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Decolorization of Malachite Green Dye Solution by Bacterial Biodegradation

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ABSTRACT

Malachite green dye is widely used in food and textile industries for various purposes and also used as biocide in the aquaculture industry to control fungal attacks and protozoan infections. Surface and ground water is contaminated by dyes due to discharge of untreated wastewater from industries. The presence of malachite green in water causes serious health effects such as mutagenesis, respiratory toxicity and carcinogenesis. Therefore, removal of malachite green from water by using various techniques is an essential concern for living beings as well as environment. In this study, the ability of isolated bacteria (from oil contaminated soil) for biodegradation of MG dye was investigated. The bacterium was able to grow in temperature range of 25 to 45°C and pH range of 5 to 9. Optimum temperature and pH for bacterial growth were determined as 37 °C and 7, respectively. Effect of temperature, initial concentration of dye and shaking condition on decolorization of dye solution was also tested. 20 ppm MG dye was efficiently degraded by bacteria in less than 2 h, and biodegradation of MB dye followed first-order kinetics model. These properties make the bacteria suitable for industrial wastewater treatment. Prog. Color Colorants Coat. 14 (2021), 79-87© Institute for Color Science and Technology.

1. Introduction

Water is one of the basic necessities for sustaining and continuation of life. It is therefore important to supply good quality water for various purposes. However, this is becoming increasingly difficult in view of large-scale pollution caused by industrial, agricultural and domestic activities. Some of the common pollutants are phenols, dyes, detergents, insecticides, pesticides, and heavy metals [1]. Dyes have become one of the main sources of water pollution due to the rapid development in many industries such as textiles, leather, paper production, and food technology [2]. With the increase of dyes usage, the discharge of dyes into water bodies is became uncontrollable [3]. In fact, the highly colored effluent released to the water bodies is also coupled with high chemical and biochemical oxygen demand (COD and BOD) and suspended solids [4]. The textile industry alone accounts for two thirds of the total dyestuff production [5, 6]. Colored wastes, in spite of causing problems for sunlight transmission, may contain chemicals which exhibit toxic effects towards microbial populations and can be toxic and/or carcinogenic to mammals [7]. Malachite Green (MG) or Basic Green 4 is a triarylmethane dye. It is also known as N,N,N',N' -tetramethyl-4,4'-diamintriphenylcarbonium. The empirical formula for MG is $C_{23}H_{25}ClN_2$ (chloride) and its molecular weight is 364.911 g/mol (chloride). Figure 1 shows the

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molecular structure of the MG. The intense green color of the cation results from a strong absorption band at 620 nm (extinction coefficient of 10^5 M^{-1} cm^{-1}). MG is a cationic dye, highly soluble in water and is widely used in textile coloring industry as well as food and paper industries [8–10]. As a synthetic dye, it is harmful to biological system and reproductive organ [11]. Both clinical and experimental observations reported so far reveal that MG is a multi-organ toxin. MB is highly cytotoxic to mammalian cells. Incidences of tumor in the lungs, breast and ovary have also been reported from rats exposed to MG [12, 13]. Decrease in RBC count (dyscrasia), Hb (anaemia) and HTC (%); increase in WBC count (leukocytosis) and delay in blood coagulation were observed after exposure to MG [14, 15]. MB causes serious public health hazards and also poses potential environmental problems.

In general, dyes are poorly biodegradable. Conventional biological treatment processes are not very effective in dye removal [16]. Conventional dye removal systems are nowadays available which depend on physical and chemical principles such as absorption, coagulation, filtration, settling, etc. The limitations and drawbacks of these methods are their high cost, high amount of sludge production, and eventually secondary pollution [17-21]. Nowadays, dye removal by bioassay has received much attention [22-24]. Biological treatment methods are an easy, permanent, inexpensive and effective solution for the treatment of wastewater contaminated by dyes. These methods use microorganisms such as bacteria, fungi, algae, actinomycetes, which have the ability to remove dyes [25]. The long growth cycle and the average decolorization rate of fungi and algae have led to their limited use in decolorization systems, as opposed to bacterial decolorization [26].

In the present study, the bacteria used for decolorization of MG were isolated from oil contaminated soil with the ability to degrade polycyclic aromatic hydrocarbons (PAHs). Effects of temperature and pH on bacterial growth was investigated. For determination of decolorization properties of the bacterium, temperature, initial dye concentration, and shaking effect on the dye degradation was investigated and the order of decolorization rate was also determined.

