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Biodegradation of dye solution containing Malachite Green: Optimization of effective parameters using Taguchi method

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Abstract

In this paper, optimization of biological decolorization of synthetic dye solution containing Malachite Green was investigated. The effect of temperature, initial pH of the solution, type of algae, dye concentration and time of the reaction was studied and optimized using Taguchi method. Sixteen experiments were required to study the effect of parameters on biodegradation of the dye. Each of experiments was repeated three times to calculate signal/noise (S/N). Our results showed that initial pH of the solution was the most effective parameter in comparison with others and the basic pH was favorable. In this study, we also optimized the experimental parameters and chose the best condition by determination effective factors. Based on the S/N ratio, the optimized conditions for dye removal were temperature 25 °C, initial pH 10, dye concentration 5 ppm, algae type *Chlorella* and time 2.5 h. The stability and efficiency of *Chlorella* sp. in long-term repetitive operations were also examined.

Keywords: Biodegradation; Optimization; Algae; Malachite Green; Taguchi method

1. Introduction

Removal of hazardous industrial effluents is one of the growing needs of the present time [1]. Dyes are synthetic aromatic water-soluble dispersible organic colorants, having potential application in various industries [2]. Dyes tinctorial value is high: less than 1 ppm of the dye produces obvious coloration [3]. Removal of color from dye bearing wastewater is a complex problem because of difficulty in treating such wastewaters by conventional treatment methods [4]. Dyes may significantly affect photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic organisms due to the presence of aromatics and metals, chlorides, etc. Dyes usually have synthetic origin and complex aromatic molecular structures, which make them more stable and more difficult to biodegrade [5]. The textile industry utilizes about 10,000 different dyes and pigments. The worldwide annual production of dyes is over 7×10^5 tonnes [6–8]. In some cases, traditional biological procedures were combined with chemical or physical treatment processes to achieve better decolorization. Ozonation, photooxidation, electrocoagulation, adsorption, reverse osmosis, membrane filtration and flocculation are applied for dye removal from textile effluents [9–13]. These chemical or physical–chemical methods are less efficient, costly and produce wastes. As a viable alternative, biological processes have received increasing interest owing to their cost, effectiveness, ability to produce less sludge and environmental benignity [14]. Mohan studied treatment of simulated reactive yellow 22 dye effluents. Their results showed that the dye–algal treatment mechanism was attributed to biosorption (sorption of dye molecules over the surface of algal cells), bioconversion (diffusion of dye molecules into the algal cells and subsequent conversion) and biocoagulation [2]. Moreover, biodegradation has potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature [2]. In recent years, a number of studies have focused on some microorganisms that are able to biodegrade and biosorb dyes in wastewaters. A wide variety of microorganisms are capable of decolorization a wide range of dyes include some bacteria: *Escherichia coli* NO_3 [15], *Aeromonas hydrophila* [14], *C. pyrenoidosa* and *Closterium lunula* [16].

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Malachite Green (MG) is most commonly used for the dyeing of cotton, silk, paper, leather and also in manufacturing of paints and printing inks. If the solution containing MG discharged into receiving streams it will affect the aquatic life and cause detrimental effects in liver, gill, kidney, intestine and gonads. In humans, it may cause irritation to the gastrointestinal tract upon ingestion. Contact of MG with skin causes irritation and redness and pain. Upon contact with eye will lead to permanent injury of human eyes and laboratory animals [17].

In this study, the biological decolorization of Malachite Green was investigated and Taguchi experimental method was used to determine optimum degradation conditions. The Taguchi method, one of the optimization, has good reappearance of experiments concerned only with the main effects of design parameters. In principle, the Taguchi's design of experiments is used to get information such as main effects and interaction effects of design parameters from minimum number of experiments. The objectives of Taguchi method for parameter design were to find out the best combination of design parameters and reduce the variation for quality [18,19].

2. Materials and methods

2.1. Dye analysis

The triphenylmethane dye used in this study was Malachite Green (Merck, C.I. 42,000) and its properties are given in Table 1. The absorbance was measured with spectrophotometer (UV/Vis spectrophotometer WPA light wave S2000) at maximum absorption wavelengths, $\lambda_{max} = 619$ nm. Samples were filtered through 0.2 μm membranes to remove algae while measuring absorbance. The efficiency of dye removal was expressed as the percentage ratio of decolorized dye concentration to that of initial one.

