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Research article

Integrated optimization, kinetic modeling, and ecotoxicological evaluation of methylene blue degradation via ozonation: toward sustainable advanced oxidation processes

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Abstract: Synthetic dyes contained in industrial wastewater have become major sources of pollution for the environment and human health owing to their persistence and difficulty in degradation through traditional wastewater treatment processes. In this study, ozonation was studied as a method for the oxidation of methylene blue (MB) dye in water as an advanced oxidation process (AOP). This research is a combination of process optimization, kinetic modeling, energy requirement assessment, and ecotoxicological evaluation. To determine the optimal conditions (effect of O_3 dose, pH, and contact time) for MB removal, mineralization (chemical oxygen demand-COD; total organic carbon-TOC), residual ozone level, and toxicity toward *Daphnia magna* and *Vibrio fischeri*, a Taguchi L9 (3^3) experimental design was utilized. Optimal conditions for MB removal achieved 98%, COD removal 88%, TOC removal 82%, a minimal level of residual ozone (0.32 mg L^{-1}), and low ecotoxicological risk. Kinetic analysis confirmed the pseudo-first-order reaction rate with $k = 0.112$ min^{-1} ($R^2 = 0.987$). The energy cost of ozonation (electrical energy per order (EEO)) was 7.06 kWh m^{-3} $order^{-1}$, which made it energy-efficient among AOPs. According to the results obtained, the key parameters for optimizing the direct ozone oxidation and OH-radical oxidation pathways included the contact time and O_3 dose under neutral pH values.

Keywords: ozonation; Methylene Blue; pseudo-first-order kinetics; Daphnia magna; wastewater

treatment

1. Introduction

The rising contamination of water with industrial effluent, agricultural, and urban waste represents a serious problem for environmental quality, public health, and socioeconomic development [1,2]. Among the many interesting classes of contaminants are synthetic dye compounds, such as Methylene blue (MB). These are complex and highly resistant to degradation and are thus challenging to manage using available environmental protection systems [3]. These contaminants arise from the widespread use of synthetic dyes in industries, including textile dyeing, paper manufacture, leather finishing, plastic, and pharmaceutical industries, without proper disposal of waste [4]. The toxicity of MB to living creatures has been well documented because it has been found to be toxic, mutagenic, and carcinogenic to humans and aquatic life forms due to its stability and solubility in water [5]. Moreover, owing to their complex structures, high stability, and resistance to degradation, these contaminants can be resistant to removal using traditional techniques such as coagulation, flocculation, adsorption, and biological treatment [6]. This explains why there is a need to develop and adopt advanced technologies that can facilitate the effective breakdown of MB and other synthetic dyes into more benign compounds. Advanced Oxidation Processes (AOPs) represent one of the novel treatments that has gained much attention recently for cleaning up wastewater contaminated with synthetic dyes [7,8]. AOPs degrade contaminants into simpler and safer molecules through the production of highly oxidative free radicals, particularly hydroxyl radicals (OH) [9,10]. Another advanced oxidation process technique that has been successfully employed for dye-containing wastewater treatment is heterogeneous catalytic oxidation. Solid catalysts are used to accelerate the production of reactive oxygen species, leading to the efficient decomposition of organic compounds. For instance, researchers have reported the use of Mn–Fe oxides for the degradation of MB via oxidant activation methods. Similarly, the use of metal–organic framework-based photocatalysts for dye decolorization and mineralization has been reported [11,12]. In particular, ozonation has become one of the most extensively applied AOPs owing to its high efficiency, ease of operation, and versatility [13]. Ozonation has become attractive as an advanced oxidation process for industrial wastewater with persistent organics, such as dyes, because of their direct oxidation by ozone or indirect oxidation by the generation of hydroxyl radicals under selected conditions [14].

Research has shown that ozonation is more efficient and capable of removing MB effectively with higher efficiency than traditional treatment methods in a shorter period of time [2,15]. Nevertheless, several factors, such as the dose of ozone, pH, treatment duration, and water matrix properties, may affect the performance of the process [9]. Consequently, these factors need to be optimized to maximize the rate of dye degradation and the economic feasibility of ozonation. Increasing the ozone dose results in an increased speed of decomposition of MB. However, this factor also affects the energy demand and level of residual oxidants. In addition, the formation of hydroxyl radicals is highly affected by the pH [16]. Kinetic analysis is also an essential tool that is widely used to improve ozonation. It can be applied to predict the performance of different cases and explain the mechanisms of dye degradation [16]. Although ozonation is a very efficient method for MB degradation, there is growing evidence that the intermediate compounds produced during this process may be more dangerous than MB [7,17]. They are usually products of incomplete oxidation processes and represent potential

ecological hazards owing to their persistence in ecosystems [17]. Consequently, toxicity evaluation is a critical part of studying ozonation and other AOP [18,19]. In contemporary toxicity assessment, there is a need for using the combination of chemical analysis and ecotoxicological tests and bioassays to identify hazardous intermediates [13,20]. This procedure must be conducted before releasing the treated waste into aquatic ecosystems [21].

Despite the advances made, there are several gaps in the understanding of ozonation processes for MB degradation. First, the major drawback is that research was conducted independently, examining individual aspects of toxicant properties, kinetics, or optimization rather than using a comprehensive approach [5,15]. In addition, many studies cover only laboratory-scale experiments, potentially failing to account for the complexity of the natural wastewater environment [9]. A vital gap is the lack of research on the effects of long-term degradation products on the aquatic environment [19]. Finally, evaluations of energy efficiency were largely overlooked, limiting the applicability of the findings from laboratory tests to larger scales and reducing costs.

In this study, we aim to investigate the ozonation process of MB as the most widely used AOP with the following three objectives: (1) Performing systematic optimization of the major variables (ozone dose, pH, and contact time) by utilizing the Taguchi L9 orthogonal array method and finding optimal operational parameters, and (2) developing a model of pseudo-first-order kinetics and energy efficiency calculations. The expected results will undoubtedly contribute to the scientific knowledge regarding AOPs and their application in wastewater purification, promoting sustainable development goal six of the United Nations [3,14].

2. Materials and methods

2.1. Research aims and synthetic wastewater preparation

Our objective of this study was to evaluate the efficacy of the ozone process, which is an AOP, as a method for removing MB from water. The research plan included the following experiments: preparation of artificial wastewater, ozonation of experimental samples, kinetic modeling, energy efficiency evaluation, and ecotoxicological tests of treated effluents to check their environmental safety. Artificial wastewater was prepared to reproduce the results obtained through ozonation.

2.2. Ozonation experimental setup

Ozonation experiments were performed using a bench-scale ozone generator with a controlled gas flow system to adjust the ozone dose level. All ozonation experiments were performed using a cylindrical glass reactor (2 L working volume) equipped with a magnetic stirrer to maintain continuous gas-liquid contact and mixing. The dissolved ozone concentration in the reactor was constantly determined using an ozone analyzer. Some key experimental parameters, such as pH, duration of reaction, and ozone dose levels, were systematically varied to different levels during the experiments. For pH determination, a calibrated pH meter was used with adjustment of the diluted NaOH or HCl solutions to change the pH value (ASTM E70 [22]; pH meter: WTW Co., Germany, InoLab 720). At regular time intervals, samples were collected, filtered, and analyzed. In terms of the mechanism of degradation, direct ozone oxidation and indirect hydroxyl radical formation via decomposition of O_3 take place, that is, $O_3 \rightarrow OH + O_2$. Degradation of the dye molecules is initiated by direct electrophilic attack of the molecular ozone on the electron-rich sites of the aromatic dye, producing some

intermediates, which can subsequently be oxidized by the very active hydroxyl radicals formed due to decomposition of O^- under the proper pH conditions. Bilateral action between direct molecular O_3 attack and the indirect oxidative mechanism based on the formation of hydroxyl radicals, •OH, explains the powerful nature of the oxidation process, which enables effective elimination of organic pollutants in the form of dyes dissolved in water and is a combination of direct and indirect mechanisms of dye destruction. Figure 1 shows a diagram of the experimental setup (A) and a schematic representation of the oxidative degradation pathway based on the action of O_3 and OH radicals (B).