2. Experimental

2.1. Instruments and chemicals

A UV-Visible spectrophotometer (PG instruments) and a Metrohm pH meter were used for the measurement of absorbance and pH, respectively. A Heidolph magnetic stirrer equipped with heater was used for stirring and shaking the samples. Bacterial cultures were grown in shaker incubator from Raimand Zist Fanavar Co. The samples were weighed with an analytical balance (Sartorius). All the reagents and chemicals were of analytical grade with the highest available purity.

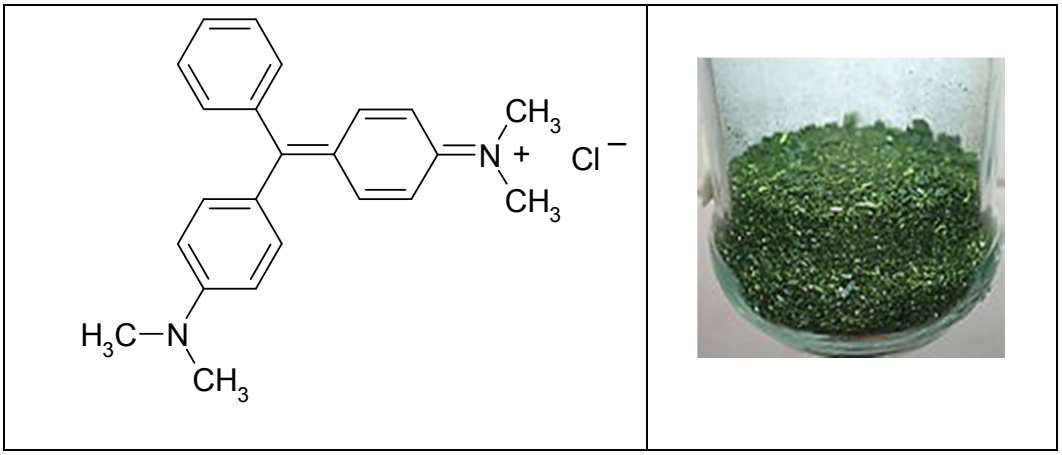

Figure 1: The structural formula and color of MG dye.

2.2. Bacterial growth

The bacterium used in this study was isolated from oil contaminated soil based on its ability to degrade polyheterocyclic aromatic compounds. The bacterium was incubated overnight at 37 °C in 10 mL Mueller-Hinton broth medium. 80 µL of an overnight culture was added to 80 mL of fresh Mueller-Hinton broth culture and incubated at 37 °C.

2.3. Optimum temperature for bacterial growth

To determine the optimum temperature for the bacterial growth, 80 µL of an overnight culture was added to 80 mL of fresh Mueller-Hinton broth culture and incubated at different temperatures (25, 37, and 45 °C) for 12 h. The growth of bacteria was investigated by measuring OD_{600}.

2.4. Effect of pH on bacterial growth

To investigate the effect of pH on bacterial growth, Mueller-Hinton broth medium was prepared and the pH of each culture was adjusted to 5, 6, 7, 8 and 9.80 µL of an overnight culture was added to 80 mL of each culture, and the OD_{600} was measured after incubation at 37 °C for 12 h.

2.5. Decolorization assay

Decolorization studies were carried out in 80 mL medium with MG under orbital shaking (150 rpm/min) and static conditions. To investigate the dye removal, 2 mL of the culture media was taken from the flask, centrifuged and the adsorption was measured at 620 nm (the maximum wavelength resulting from the spectrum analysis) using UV-Vis Spectrophotometer. Medium without dye and inoculum was used as blank while Medium with dye but without inoculum was used as control. The decolorization efficiency was expressed as the percentage ratio of the decolorized dye concentration to the initial dye concentration based on equation 1.

$$\text{Decolorization } (\%) = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 is the initial dye concentration and A is the dye concentration after predetermined incubation time.

2.6. Effect of temperature on decolorization

To determine the effect of different incubation temperatures on the decolorization of MG, three bottles containing Mueller-Hinton broth medium were prepared and the pH was adjusted to 7.80 µL of an overnight culture was added and incubated at 37 °C for 12 h. The MG was added to each bottle in final concentration of 10 ppm and incubated at 25, 37 and 50 °C for 2 h. Then 2 mL of each bottle was centrifuged at 10,000 rpm/min for 20 min and the absorption was measured at 620 nm.

