Deng, H. Rong and C. Li, "Efficient biodegradation of malachite green by a newly isolated *Klebsiella pneumoniae* strain WA-1", ***Environ Prog Sustain Energy***, 39(4), e13346 (2020).
41. M. A. El-Bendary, M. E. Fawzy, M. Abdelraof, M. El-Sedik and M. A. Allam, "Efficient malachite green biodegradation by *Pseudomonas plecoglossicide* MG2: process optimization, application in bioreactors, and degradation pathway", ***Microb Cell Fact***, 22(1), 192 (2023).

Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 629-643.

نشرة العلوم الصيدليـــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تحسين كفاءة *Rhodotorula mucilaginosa* *MH341115* (AUMC13570) في التحلل البيولوجي للصبغة السامة الخضراء المالاكيتية من المياه الملوثة

سميه محمود نصار* – نفين علام نفادى – مايسه محمد احمد على – ايمان مصطفى محمد

قسم النبات والميكروبيولوجى، كليه العلوم، جامعه اسيوط، اسيوط، مصر

تعتبر صبغة الأخضر المالاكيت ملوثا بيئيًا واسع الانتشار تعرض صحة الإنسان واستقرار الغلاف الحيوي للأرض للخطر. قام هذا البحث بتقييم كيفية تأثير الظروف الغذائية والبيئية على إزالة اللون الأخضر المالاكيت بواسطة *Rhodotorula mucilaginosa* AUMC13570. تم تغيير عامل واحد لكل تجربة مع الحفاظ على ثابت المتغير المُحسَّن مسبقًا في طريقة التحسين بخطوة واحدة المستخدمة لتحسين العملية مع مراعاة ستة متغيرات رئيسية: نوع التحضين (ثابت واهتزاز)، وتركيب الاوساط الغذائيه (أ و ب و ج) ودرجة الحرارة (٢٥ و٢٨ و٣٠ و٣٧ درجة مئوية)، وسرعة الاهتزاز (٥٠ و١٠٠ و١٢٠ و١٥٠ دورة في الدقيقة)، وعمر العزله الميكروبيه (يوم واحد ويومان وثلاثة وأربعة وخمسة وستة) أيام، وتركيز الأولي للصبغة (٥٠ و١٠٠ و٢٠٠ و٣٠٠ و١٠٠٠ ملجم/لتر). تم تقييم قدرة إزالة اللون من خلال ملاحظة انخفاض امتصاص الصبغة كل ساعتين خلال فترة الحضانة التي تبلغ ١٢ ساعة. أظهرت نتائجنا أن تركيز الصبغة ٥٠ مجم / لتر، والتحضين مع التحريك، وعمر العزله الميكروبيه لمدة أربعة أيام، وسرعة التحريك ١٢٠ دورة في الدقيقة، ودرجة حرارة ٣٧ درجة مئوية هى الظروف المثاليه التى بها أنتجت *Rhodotorula mucilaginosa* AUMC13570 أعلى معدل إزالة اللون. سلط تحليل البيانات الضوء على العوامل المختلفة التي أثرت على فعالية إزالة اللون.