2.2. Algal biomass

Four algal genera (*Cosmarium*, *Chlorella*, *Chlamydomonas* and *Euglena*) were acquired from natural lake and used immediately. The composition of growth medium for *Cosmarium* and *Chlorella* species is given in Table 2, which obtained from Merck, Germany. The medium contained following components used for *Chlamydomonas* and *Euglena*: $Ca(NO_3)_2$, 6.5 g l^{-1}; K_2HPO_4, 1.5 g l^{-1}; KNO_3, 1.5 g l^{-1}; $FeCl_2$, 0.01 g l^{-1}. Alga were grown in several 1 l glass jars containing growth media in order to obtain algal stock culture to be used during the experiments. The pH of media was adjusted with diluted H_2SO_4 and NaOH solutions and then the pH values were measured with pH meter (654 pH meter Metrohm Switzerland) before sterilization. Cells were cultivated at 25 °C under static incubation condition for a maximum 15 days exposure period. The jars were placed near the window and exposed to natural light. Algal biomass were measured by counting the number of cells by optical microscopy (Olympus, Japan) using a Neubauer Hemocytometer (Germany).

Table 1
Properties of Malachite Green

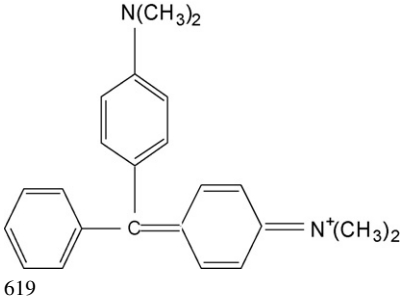
Dye	Malachite Green
Structural	
λ_{max} (nm)	619
Chemical class	Triphenyl methane
M_w (g mol^{-1})	927.02
C.I. number	42000

Table 2
Formulation of medium used in this study

Ingredient	Concentration (mg/l)
$NaNO_3$	95.2
$CaCl_2 \cdot 2H_2O$	25
$MgSO_4 \cdot 7H_2O$	75
K_2HPO_4	75
KH_2PO_4	175
NaCl	25
$FeSO_4 \cdot 7H_2O$	4.98
H_2SO_4	0.001 ml/l
H_3BO_3	11.42
EDTA	50
KOH	31
$ZnSO_4 \cdot 7H_2O$	8.82
$MnCl_2 \cdot 7H_2O$	14.4
$MoO_4Na_2 \cdot 2H_2O$	0.71
$CuSO_4 \cdot 5H_2O$	1.57
$CoCl_2$	0.49

2.3. Batch decolorization operation

The experiments were conducted in 250 ml Erlenmeyer flasks containing 100 ml of dye synthetic solution and algal biomass. To evaluate the effects of operation and environmental factors on the efficiency of dye removal, the batch decolorization experiments were carried out at different initial dye concentrations (2.5–12.5 ppm), temperatures (5–35 °C) and pH values (2.5–10) and different alga types. The pH was adjusted using diluted NaOH and HCl solutions. The batch decolorization experiments were performed under a static-incubation condition.

2.4. Orthogonal array and experimental factors

The conventional approach of experimenting with one variable (or one factor) at a time is labor-intensive and time consuming. Based on our pervious work from color removed process by biodegradation system the main operational parameters and their levels were selected and showed in Table 3 [20]. The orthogonal array of L_{16} type was used, and is represented in Table 4. L and 16 mean Latin square and the replication number of the experi-

Table 3
Parameters and their values corresponding to their levels to be studied in experiments

Parameter	Levels			
	1	2	3	4
A. Temperature (°C)	5	15	25	35
B. Initial pH	2.5	5	7.5	10
C. Dye concentration (mg l^{-1})	2.5	5	10	12.5
D. Algae type[a]	a	b	c	d
E. Time (h)	0.5	2.5	5	7

[a] Algae type: a, *Chlamydomonas*; b, *Euglena*; c, *Cosmarium*; d, *Chlorella*.

ment, respectively. Five–four level factors can be positioned in an L_{16} orthogonal array table. The number in table indicates the levels of a factor [18].