2.7. Effect of initial concentration of MG on decolorization

The influence of the MG initial concentration in the solution on the degradation rate was investigated. MG was added to bottles containing 12 h bacterial cultures in final concentration of 5, 10 and 20 ppm and incubated at 37 °C for 2 h. Then 2 mL of each bottle was centrifuged at 10,000 r/min for 20 min, and the absorption was measured at 620 nm.

2.8. Determination of decolorization kinetics

Two common kinetic models were selected in this study to describe the decoloration process: first-order and second-order. The linear form of the first-order and the second-order models is described as equations (2) and (3), respectively [27].

$$\ln \frac{C_o}{C_e} = k_1 \, t \quad (2)$$

$$\frac{1}{C_e} - \frac{1}{C_o} = k_2 \, t \quad (3)$$

where C_o and Ce (mg/L) are the dye concentrations at the initial time and at time t, k_1 (L/min) is the rate constant of the first-order kinetic, k_2 (Lmg/min) is the rate constant of the second-order kinetics, and the k value is evaluated from the slope of the straight line between the left side of equations (2) and (3) versus time.

3. Results and Discussion

Malachite green has been widely used for dyeing of leather, wool, jute, and silk, in distilleries, and as fungicide and antiseptic in aquaculture industry to control fish parasites and disease [31]. Although the use of this dye has been banned in several countries and not approved by US Food and Drug Administration, it is still being used in many countries due to its low cost,

availability, efficacy, and lack of proper alternatives [32, 33]. Therefore, effective color removal is very important and should be emphasized in the treatment of wastewater before it can be released into the environment. Compared to chemical degradation processes, biological technique has been described as a promising route for the decolorization of most problematic contaminants originating from dyes. Microbial decolorization is particularly environmentally friendly and economically feasible [34]. Several microorganisms have been selected for dye decolorization. Their effectiveness was dependent on their activities and adaptability to the environment. Bacteria were reported to be more efficient than fungi in most studies on dye biodegradation [35]. MG has properties that make it difficult to remove from aqueous solutions and it is also toxic to major microorganisms. Most bioremediation studies focus on adsorption. There is a little study on biodegradation of MG because of the toxicity of MG for bacteria [36-43]. Some of them used enzymes or crude extracts for degradation of MG [36-40]. Bacterium used in this study showed short-time dye degradability. Degradation of MG was more than 97% in all concentration at 37 °C at static condition (Figure 2).

3.1. Optimum temperature for bacterial growth

Due to the toxicity of malachite green, the bacterium was unable to grow in the presence of dye from the beginning of culture. According to the bacterial growth curve, 12 hours bacterial growth was appropriate for color addition. Incubation temperature is one of the parameters that influence the bacterial growth. Bacteria are more tolerant against environmental conditions than other organisms. However, each species has its own characteristics and particular range of values for its best growth and reproductions. As shown in Figure 3, the bacterium can growth in wide range of temperature but after 12h incubation the maximum absorption on 600 nm was achieved at 37 °C and the bacterial growth was so slowly at 50 °C. Therefore, bacterial cultures were incubated at 37 °C for 12 h for next experiments.

3.2. Effect of pH on bacterial growth

Apart from complete nutritional composition, appropriate and stable pH is another important requirement for optimum bacterial growth in culture media. The pH of a culture medium should be suitable for the growing bacteria. As a result, the bacteria could grow at pH range of 5–9 and the best growth was at pH 7 (Figure 4). So, for the next experiments the pH of culture media was adjusted to 7.

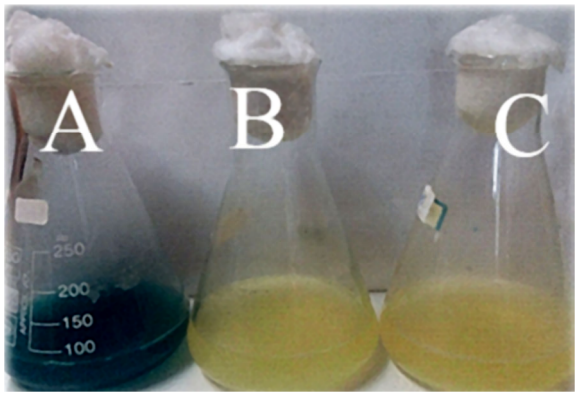

Figure 2: Complete degradation of MG under shaking condition after incubation at 37 °C for 2h (A. media containing 20 ppm MG, B, after 2h treatment with bacteria, C. bacterial culture without dye).