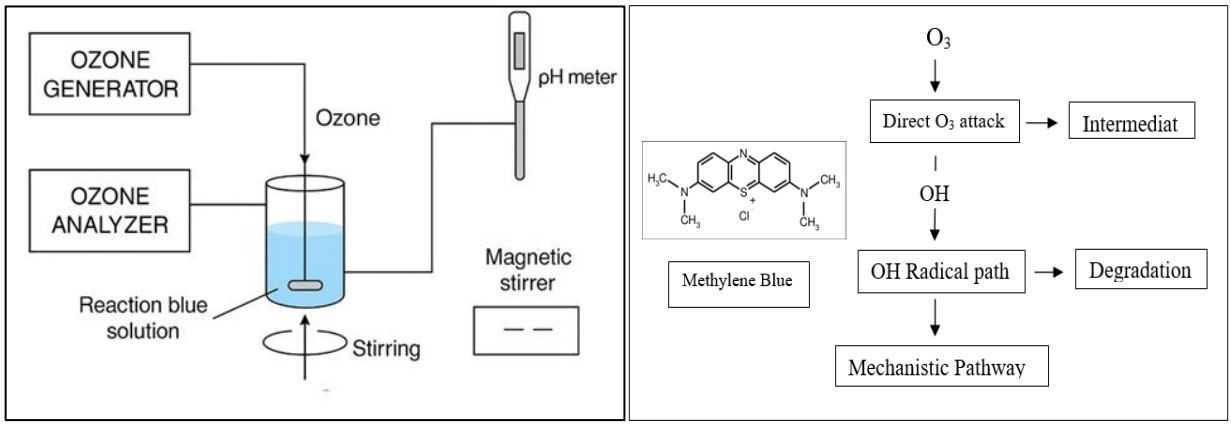


Figure 1. (A) Ozonation reactor setup. (B) MB degradation pathways via O_3 and •OH.

2.3. Operational parameters and experimental design

The three major operational parameters that were changed to evaluate their impact on MB breakdown were the ozone dose (O_3), pH (P), and contact time (t). The tests involved three levels of each variable, and hence there could have been 27 experiments (3 × 3 × 3). To avoid changing the reactor volume, temperature, mixing speed, gas flow rate, and diffuser type during the tests, only the initial MB concentration was varied. The Taguchi L9 (3^3) orthogonal array design methodology was used to reduce the number of experiments to nine while ensuring orthogonality between factors. A list of all operating factor levels can be found in Table 1. To identify the optimal levels of the factors based on the S/N ratio criteria, where larger values are desirable for removal efficiency and smaller values are desirable for toxicity indicators, the ANOVA analysis method was selected to determine the significance of each factor. After the application of S/N analysis and selection of the optimal combination, a test at an increased influent MB concentration (Series B, C_2 = 20 mg L^{-1}) was carried out.

Table 1. Independent variables and factor levels applied in the Taguchi L9 (3^3) orthogonal array design.

Factor	Level 1	Level 2	Level 3
Ozone concentration (mg L^{-1})	0.5	1	1.5
pH	5	7	9
Contact time (min)	10	20	30

2.4. Analytical procedures

The efficacy of MB degradation was determined spectrophotometrically using the absorbance of the sample at 664 nm on a UV-visible spectrophotometer. The color was measured according to ASTM D1209 [23]. The percentage of dye removal (discoloration) at the end of the degradation process was defined as follows:

$$\text{Dye removal (\%)} = [(A_0 - A_e) / A_0] \times 100 \quad (1)$$

where A_0 and A_e are the absorbances before and after the ozonation process, respectively. This equation is a direct indication of the extent to which the chromophore pattern of the MB is broken or damaged.

COD was calculated based on ISO 15705 [24] using a COD photometer (model RD-125, Lovibond Company, Germany) using the closed reflux titrimetric method. This parameter measures the decrease in organic load and represents the extent of oxidation beyond that possible with simple decolorization during ozonation. The pH of each solution was first brought to the desired initial pH by the addition of a diluted solution of NaOH or HCl and then measured using a calibrated pH meter (WTW Co., Germany, INOLAB 720).

TOC was measured according to ASTM D7573 [25] using a TOC analyzer (model TOC-L, Shimadzu Company, Japan). This experiment consisted of the oxidation of organic carbonaceous materials in a combustion reactor and the measurement of the resulting CO_2 in the infrared form. The TOC results showed direct quantitative information on the degree of mineralization of MB and the resultant oxidation products, and COD enabled stepwise monitoring of the process of chromophore cleavage to complete mineralization.

The remaining dissolved ozone concentration was determined using the indigo colorimetric method according to Standard Methods 4500-O_3 [26] at the end of each experiment to ensure that adequate oxidant levels were maintained during the reaction, assess the extent of degradation, and evaluate any potential interference with downstream bioassays. Monitoring residual ozone is essential to confirm that the oxidant is effectively utilized for meaningful mineralization rather than being lost through self-decomposition.

2.5. FTIR spectroscopic analysis

To determine the changes that occur in the structure of the dye molecule during the ozonation process, Fourier Transform Infrared (FTIR) spectroscopy of the MB samples was carried out before and after ozonation treatment. Liquid samples were either prepared by depositing a thin film on a KBr window and air-drying or prepared as KBr pellets. An FTIR spectrometer was used to record spectra at a spectral resolution of 4 cm^{-1} and 32 accumulative scans/sample in the wavenumber range of 400-4000 cm^{-1} to determine a sufficient signal-to-noise ratio. All the spectra were taken under optimal ozonation conditions (O_3 = 1.5 mg L^{-1}, pH = 7, t = 30 min) to determine the disappearance of the characteristic functional groups of the parent dye and the appearance of new absorption bands, which can be attributed to ozonation byproducts. All spectra were baseline-corrected and normalized to the strongest peak before interpretation, so that the pre- and post-treatment samples could be directly compared.

2.6. Ecotoxicological assessment

The acute toxicity of the treated effluents was assessed according to OECD Guideline 202 [27], which assesses the immobilization of *Daphnia magna* neonates after 48 h of exposure. *Daphnia magna* is a popular sentinel crustacean in freshwater, whose sensitivity to organic and oxidation byproducts of treated wastewater qualifies it as an ecologically relevant endpoint in the assessment of the safety of treated wastewater. Oxidant interference was avoided by quenching residual ozone before testing according to the OECD 202 protocol. The outcome was reported as the percentage of immobilization in comparison with unexposed controls.

The ISO 11348-3 [28] bioluminescence inhibition test of freeze-dried *Vibrio fischeri* was used to determine microbial toxicity. Luminescence was obtained at the end of 15 min of contact at 15°C, and results were given in terms of percentage inhibition with respect to the control. The reduction in the intensity of bioluminescence is a sensitive parameter for acute microbial toxicity owing to the remaining organic effluents in the treated effluents. The correct sample preparation, such as the residual ozone control according to ISO 11348-3, ensured that the measured inhibition was due to organic by-product bioactivity and not oxidant artifacts.