3. Results and discussion

3.1. Determination of optimal conditions using Taguchi method

A Taguchi method was used to identify the optimal conditions and to select the parameters having the most principle influence on the dye removal. The structure of Taguchi's L_{16} design and the results of measurement are shown in Table 4. In the Taguchi method, the terms 'signal' and 'noise' represent the desirable and undesirable values for the output characteristic, respectively. Taguchi method uses the S/N ratio to measure the quality characteristic deviating from the desired value. The S/N ratios are different according to the type of characteristic. In the case that bigger characteristics are better, the S/N ratio is defined as [18]

$$\frac{S}{N} = \frac{-10\log(1/y_1^2 + 1/y_2^2 + 1/y_3^2 + \cdots + 1/y_n^2)}{n} \quad (1)$$

Table 4
Experimental layout using the L_{16} orthogonal array and experimental results for results for percent of dye removal

Experimental number	A	B	C	D	E	Dye removal (%)			
						1	2	3	S/N
1	1	1	1	1	1	0.1	0.2	0.0	−109.654
2	1	2	2	2	2	34.6	30.1	34.4	30.324
3	1	3	3	3	3	33.1	35.8	35	30.775
4	1	4	4	4	4	61.3	59.3	61.4	35.655
5	2	1	2	3	4	16.2	16.5	16.8	24.346
6	2	2	1	4	3	73.8	73	69	37.127
7	2	3	4	1	2	44.8	46.5	44.2	33.09
8	2	4	3	2	1	28.6	31.4	30.5	29.57
9	3	1	3	4	2	22.3	21.6	23	26.957
10	3	2	4	3	1	17.9	20.1	18.3	25.435
11	3	3	1	2	4	78.7	80.5	79.7	38.02
12	3	4	2	1	3	93.9	93.5	93.3	39.422
13	4	1	4	2	3	1.2	1.3	1.9	2.831
14	4	2	3	1	4	21.9	23	20.2	26.691
15	4	3	2	4	1	56.6	53.5	54.8	34.795
16	4	4	1	3	2	79.4	79.8	79.5	37.014

Table 5
Response for the Taguchi analysis of dye removal data

Parameter	Mean S/N ratio			
	Level 1	Level 2	Level 3	Level 4
A. Temperature (°C)	−3.225	31.033	32.458	25.064
B. Initial pH	−14.399	29.894	34.17	35.665
C. Dye concentration (mg l^{-1})	0.877	32.222	28.498	23.734
D. Algae type	−2.613	24.667	29.643	33.633
E. Time (h)	−4.964	32.096	27.02	31.178

where y_i is the characteristic property, n is the replication number of the experiment. The unit of S/N ratio is decibel (dB), which is frequently used in communication engineering. Table 4 shows the S/N ratio for decolorization of the solution containing MG calculated using Eq. (1). The mean S/N ratio for each level of the parameters was summarized as S/N response, which was shown in Table 5. Fig. 1 shows the S/N response graph for decolorization of MG solution. Therefore, the optimum condition is A3, B4, C2, D4 and E2. In other words, based on the S/N ratio, the optimal parameters (conditions) for dye removal are A (temperature) at level 3 (25 °C), B (initial pH) at level 4 (10), C (dye concentration) at level 2 (5 ppm), D (algae type) at level 4 (*Chlorella*) and E (time) at level 2 (2.5 h). Finally, in this condition 80.7% dye removal can be obtained. In order to conduct an analysis of the relative importance of each factor more systematically, an analysis of variance (ANOVA) was applied to the data. The main objective of ANOVA is to extract from the results how much variations each factor causes relative to the total variation observed in the result. From the results of ANOVA in Table 6, the initial pH had the largest variance. The temperature of reactor indicated the second, respectively. Consequently, it can be concluded that the most influential factor was in the order of the pH. On the other hand, the degree of freedom (DOF) for each factor was 3 and total DOF was 15, so the DOF for error term was 0, and finally the variance for the error term (V_e), obtained by calculating error sum of squares and dividing by error degrees of freedom, could not be calculated. Henceforth, it was impossible to calculate the F-ratio, defined as the variance of each factor dividing by V_e. In order to eliminate the zero DOF from the error term, a pooled ANOVA was applied. The process of ignoring a factor once it was deemed insignificant was called pooling.