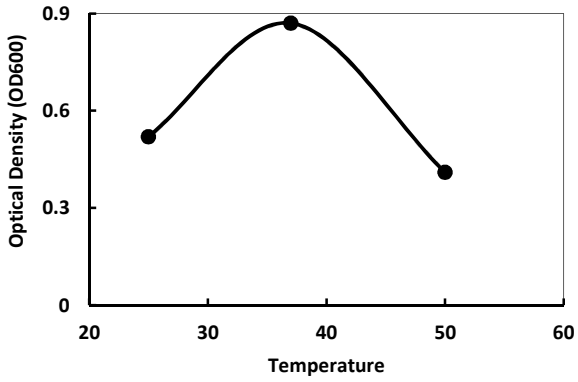

Figure 3: Effect of temperature on bacterial growth.

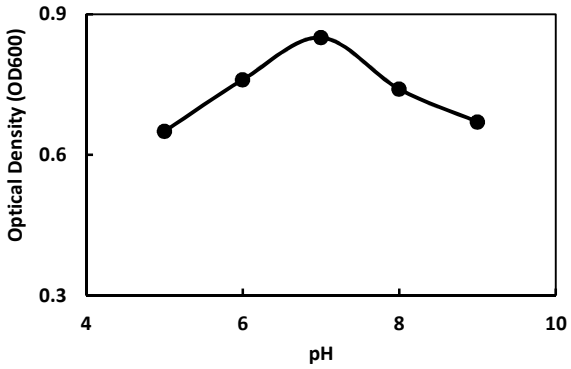

Figure 4: Effect of pH on bacterial growth.

3.3. Effect of temperature on decolorization

The influence of temperature on dye decolorization was evaluated by incubation of dye and bacteria at temperatures between 25-50 °C and the results are presented in Figure 5. The results demonstrated that the amount of dye decolorization increased with the increase in temperature, but the percentage of decolorization decreased gradually. The optimal temperature for decolorization was found to be 37 °C. At temperature beyond 40 °C, there was a decrease in decolorization rate. This observation can be attributed to the thermal denaturation of the enzyme molecules. In addition, this can be explained by cell viability reduction or inactivation of the enzymes responsible for decolorization, at high temperatures. Similar results have been reported previously [28].

3.4. Effect of initial concentration of MG on decolorization

Concentration of substrate in the aqueous phase has significant influence on any enzyme-mediated reaction [29]. Effect of initial MG dye concentration was investigated at different concentrations (5, 10 and 20 ppm), while keeping all the other parameters constant. The amount of the removed dye increases with increasing the initial MG dye concentration (Figure 6). This can be attributed to the presence of more dye molecules available for decolorization and subsequent degradation. However, decolorization efficiency decreased with increasing the MG dye concentration. It can be explained in view of the toxic effect of MG on bacterial growth at high concentrations. The observed trend is consistent with the literature [30].

3.5. Determination of decolorization kinetics

The kinetics of the decolorization processes provides useful information on the efficiency of enzymatic degradation and feasibility of scale-up operations. The decolorization data obtained from the experimental results were analyzed by first and second order kinetic model equations given in Table 1.

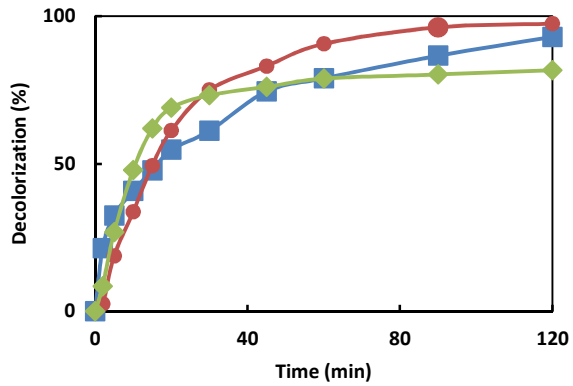

Figure 5: Effect of temperature on decolorization of MG in final concentration of 10 ppm (25 ■, 37● and 50 °C ♦).