2.7. Experimental replication and quality control

Each experiment was performed three times to minimize errors and increase reproducibility. Each instrument used during analysis, such as the spectrophotometer, ozone analyzer (measured using the indigo method (Standard Methods 4500-O_3)), total organic carbon analyzer (Shimadzu TOC-L), chemical oxygen demand photometer (Lovibond RD-125), and pH meter (WTW INOLAB 720), was calibrated before each series of experiments. The accuracy and scientific validity of the results obtained were assured through rigorous adherence to the established analytical standards (ISO, ASTM, and OECD). This research methodology is reliable for analyzing the ozonation process, and the results are scientifically valid and can be implemented in wastewater treatment processes.

2.8. Kinetic modeling

The ozonation of MB was investigated using pseudo-first-order kinetic analysis [29]. In circumstances where ozone predominates over the dye, the rate of the reaction is determined mainly by the dye concentration, and the integrated rate expression is

$$kt = \ln (C_0 / C_t) \quad (2)$$

where C_0 and C_t represent the concentrations (in mg L^{-1}) of MB at times 0 and t (min), respectively, whereas k (unit min^{-1}) represents the pseudo-first-order rate constant for the reaction under study. It is presumed that the ozone concentration during MB oxidation remains quasi-steady, which is a realistic assumption in view of the fact that under the studied conditions, the ozone dose exceeded the dye amount [14,16].

The rate constant k was directly proportional to the regression of $\ln(C_0/C_t)$ on reaction time by means of linear regression analysis, with the coefficient of determination R^2 being used as the goodness of fit criterion. Under optimized conditions (O_3 = 1.5 mg L^{-1}, pH = 7, t = 30 min), the linear regression analysis yielded k = 0.112 min^{-1} with R^2 = 0.987, which indicated an outstanding agreement between theoretical and experimental curves and confirmed first-order kinetics for MB degradation. For this

rate constant value, the half-life of the process was $t_{½} = \ln 2/k \approx 6.2$ min.

As the O_3 dose was increased, a rise in k was observed; however, above 1.5 mg L^{-1}, the rates started leveling off, thus signaling the transition of the process to the regime controlled by mass transfer of ozone from the gas phase to the liquid one (k_la-controlled regime). This behavior may be explained by the fact that the ozone dissolution rate becomes a bottleneck under these conditions [16]. Such results have been demonstrated in the literature [9,14] and indicate the transition of the process to a mass-transfer-controlled regime at high oxidant doses.

2.9. Energy efficiency analysis

The ozonation process was also tested in terms of its energy performance by determining the EEO, which is the amount of electrical energy needed to obtain one log-order of MB concentration reduction per volume of the treated solution:

$$EEO\ (kWh\ m^{-3}\ order^{-1}) = (P \times t) / (V_m^3 \times \log (C_0 / C_t)) \quad (3)$$

where P (kW) is the electrical power of the ozone generator, t (h) is the treatment time, V (L) is the volume of the aqueous solution, and $\log(C_0/C_t)$ is the log reduction in the MB concentration. During all experiments, the total electrical power consumption included the ozone generator (35 W), magnetic stirrer (5 W), and air pump (8 W), resulting in a total power input of 48 W.

Further, to facilitate a more convenient comparison with values of EEO from the literature on ozonation processes, EEO (kWh m^{-3} $order^{-1}$) for ozonization of aqueous MB solutions was calculated. In our case, the ozone utilization efficiency represented the ratio of the quantity of productively consumed ozone to the residual ozone in the effluent, and was thus an additional parameter characterizing the economic efficiency of the ozonation process. With the optimum values (O_3 = 1.5 mg L^{-1}, pH = 7, t = 30 min, removal efficiency = 98%), the energy consumption was equal to 48 W (35 W for ozone production, 5 W magnetic stirring, and 8 W air pumps). Hence, EEO could be calculated as: EEO = (0.048 × 0.5)/(0.002 × log(10/0.2)) = 7.06 kWh m^{-3} $order^{-1}$. The obtained EEO value fit well into the average range (2–40 kWh m^{-3} $order^{-1}$) of EEO for direct ozonation processes (ozonation of waters containing dye) [14] and was noticeably lower than those for UV/H_2O_2 AOPs (20–100 kWh m^{-3} $order^{-1}$).

3. Results and discussion

The experimental findings are presented in this section, and the suitability of ozonation for the degradation of MB is discussed. We not only discuss the discovery of kinetic behavior, toxicity analysis, and water quality analysis, but also explain the behavioral patterns, compare the results with the prediction of expected behavior, and explain the implications of the results for wastewater treatment practices.

3.1. Process performance and Taguchi Optimization

The main-effects analysis determined that O_3–P_2–t_3 (1.5 mg L^{-1} ozone, pH 7, 30 min) was the optimal operating condition using the Taguchi L9 design for ozone dose (O), pH (P), and contact time (t). The results of the experiments and the predicted optimum and confirmation data are summarized in tables 2-4.

Table 2. Taguchi L9 design and experimental results.

Run ID	O_3 (mg/L)	pH	t (min)	Y1 MB (%)	Y2 Final pH	Y3 COD (%)	Y4 TOC (%)	Y5 O_3 residual (mg L^{-1})	Y6/Y7 Tox (%)
A-R01	0.5	5	10	77	5.3	64	52	0.26	18 / 23
A-R02	0.5	7	20	87	7	74	65	0.2	12 / 17
A-R03	0.5	9	30	86	8.7	75	65	0.14	12 / 17
A-R04	1	5	20	89	5.3	78	69	0.28	11 / 15
A-R05	1	7	30	95	7	84	77	0.22	6 / 10
A-R06	1	9	10	81	8.7	69	57	0.4	16 / 20
A-R07	1.5	5	30	95	5.3	86	79	0.3	6 / 9
A-R08	1.5	7	10	88	7	76	67	0.48	11 / 14
A-R09	1.5	9	20	91	8.7	81	72	0.42	10 / 13

Table 3. Predicted optimum and confirmation results.

Combination	Y1 MB (%)	Y3 COD (%)	Y4 TOC (%)	Y5 (mg/L)	Y6 Daphnia (%)	Y7 Vibrio (%)
O_3-P_2-t_3 Predicted Optimum	98	88	82	0.32	4	7
B-CONF (C_2 = 20 mg/L)	94	84	79	0.27	6	9

Table 4. Optimal conditions and performance summary.

Parameter	Unit	Range Tested	Optimum	Result at Optimum
Ozone dose (O_3)	mg L^{-1}	0.5–1.5	1.5	98% MB removal
pH	——	5–9	7	Balanced O_3 / •OH oxidation
Contact time (t)	min	10–30	30	Complete decolorization and mineralization
COD reduction	%	60–90	——	88%
TOC reduction	%	50–85	——	82%
Residual O_3	mg L^{-1}	0.14–0.48	——	0.32 mg L^{-1} (efficient utilization)
Daphnia immobilization	%	6–18	——	4–5% (environmentally safe)
Vibrio inhibition	%	9–23	——	7–8% (environmentally safe)

Contact time and dose of ozone had a dominant effect on all the performance parameters, whereas pH was had a secondary effect on the ozone process. The duality of the oxidant and precursor of ozone as direct electrophilic oxidants and precursors of ozone was observed in the steady increase in decolorization and organic load removal (Y1, Y3, and Y4) with increasing ozone dose. The system produced enough oxidizing species to eliminate kinetic and mass transfer constraints at the optimum level of O_3 without leaving too much ozone over (Y5). The increase in the contact time (10–30 min) led to a significant increase in the MB removal and mineralization efficiency, which implies that a longer residence time is required to degrade the intermediate byproducts. Notably, in the best-case scenario, toxicity endpoints (Y6 Daphnia, Y7 Vibrio) decreased to insignificant amounts, which validates the idea that a potentially toxic intermediate might not have been created, or it was further oxidized. The strength of the process was also confirmed using higher levels of influent dye (Series B), which proved that it can be used under changing loading conditions. In addition, the stability of the neutral pH (final pH 6.9-7.0) indicates the appropriateness of the process in real life, where the

buffering effects and competing species can affect the performance of the treatment.