Table 6
Analysis of variance

Factor	DOF (f)	Sum of square (S)	Variance
A	3	3339.543	1113.181
B	3	6881.043	2293.681
C	3	2376.6514	792.171
D	3	3219.474	1073.158
E	3	3746.528	1248.842
Other/error	0		
Total	15	19563.104	

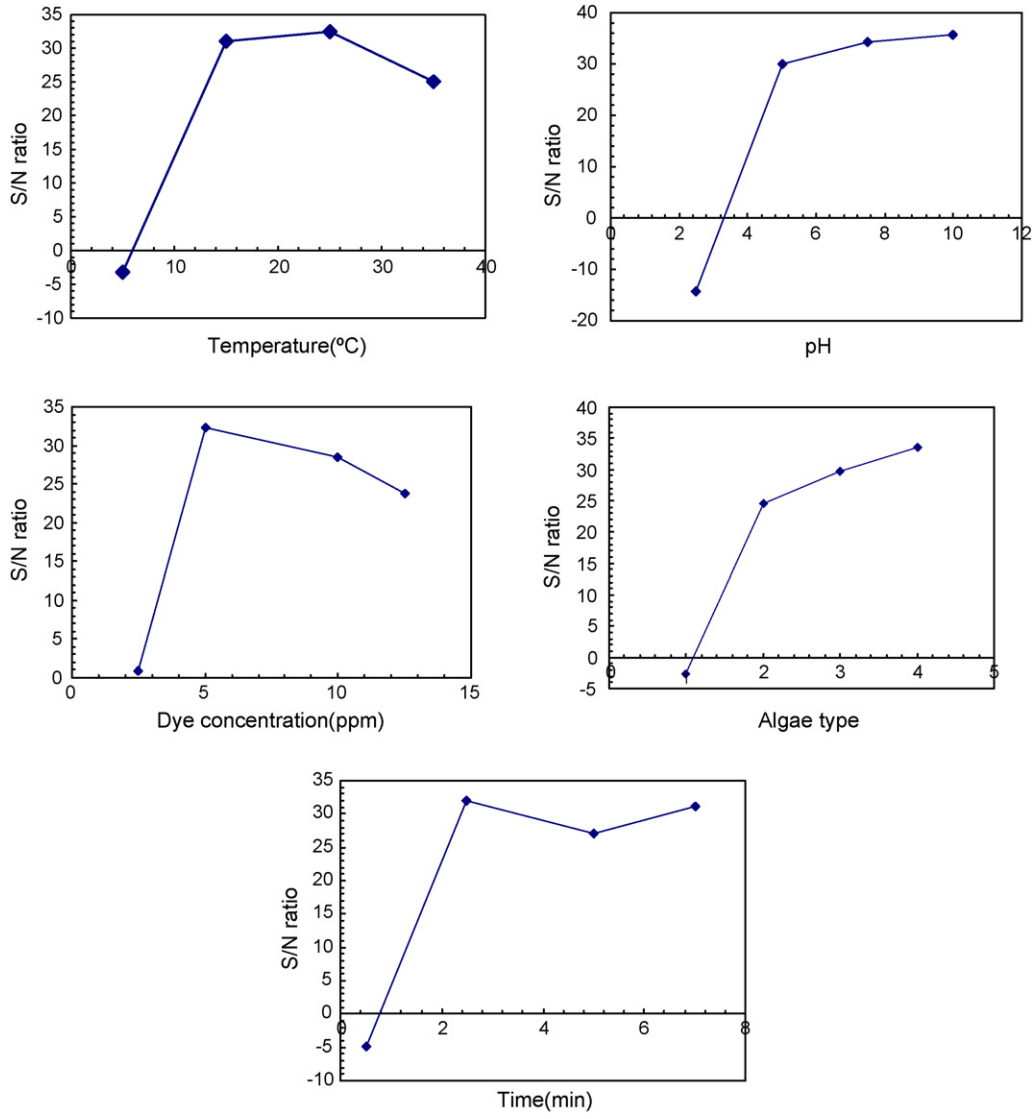


Fig. 1. Effect of each parameter on dye removal.

3.2. Effect of repeated uses

Our results showed that *Chlorella* sp. had the best effect in comparison with other type of alga. So the effect of repeated uses of *Chlorella* sp. was investigated. *Chlorella* sp. was cultivated for 43.5 h in batches and each batch lasted for 7 h to investigate the successive reuse of algal cells for the degradation of MG. Fig. 2 shows the results of repeated batch decolorization. During five repeated runs *Chlorella* sp. showed the same decolorization rate, since the algal cells were repeatedly exposed to the dye. The results indicated that *Chlorella* sp. hold excellent reusability and persistence in repetitive biological decolorization operation. Chang and Kuo performed repeated batch decolorization of C.I. Reactive Red 22 by *E. coli* NO_3. Their results showed that, after the first batch run, the decolorization rate of *E. coli* NO_3 increased dramatically for runs 2–5. This might be attributed to an adaptation effect, since the *E. coli* NO_3 cells were repeatedly exposed to the azo dye [15].