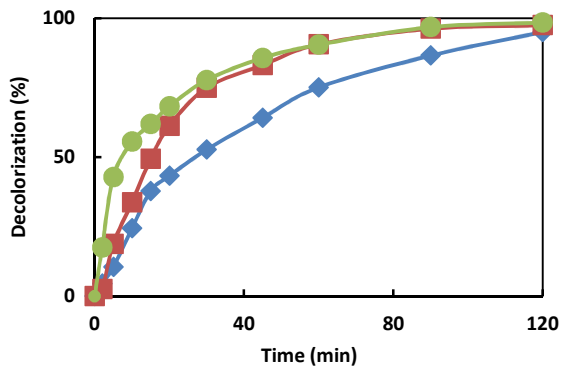

Figure 6: Effect of MG dye concentration on decolorization at 37 ˚C (5 ●, 10 ■ and 20 ppm ♦).

Table 1: First and second order kinetics model equations.

Kinetic models	Linear form	Plot	Rate constant
First order	$\ln C_0/C_e = k_1 t$	$\ln C_0/C_e$ vs time	k_1 (min^{-1})
Second order	$1/C_e - 1/C_0 = k_2 t$	$1/C_e - 1/C_0$ vs time	$k_2 mgL^{-1}min$

where C_e is the dye concentration in the solution (mgL^{-1}) at any time t, and C_0 is the initial concentration of the dye in the solution (mgL^{-1}). Different initial MG dye concentrations were used to determine the order of dye decolorization. Figure 7 shows a plot for the First-order kinetics model for different initial concentrations of MG dye.

The rate constants of degradation reaction and coefficients of least square method analysis are tabulated in Table 2. The correlation coefficients (R^2) were highest in the first-order model (in the range of 0.97-0.99) as compared to the second order kinetics model (Figure 7). The results show that biodegradation of MB dye follows first-order kinetics model. Experimental data showed good accordance with the first order kinetics model, confirming degradation of MG dye. The results show that decolorization process depends on MG dye concentration. This finding is consistent with earlier results reported in pervious paper [27].

3.6. Effect of shaking on decolorization

To investigate the effect of shaking on MG decolorization, several dye concentrations (5, 10 and 20 ppm) were incubated at 25, 37 and 50 °C under shaking (150 rpm) and static conditions for 2h. At 25 and 37 °C, the shaking had no significant effect on decolorization. While at 50 °C, about 99.8, 99.9 and 71.8% of was removed, respectively, under shaking condition. But under static condition, the rate of decolorization was decreased to 97.8, 81.6 and 52.3% for the initial dye concentration of 5, 10 and 20 ppm, respectively (Figure 8). It can be explained by considering the toxic effect of MG on bacterial growth at high concentration and harsh conditions (50 °C). To investigate the toxic effect of the dye, 50 µL of all cultures was transferred to 80 mL fresh media without dye, and then incubated at 37 °C for 24h. In all cultures, bacterial growth was observed except the culture from the flask that contained 20 ppm MG and treated at 50 °C for 2 h. Shaking increases the mixing of the oxygen in the medium and resulting bacterial growth. The same results were obtained by Kumar et al. [31], who found that shaking was beneficial for achieving maximum decolorization of brilliant green dye as a result of better oxygen transfer and nutrient distribution.

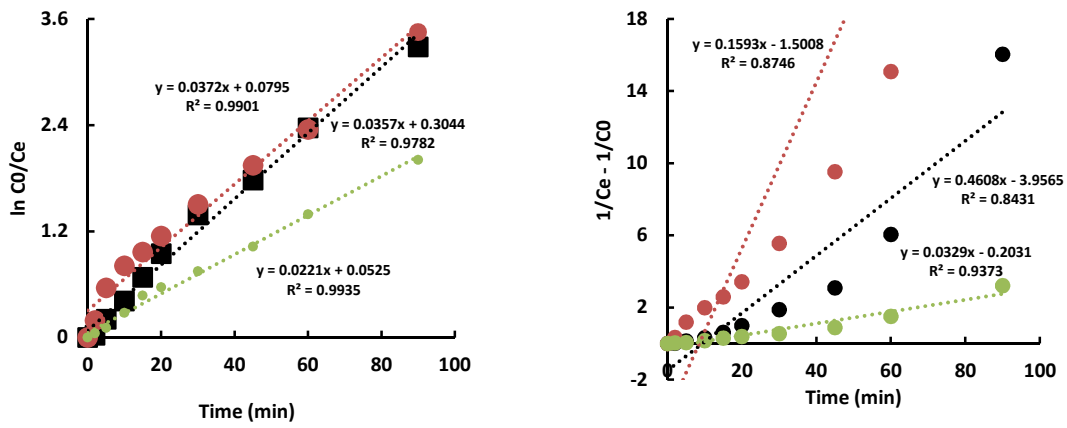

Figure 7: First and second order decolorization kinetics graphs at different initial concentrations of MG dye at 37 °C (5 ●, 10 ■ and 20 ppm ♦).