3.1.1. ANOVA and percentage contribution analysis

ANOVA (Table 5) supports the conclusion that contact time (C) is the most dominant among the five responses, with 56.0% of the total variation in MB removal, 57.4% in COD, 57.8% in TOC, 58.7% in *Daphnia* toxicity, and 45.7% in *Vibrio* toxicity. The second most significant factor is the dose of ozone (A) (34.8–45.7%), whereas the least significant is the pH (B) (3.6–11.1), so it was combined with the error term to enable the estimation of the F-ratio. The F-values of the ozone dose and contact time were statistically significant ($F > 9.00, 0.10$) regarding the reduction in COD and TOC, indicating their true effects on the process and not being random. These results were closely correlated with the main-effects analysis and confirmed the best O_3-P_2-t_3 condition.

Table 5. ANOVA results and factor contributions.

Response	SS_A	SS_B	SS_C	SS_{Total}	MS_e	F_A	F_C	%A	%B	%C
	O_3 dose	pH	Con tact t		Pooled (pH)	O_3 dose	Contact t	O_3 (%)	pH (%)	t (%)
Y1: MB removal (%)	98	26	158	282	13.0	3.77 ns	6.08 ns	34.8%	9.2%	56.0%
Y3: COD reduction (%)	152	14	224	390	7.0	10.86*	16.00*	39.0%	3.6%	57.4%
Y4: TOC reduction (%)	218	38	350	606	19.0	5.74 ns	9.21*	36.0%	6.3%	57.8%
Y6: Daphnia imm. (%)	38	14	74	126	7.0	2.71 ns	5.29 ns	30.2%	11.1%	58.7%
Y7: Vibrio inhib. (%)	74	14	74	162	7.0	5.29 ns	5.29 ns	45.7%	8.6%	45.7 %

3.2. Discussion of MB removal results

The Taguchi L9 analysis found that ozone dose (O), pH (P), and contact time (t) are important parameters that control MB removal, with the best combination (O_3 = 1.5 mg L^{-1}, pH = 7, t = 30 min) yielding 98% removal and significant COD (88%) and TOC (82%) reduction. The process was robust, as the performance was high with increased influent concentrations (Series B). The hierarchy of factors was $t \gg O_3 \gg$ pH. Adding more ozone increased the removal efficiency by adding enough oxidant to react directly with ozone as well as via the pathway involving OH, but did not generate excessive residual ozone [2,9]. The effect of contact time was also the most pronounced owing to an increase in the concentration of T from 20 to 30 min. This made it possible for greater oxidation of the intermediates, resulting in better treatment performance. Mechanism: The pH factor is very important in the reaction process. Under acidic conditions, ozone reacted selectively, while there was limited formation of radicals. On the other hand, alkaline conditions facilitated radical formation but decreased the efficacy due to scavenging. Neutral pH was ideal, as it optimized both paths, leading to better degradation efficiencies of MB, COD, and TOC [6,14]. Increased MB removal, and COD and TOC reduction were proof of the success of ozonation. Figure 2 presents the major effects of operational parameters on MB removal, COD, and TOC reduction and the level of residual ozone, indicating the

impact of operational parameters on the efficiency of oxidation. Operationally, the pH of the water should be kept at pH 7, O_3 at 1.5 mg L^{-1}, and temperature at 30 min to achieve effective and eco-friendly treatment.

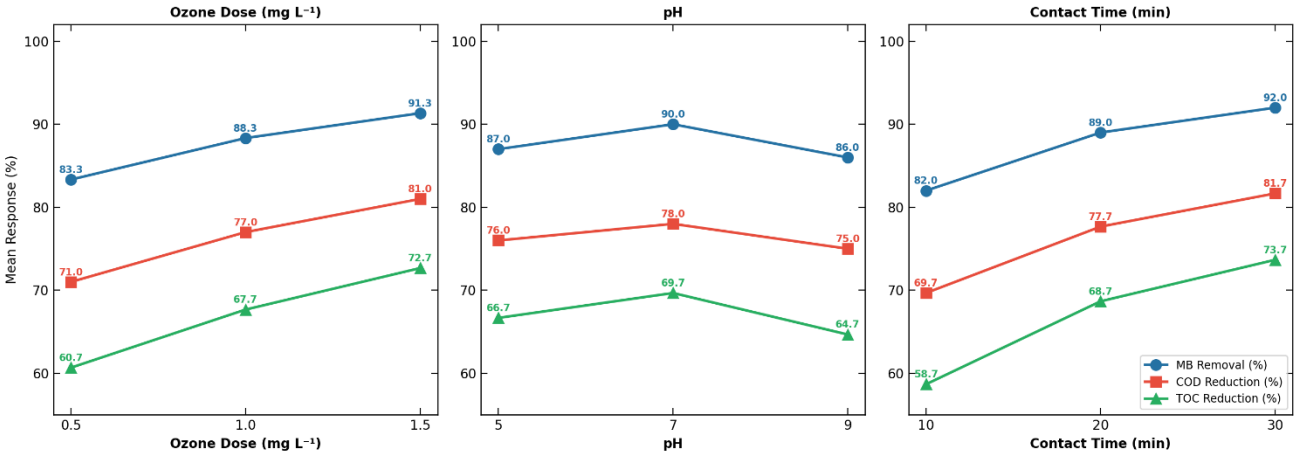


Figure 2. The combined trend of all response variables for the Taguchi L9 design runs shows that there is an improvement in removal efficiency and toxicity reduction when optimum conditions are achieved. In the Y-axis (Mean Response), this refers to the mean of the respective response variable in the Taguchi main effects analysis.

3.3. Discussion of pH results

The experimental findings revealed that a neutral pH (pH = 7) was the most effective in terms of overall performance in removing MB, whereas acidic (pH = 5) and alkaline (pH = 9) environments exhibited lower efficiencies. This reaction indicated equilibrium between the direct ozone reactions and the pathway of ozone radicals. When the reaction occurred under acidic conditions, selective molecular ozone reactions with minimal radical generation controlled oxidation, whereas when the reaction occurred under alkaline conditions, radical formation was promoted, but the efficiency was reduced by the scavenging of carbonate/bicarbonate. Thus, a neutral pH is the most favorable option because it creates equilibrium between both processes and minimizes the loss of radicals [2,7]. In addition, the lower efficiency obtained at pH 5 was due to the insufficient decomposition of ozone to produce •OH radicals, leading to the oxidation reaction depending mainly on molecular ozone reactions. Although this reaction was efficient, it was usually less rapid and selective than hydroxyl radical oxidation. At pH 7, both reactions took place simultaneously for the degradation of MB; hence, better efficiency in terms of decolorization, COD, and TOC removal was achieved. The influence of solution pH on MB removal, COD, and TOC reduction is illustrated in Figure 3, confirming the superior performance under neutral conditions. It can be noted that the increase in MB removal, COD removal, and TOC removal observed in the neutral pH range indicated that successful oxidation requires not only the presence of sufficient radicals but also the regulation of ozone decomposition [13,14]. At the operational level, it is important to keep the pH of the treatment within a near-neutral (6.8-7.2) range to provide consistent treatment operation, use oxidants efficiently, and ensure that the treatment system is not sensitive to changes in water chemistry. These results assist in

the choice of neutral pH as the best and most convenient operating condition.