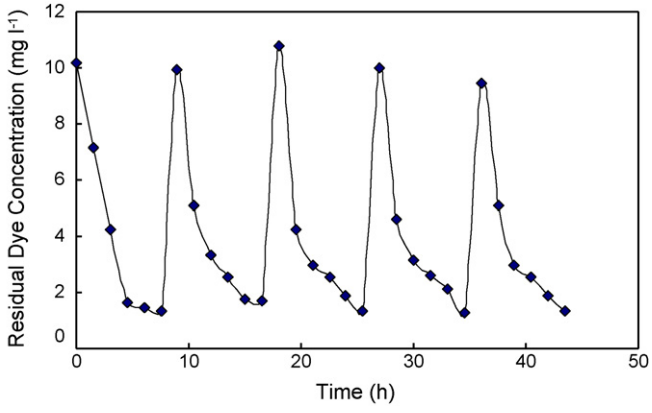


Fig. 2. Biological decolorization profiles during repeated batch operations. $T = 25\,^{\circ}C$; [MG] = 10 ppm; $[Chlorella]_0 = 9 \times 10^6$ cells ml^{-1}; pH 9.

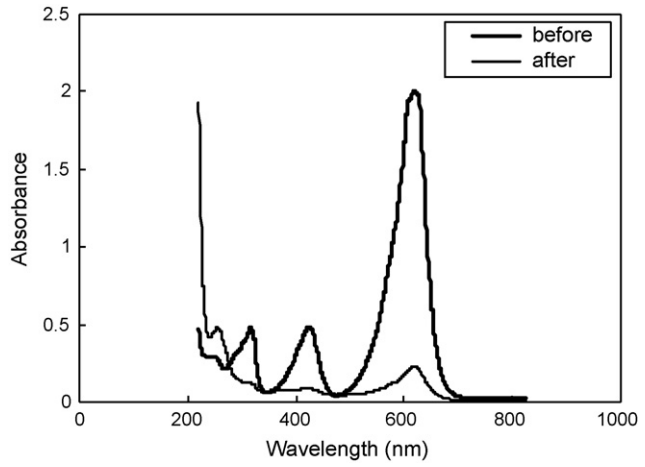


Fig. 3. UV–vis spectra of MG biodegraded by algae before and after optimized condition. $T = 25\,^{\circ}C$; [MG] = 10 ppm; $[Chlorella]_0 = 9 \times 10^6$ cells ml^{-1}; pH 9.

3.3. UV–vis spectra changes

Fig. 3 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 619 nm. The decrease of absorbance peak of MG at $\lambda_{max} = 619$ nm in this figure indicated a rapid degradation of the dye. According to the pervious literature [14] biodecolorization of dyes can be due to adsorption to biomass or biodegradation. In adsorption examination, the absorption spectrum will reveal that all peaks decrease approximately in proportion to each other. If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will disappear or a new peak will appear. In addition, extra absorbance peaks appeared in decolorized solution, probably resulting from the absorbance of metabolites or degraded fragments of dye molecules. These results indicated that the dye removal by algae might be largely attributed to biodegradation. We prepared another experiment to know whether autoclaved cells decolorize MG or not. The results showed that under the same condition, at the reaction time of 210 min, both dead and live cells could remove MG, 78% and 82%, respectively. Decolorization of dead cells may be due to increase of the cell wall area that ruptured during autoclaving and also may be due to revealing of special sites on cell wall [14]. The removal of MG by live cells approximately attributed to bioconversion.

3.4. Conclusion

The results indicated that utilization of Taguchi method was suitable for optimization of biodegradation of Malachite Green. Results obtained from this work showed that the algae specie possessed high decolorization efficiency. The decolorization was dependent on the dye concentration, pH, type of algae and temperature. The optimal conditions for dye removal were A (temperature) at level 3 (25 °C), B (initial pH) at level 4 (10), C (dye concentration) at level 2 (5 ppm), D (algae type) at level 4 (*Chlorella*) and E (time) at level 2 (2.5 h). The initial pH had largest effect and contribution in dye removal. The repeated use of *Chlorella* sp. for Malachite Green degradation was performed and the results revealed that algal cells were stable for 43.5 h in the repeated batch cultivation for Malachite Green degradation.

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