Table 2: First and second order kinetics constants and correlation coefficients o obtained for degradation of MG dye.

Kinetics model	Constant	5 mgL^{-1}	10mgL^{-1}	20 mgL^{-1}
First order	k_1 (min^{-1})	0.9901	0.9782	0.9935
Second order	k_2 $mgL^{-1}min$	0.8746	0.8431	0.9373

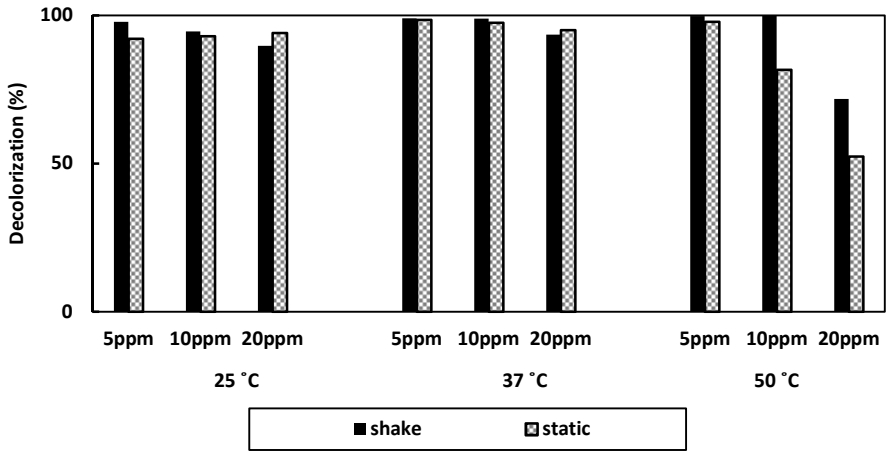

Figure 8: Effect of shaking on decolorization of MB dye after 2h incubation.

Mnif et al. [41] reported 83 and 40 % elimination of MG with initial concentration of 1000 mg/L under shaking and static conditions, respectively, by *CitrobacterSedlakii* RI11. They demonstrated that dye removal was done by adsorption on the microbial biomass and biodegradation. Chaturvedi et al. [42] reported 90 % degradation of MG (400 mg/L) after 96 h by *Ochrobactrumpseudogrignonens*e strain GGUPV1 isolated from copper mine waste water. In this study, degradation of MG was more than 97% in all concentrations at 37 °C under static condition. Because of toxic effect of malachite green on bacterial growth, concentration of 20 ppm at 50 °C was decolorized about 52% under static condition after 2h incubation, while under shaking condition was decolorized completely after 5h. Since decolorization did not occur after bacterial death (at 50 °C, 20 ppm of dye after 2h incubation under static condition), indicates that bacterium degrades MG instead of adsorption. To confirm the degradation process, after 12h of cultivation, the bacteria were inactivated by autoclave and then dye removal ability was investigated. It was observed that the efficiency of color removal was greatly decreased from 4 to 8%. The ability of bacteria to grow at wide range of temperature and pH values and also the ability for degradation of several concentration of MG in wide range of temperature under shaking and static conditions in short time (about 2 h) made it suitable for wastewater treatment.

5. Conclusions

This study investigated the ability of bacterium for degradation of Malachite Green. The bacterium was able to grow at temperatures between 25 to 50°C and pH between 5 to 9, respectively. Optimum temperature and optimum pH for bacterial growth was determined as 37 °C and 7, respectively. Results showed that degradation of 5 and 10 ppm concentrations of the MG was done completely in the wide range of temperature after 2h at shaking condition. Also dye can be degraded efficiently in the same time at static condition. 20 ppm concentration of the MG dye was efficiently degraded by bacteria in less than 2 h. Biodegradation of MB dye followed first-order kinetics model. Increase in dye concentration resulted in decrease in decolorization rate while the amount of degradation was increased. The results also showed that the bacterium decolorized the MG dye solution by degradation of MG instead of bacterial adsorption. All of these properties make the bacterium suitable for industrial wastewater treatment.

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