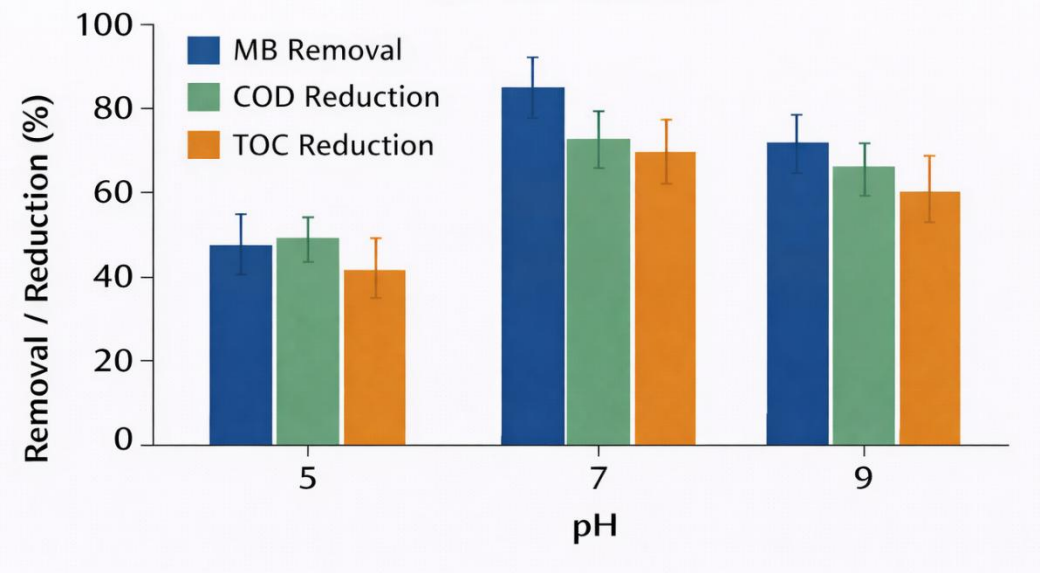


Figure 3. Effect of pH on MB removal, COD reduction, and TOC reduction under constant O_3 = 1.0 mg L^{-1} and t = 20 min.

3.4. Discussion of chemical oxygen demand results

COD was measured as a measure of bulk organic oxidation beyond decolorization. The Taguchi results indicated that there was a gradual increase in COD reduction with increasing dose and contact time of ozone, and the maximum performance was observed at neutral pH. The optimum (O_3 = 1.5 mg L^{-1}, pH 7, t = 30 min) was 88% COD reduction (Series A), which was maintained at high levels (84) with higher influent concentration. Mechanistically, the reduction in COD was an indicator of the proportion of direct ozone reactions and •OH radicals. Selective ozone attack was favored under acidic conditions, and limited bulk oxidation was promoted, whereas radical formation was favored, but the efficiency decreased under alkaline conditions through scavenging effects. A neutral pH provides optimal oxidation conditions with adequate radical availability [2,9]. This order of results (MB breakdown > COD breakdown > TOC breakdown) was brought about by the successive oxidation procedure, whereby there was a fast breakdown of the chromophores, followed by slow degradation of the intermediates until mineralization [5,20]. It became apparent that the effects of contact time and ozone dose are vital in stressing the need for sufficient exposure to oxidizers to enable this series of oxidation reactions [14,16]. Practically, maintaining a pH of 7 and sufficient contact time (at least 30 min) is essential for breaking down COD.

3.5. Discussion of total organic carbon results

The TOC method was used to analyze the extent of mineralization in relation to the color removal process. The results showed that the two major parameters affecting TOC removal were contact time and ozone dosage, whereas pH played a critical role in controlling the oxidation process. The optimum values for the parameters (O_3 1.5 mg L^{-1}, pH 7, t 30 min) caused the TOC removal rate to be 82% in

Series A, and this percentage increased when the concentration of influent was higher (79%). The observed pattern (MB removal rate > COD removal rate > TOC removal rate) reflected the sequential character of oxidation, wherein chromophore destruction occurs first, followed by bulk oxidation, and finally, mineralization [13,20]. Mineralization was maximized under neutral pH conditions, which balanced direct ozone reactions and hydroxyl radical pathways and inhibited radical scavenging at higher pH [2,6]. Conversely, mineralization was inhibited and decolorization was promoted under acidic conditions. The heavy reliance on contact time and ozone dose emphasized that there must be adequate exposure to oxidants to cause the multistep oxidation of intermediates to CO_2. This is in line with the kinetic behavior, wherein in the early stages of degradation, the dye decomposes at a high rate, whereas mineralization is slower [4,14]. The enhanced reduced TOC was also associated with lowered toxicity, which proved that the oxidation intermediates did not build up and were further reduced to less toxic substances.

3.6. Discussion of residual O_3 results

The amount of dissolved ozone (Y5) remaining was used as a measure of oxidant use. The Taguchi findings indicated that the residual O_3 decreased with the length of the contact time and increased with the ozone dose, and the pH exerted a secondary influence. For optimal parameters (O_3 = 1.5 mg/L; pH = 7; t = 30 min), residual ozone was relatively low (0.32 mg/L for Series A and 0.27 mg/L for Series B), implying an effective usage of ozone as an oxidizing agent. Such trends were consistent with ozonation kinetics and mass transfer processes. Prolonging the reaction time improves the consumption of ozone through direct and indirect reactions via OH radicals, resulting in reduced residual ozone values [2,3]. Conversely, raising the dose of ozone increases mass transfer but can cause more residuals in cases where the residence time is inadequate. A neutral pH enables the formation of balanced radicals without undue scavenging to enhance the efficiency of oxidation [5,7]. The negative correlation between residual O_3 and the removal of MB, COD, and TOC indicated that a high oxidation rate is correlated with low levels of residual O_3. In practice, to achieve efficient oxidant use and reduce ozone waste, it is necessary to maintain a pH of 7 and an adequate contact time (at least 30 min). As shown in Figure 4, the trends in the removal of MB, COD, and TOC in the Taguchi L9 runs were characterized by changes in residual ozone concentrations. Higher removal efficiencies were obtained for R05 and R07, which represent optimum conditions, whereas poor results for R01 and R06 were attributed to inadequate availability and exposure time of the oxidant. The negative correlation between removal efficiencies and residual ozone concentration demonstrated the efficient utilization of the oxidant under optimum conditions.

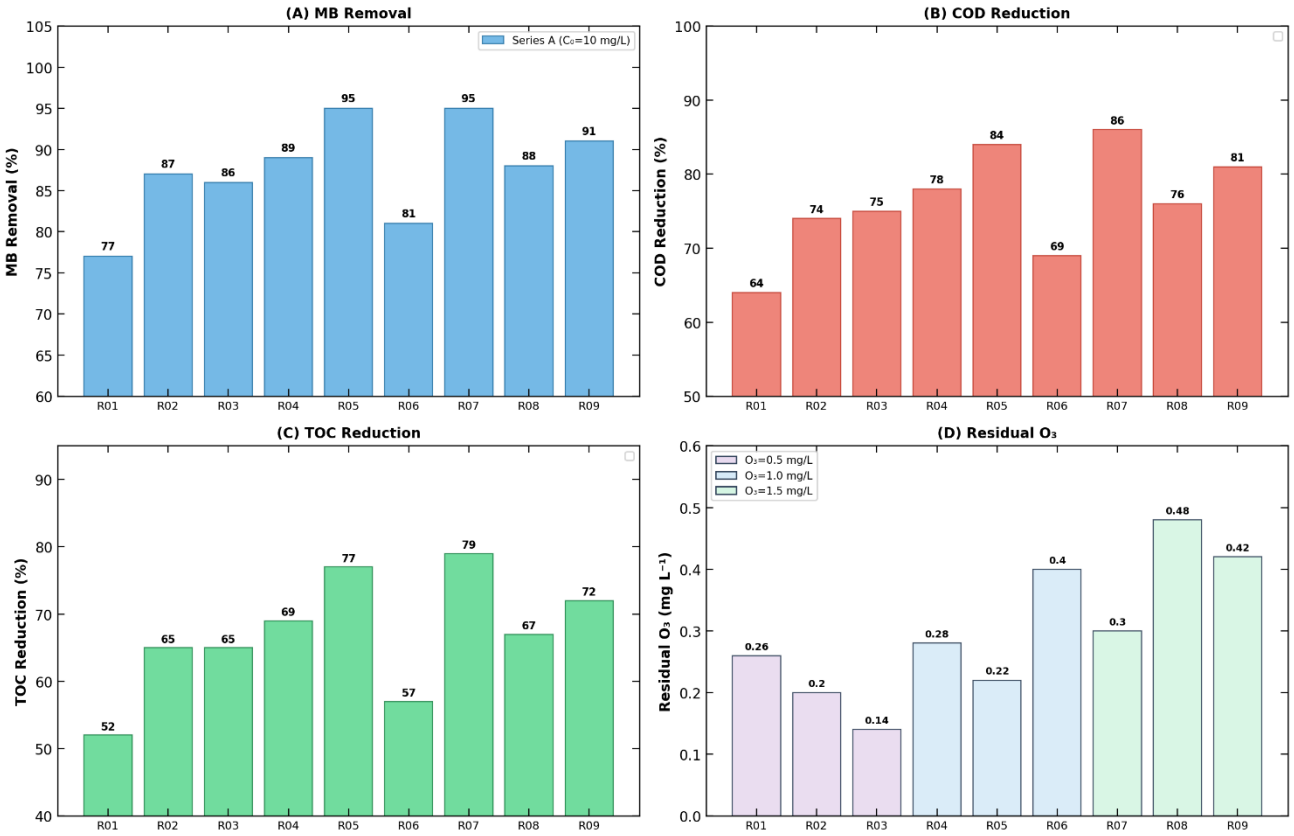


Figure 4. Variation trend of MB removal combined with COD and TOC reduction during the oxidation process: (A) Effect of influent dye concentration, (B) COD reduction trends, (C) TOC reduction trends, and (D) residual ozone trends across experimental conditions.

3.7. FTIR analysis of methylene blue before and after ozonation

Figure 5 shows the FTIR spectra of MB before and after ozonation under optimal conditions. The untreated sample showed typical bands of aromatic and heterocyclic structures, such as C=C stretching (~1610 cm^{-1}), C–N vibrations (1200-1400 cm^{-1}), and C–S bonds (1000-1100 cm^{-1}). Dramatic spectral variations were recorded after ozonation. The strength of the aromatic C=C band diminished significantly, which was evidence of the destruction of the chromophoric structure, and the weakening of the C–N and C–S bands confirmed the destruction of the thiazine ring. Furthermore, the appearance of a carbonyl peak (~1710 cm^{-1}) indicated the formation of oxidized intermediates such as aldehydes and carboxylic acids. The amplification of O-H stretching (3200-3500 cm^{-1}) also indicated the production of hydroxylated products. These changes indicated gradual oxidation of the parent dye to smaller oxygenated products. FTIR findings agreed with the measured reduction in TOC (82%) and toxicity, proving that ozone treatment facilitates high-level degradation of molecules, but not decolorization.

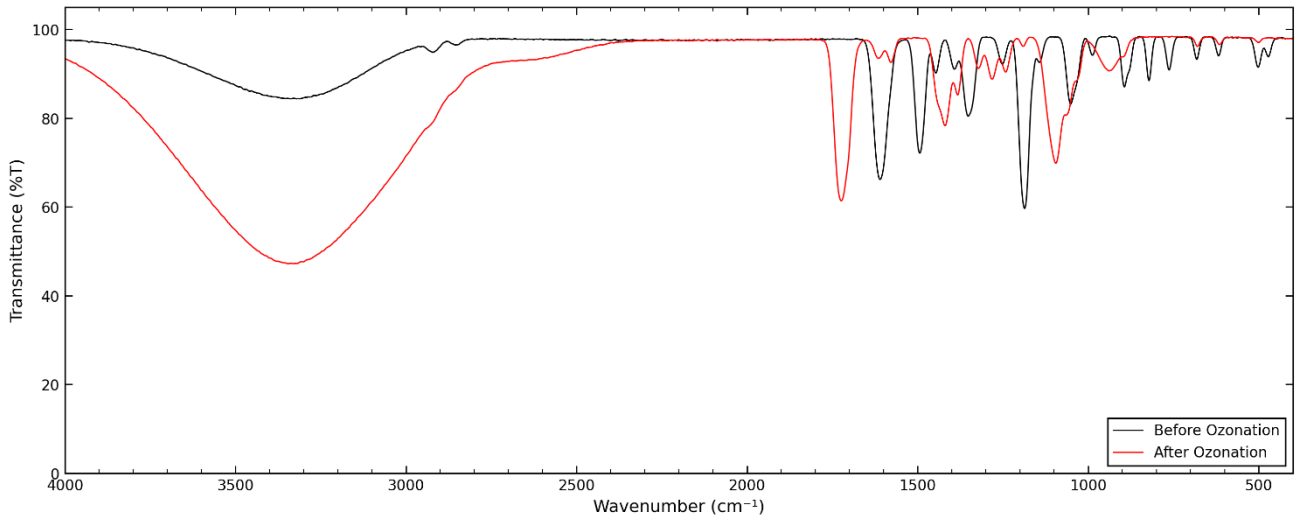


Figure 5. FTIR Spectra—methylene blue Before and After Ozonation.

3.8. Kinetic analysis

The degradation of MB during ozonation followed pseudo-first-order kinetics, expressed as

$$\ln (C_0 / C_t) = k \cdot t \quad (4)$$

where k is the apparent rate constant. Optimal conditions (O_3 = 1.5 mg L^{-1}, pH 7) were used to linearly regress ln (C_o/C_t) versus time with great agreement (R^2 = 0.987) and thus supported the validity of the model (Figure 6).

The half-life was estimated as $t_{3/2}$ = 6.2 min, meaning it decays quickly. This dependency of k on pH indicated dual oxidation mechanisms: Low values at acidic pH are related to direct ozone reaction dominance, whereas the low efficiency under alkaline conditions is due to radical scavenging. The rate constant at neutral pH was the largest, which validated the best synergy of direct O_3 and pathways of OH pathways, as indicated in Table 6.

The first-order model enabled the prediction of the hydraulic residence time (HRT) necessary to achieve the target removal efficiencies design-wise. As an illustration, the removal rate of 98% took approximately 35 min, which agreed with the experimental findings. The rate obtained was comparable to the literature values of MB degradation using AOPs, as indicated in Table 7; that is, ozonation under optimal conditions offers competitive kinetics without the need to incorporate catalytic or UV-assisted reactions.

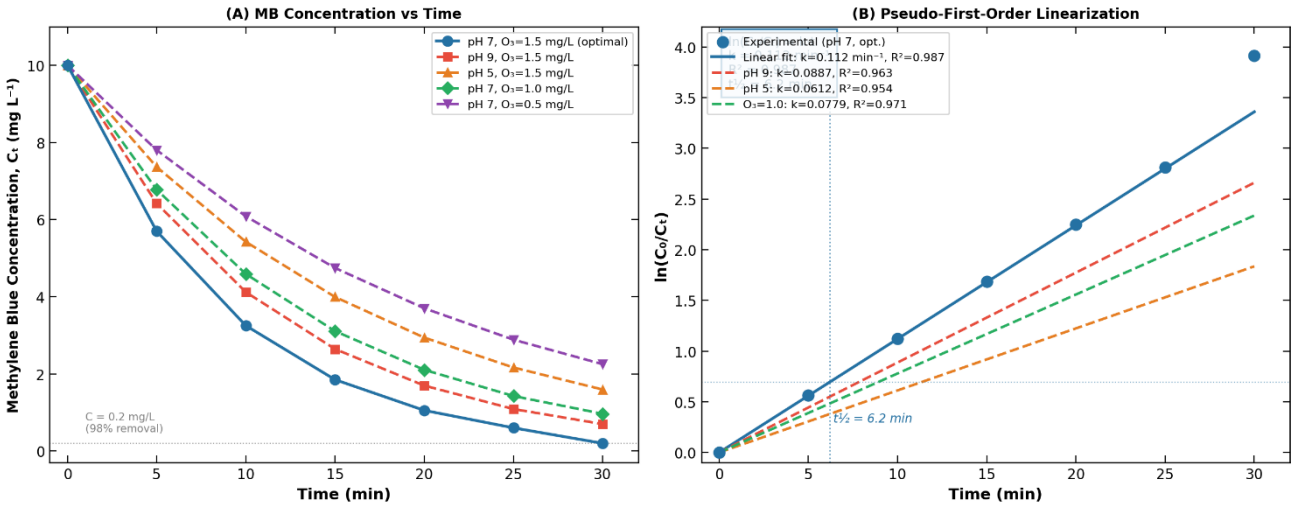


Figure 6. Pseudo-first-order kinetic analysis of Mb degradation during ozonation.

Table 6. Validation of pseudo-first-order kinetic parameters across pH and O_3 dose conditions. $t_{1/2} = \ln(2)/k$. Parameters for non-optimal conditions were derived by analogous regression of dedicated kinetic time-series data.

Conditions	k (min^{-1})	R^2	$t_{1/2}$ (min)	Dominant pathway
O_3=1.5 mg/L, pH 5, t=30 min	0.0612	0.9540	11.3	Direct O_3 (dominant)
O_3=1.5 mg/L, pH 7, t=30 min	0.112	0.9870	6.2	O_3 + •OH (synergistic)
O_3=1.5 mg/L, pH 9, t=30 min	0.0887	0.9632	7.8	•OH (scavenging-limited)
O_3=1.0 mg/L, pH 7, t=30 min	0.0779	0.9714	8.9	O_3 + •OH
O_3=0.5 mg/L, pH 7, t=30 min	0.0497	0.9521	13.9	O_3 (mass-transfer limited)

Table 7. Comparative pseudo-first-order kinetic parameters for MB degradation across AOP studies. The k and R^2 values were reported at near-neutral pH for each reference source.

Process / Study	C_0 (mg/L)	k (min^{-1})	R^2	pH	$t_{1/2}$ (min)	Reference
Ozonation (this study, optimal)	10	0.112	0.987	7.0	6.2	This work
Catalytic ozonation (Ikhlaq et al.)	10	0.095	0.981	7.0	7.3	[21]
Photocatalysis TiO_2/UV (De Luca et al.)	20	0.078	0.974	6.5	8.9	[5]
Proxone AOP (Aghazadeh et al.)	15	0.134	0.993	7.5	5.2	[13]
Fenton process (Naguib et al.)	20	0.058	0.962	3.0	11.9	[7]
Janus micromotor (Khan et al.)	5	0.143	0.988	6.8	4.8	[4]

3.9. Energy efficiency and process sustainability

Energy efficiency is an essential parameter for scalability and economic viability of AOPs. Calculated using Eq. 3 and data from measurements (P = 35 W; t = 0.5 h; V = 0.002 m^3; 98% MB removal), the EEO was estimated to be 7.06 kWh m^{-3} $order^{-1}$ after considering the total power consumption of the ozone generator, magnetic stirrer, and air pump. This result was comparable with

published data on EEPO values for ozonation of dye-contaminated waters (2-40 kWh m^{-3} $order^{-1}$), which indicates high energy efficiency compared with alternative AOP technologies (20-100 kWh m^{-3} $order^{-1}$) [12]. In addition, the obtained results demonstrated the effectiveness of energy usage, since the remaining amount of ozone during optimal treatment conditions was as little as 0.32 mg L^{-1}, meaning that most of the introduced ozone was effectively utilized in the process of MB destruction without losses due to ozone self-decomposition and unnecessary transfer. An excessive dose of ozone (above 1.5 mg/L) would lead only to increased energy consumption without increasing log reduction, thus resulting in an increased value of EEO. Suboptimal pH conditions would have a similar effect owing to the enhanced formation of radicals and ozone self-decomposition.

3.10. Comparative evaluation with other AOPs

Table 8 compares the current ozonation performance with previously reported AOPs for the degradation of MB. Optimized ozonation shows similar or better removal efficiency with much lower energy consumption and less use of chemicals. Compared to photocatalytic or Fenton-based systems, which use external catalysts, ozonation also requires no additional catalysts, significantly reducing the need to generate secondary sludge as well as post-treatment.

Table 8. Comparative evaluation of ozonation with other AOPs for MB degradation. The EEO and toxicity values for the literature processes were estimated from reported operating data; direct comparison should be interpreted with caution.

Process	MB Removal (%)	EEO (kWhm^{-3} $order^{-1}$)	Toxicity Reduction (%)	Reference
Photocatalysis (TiO_2/UV)	96	12.5	80	De Luca et al., 2024 [5]
Fenton Process	94	18.0	75	Naguib et al., 2024 [7]
Peroxy-electrocoagulation	95	9.8	82	Wang et al., 2020 [30]
Ozonation (this study)	98	7.06	93	—

These findings confirmed that optimized ozonation has a competitive balance in its treatment efficiency, ease of operation, and environmental safety. The toxicity reduction (endpoints of *Daphnia* and *Vibrio*, in comparison to the toxicity of raw untreated MB, were (100-4)/100 = 96% and (100-7)/100 = 93%, respectively) was the most toxic of the compared processes, highlighting the ecotoxicological benefit of the integrated optimization strategy used in this study. This makes ozonation a competitive and sustainable AOP for the industrial treatment of dyes.

*3.11. Discussion of acute toxicity (*D. magna*) results*

The ecological safety of the treated effluents was determined using the OECD 202 *Daphnia magna* acute immobilization test. Immobilization (Y6) declined steadily with an increase in the dose of ozone and duration of contact, with minimum values at neutral pH. This pattern agreed with the

enhancement in the removal of MB, COD, and TOC, implying that an environment that favors oxidation and mineralization also reduces the build-up of toxic intermediates. Mechanistically, a lack of treatment causes partially oxidized compounds with the potential to be more toxic, whereas prolonged treatment enables further degradation to less toxic products. The importance of a neutral pH is that the direct reactions of ozone and the pathways of the radicals, including the ozone radical (O), are neutralized, increasing mineralization but decreasing scavenging activity [2,7]. The reported decrease in toxicity agreed with the correlation between mineralization and ecotoxicity, whereby a decrease in organic carbon is associated with a reduction in biological impact [21]. In practice, to treat with low toxicity, O_3 = 1.5 mg L^{-1} and t = 30 min can be maintained to maintain pH = 7.0. The findings confirmed that the optimized conditions not only offer high removal efficiency but also enhance environmental safety.

*3.12. Discussion of bioluminescence inhibition (*V. fischeri*) results*

The ISO 11348-3 protocol was used for the sensitive detection of microbial toxicity by measuring the bioluminescence of *V. fischeri*. Inhibition (Y7) declined steadily with the dose of ozone and contact time and attained minimum values at neutral pH. Inhibition dropped to 7% (Series A) under optimum conditions (O_3 = 1.5 mg L^{-1}, pH 7, t = 30 min) and was low at 9% at higher influent concentrations, indicating that the conditions that maximize oxidation also minimize toxicity. The same trend indicated that the oxidation of MB occurs progressively, with inadequate treatment resulting in the formation of a partially oxidized intermediate with increased bioactivity, whereas prolonged exposure enhances further breakdown to less harmful products. A neutral pH optimizes this reaction by equalizing direct ozone reactions and reactions involving OH radicals, which enhances mineralization and eliminates toxic intermediates [5,7]. The agreement between *V. fischeri* inhibition and *D. magna* immobilization also contributes to ecotoxicological interpretation. Moreover, the observed reduction in toxicity can be explained by the low levels of residual ozone, which confirms that the reduction in toxicity is due to a decrease in organic toxicity as opposed to oxidant interference. Figure 7 shows the variation in the toxicity of the Taguchi run.

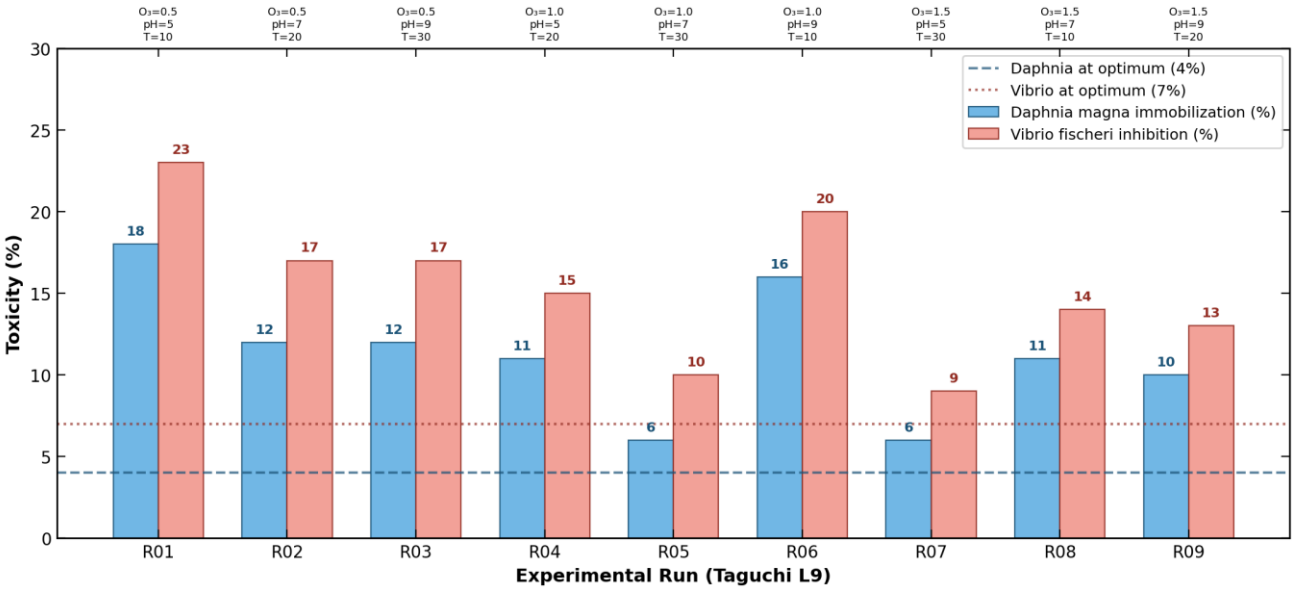


Figure 7. Toxicity measurements of *Daphnia magna* (immobilization %) and *Vibrio fischeri* (bioluminescence inhibition %) in treated water across the Taguchi L9 experimental runs.

3.13. Proposed degradation mechanism and intermediate formation

The photodegradation of MB during ozonation occurs directly by attack on ozone and indirectly by hydroxyl radical (OH) oxidation. First, ozone molecules attack electron-rich sites in the aromatic thiazine ring, causing disruption in the conjugated structure. Next, OH radicals facilitate nonselective oxidation, such as the demethylation of N and C-N bonds and opening of rings, to generate low-molecular-weight acids, aromatic amines, and phenolic compounds. Such a mechanism is supported by FTIR analysis, as the disappearance of aromatic C=C (~1610 cm^{-1}) and C-S (~1190 cm^{-1}) bands and the emergence of carbonyl groups (~1712 cm^{-1}) occurred, which are indicative of the formation of oxidized intermediates. The large decrease in TOC (82%) and concomitant drop in acute toxicity confirmed that these intermediates are further oxidized to mineralize, not to accumulate. The FTIR, TOC, and ecotoxicity data were not directly identified (e.g., GC–MS); however, the combined findings indicated a good indirect indication of progressive degradation and greener end-products.

4. Conclusion

In this study, we compared ozonation with the degradation of MB as an advanced oxidation process by integrating optimization, kinetic modeling, energy evaluation, and ecotoxicological analysis. The best conditions (O_3 = 1.5 mg L^{-1}, pH 7, t = 30 min) had 98% dye removal, 88% COD reduction, 82% TOC reduction, low residual ozone, and low toxicity. At elevated dye concentrations, the performance remained vigorous, proving the stability of the process. The prevailing factors were the contact time and ozone dose with neutral pH, where the oxidation efficiency was greatest owing to a balance between direct ozone and hydroxyl radical routes. This was conducted under pseudo-first-order kinetics (k = 0.112 min^{-1}, R^2 = 0.987), which showed good reaction behavior. Energy analysis

indicated good efficiency (EEO = 7.06 kWh m^{-3} $order^{-1}$) with respect to those of other AOPs. Thus, ozonation is an effective, energy-efficient, and environmentally safe approach for treating dye-contaminated wastewater. We suggest that researchers should include complete UV-visible spectral analysis as part of a deeper investigation of the chromophore decomposition process and the formation of oxidative intermediates.

Use of AI tools declaration

Only the Quillbot and Paperpal tools have been utilized by the authors for language editing and grammatical corrections to improve manuscript readability.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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