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by Gunawan Gunawan

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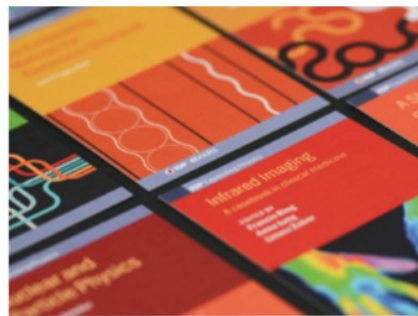
Ozonation of methylene blue and its fate study using LC-MS/MS

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Abstract. In this study, ozone was used to degrade an organic compound of methylene blue dye. The methylene blue was treated with ozonation using ozone generator and the degradation was observed as dye decolorization. Various parameters of ozonation such as contact time, pH, and temperature were chosen. The maximum ozonation of methylene blue degradation was at pH and contact time of 11 and 13 min, respectively. The effect of temperature on the destruction of methylene blue with ozone was obtained the highest result at temperature 35 °C. The LC-MS/MS results showed that ozonation caused degradation of methylene blue producing a compound with molecular weight of 208 g/mol as the unstable compound of N-(4-nitrophenyl) butanamide ($C_{10}H_{12}N_2O_3$). Finally, it formed stable fraction compound of 1-nitrocyclohexene ($C_6H_9NO_2$) with molecular weight of 127 g/mol which much safer and reduce the harmness of methylene blue in waste.

1. Introduction

The development of industries that grow fast with amount of waste will be generated. Many industries can`t manage their waste properly yet so that this waste is often found to accumulate especially in watersheds. Coloring compounds from a large quantity of textile waste pose challenges for the textile industry. The use of a number of dyes up to 50 % produces colored liquid waste, which poses not only aesthetic problems but also important environmental problems, such as its toxicity and potential carcinogenicity of certain inorganic dyes [1]. Dyes are colored compounds widely used in textile, printing, rubber, cosmetics, plastics and leather industries to color a product. The dyes can be classified as anionic, cationic and nonionic dyes [2]. Methylene blue is one of the cationic dyes often used for dyeing cotton, wood and silk [3]. Methylene blue is not too dangerous in small doses, but long exposure to methylene blue can have several adverse effects, including increased heart rate, vomiting, cyanosis, jaundice, and necrosis [4].

Methylene blue released from staining process can be harmful to the environment if it is discharged directly into the environment. Researchers around the world are still looking for ways to optimize the impact of industrial waste on the environment and ecosystems [5]. Therefore, it is important to remove dyes from waste or treat them in a way that minimizes environmental damage and decolorizes water. Different methods have been used to remove dyes from aqueous solutions [6]. Since dyes are chemically and microbiologically stable, conventional biological wastewater treatment methods are not effective in removing color [1] Many new organic products (such as alizarin red and methylene blue) can't be degraded effectively by conventional methods because the molecular structure of organic substances is stable [7]. Physical and chemical methods such as adsorption,

electrodécolorization, extraction, photocatalysts, dye-degrading membranes have been used, but most methods have weaknesses, such as high prices and low efficiency [6].

Ozone, because of its high reactivity, has been widely used for the treatment of water. This is applied to remediate water taste and color and to remove organic substances in the water. Ozonation is known as one of the most promising processes for wastewater treatment [8]. In the process of ozonation, destruction and decolorization occur. Destruction is a process of breaking down compounds into elements for analysis. The method is used to eliminate the effect of the matrix on the sample [7]. decolorization is a decrease in the intensity of the color in a solution. The term decolorization is very closely related to adsorption, but in decolorization, dyes are absorbed, while in adsorption they are more likely to absorb heavy metals [9].

The ozonation process is generally assisted by certain compounds, catalysts. UV-Vis spectrophotometric measurement showed that nanocomposite added to the system exhibited excellent photocatalytic activity against degradation of methylene blue [10]. Although Kamel et al. [1] provide good solution to environmental problems associated with artificial methylene blue sample in aqueous solutions by ozonation in continuous reactors. Other researchers [11] used an improved ozonation process to treat MnO_2/O_3 treated methylene blue wastewater with kaolin. The project result in efficient remediation of methylene blue contained wastewater, chemical oxygen demand (COD) of 88.3% and the decolorization degree of 99.9 % were obtained in 10 minutes at pH 11. Zhang et al. [12] also investigated the effect of pH on ozonation and carbon assisted ozonation processes, with methylene blue (MB) being used as a model compound. Quantitative kinetic studies and descriptions have been performed to evaluate the effect of pH on the control of the ozonation process. In this study, ozonation is used to degrade methylene blue compounds and the fate of the products are observed with LC-MS / MS.

2. Experimental

2.1. Materials
Methylene blue p.a (Merck), aquadest, NaOH 99 % p.a (Merck), H_2SO_4 98% p.a (Merck), and universal pH (Merck).

2.2. Instrumentation
Ozone Generator (X-Troy), ozone meter (CPR), UV-Vis spectrophotometer (Shimadzu 128), LC-MS/MS (UPLC: ACQUITY UPLC@BEH C18, MS: Waters Xevo TQD Triple Quadrupole), Stirring Hot Plate (IKA C-MAG HS 7), analytical balance (Ohauss), Thermometer (Pyrex), glassware (Herma, pyrex).

2.3. Procedures

2.3.1. Preparation of methylene blue. One gram of methylene blue was dissolved in 1000 mL of distilled water to obtain 1000 mg/L of methylene blue stock solution, then each dilution was carried out to 10 mg/L. The sample solution used for ozonation was 50 mL.

2.3.2. Determination of maximum wavelength. The maximum wavelength of methylene blue solution was determined by measuring the absorbance of the sample solution at different wavelengths from 400 nm to 700 nm using UV-Vis spectrophotometer.

2.3.3. Determination of ozone capacity. Ozone generator producing certain concentration ozone was measured using ozone meters (calibrated by titration method) which corresponds to ozone concentration of 46.32 mg/L. The air flow (measured by a gas flow meter) was 3 L/min. The ozone capacity = ozone concentration x its air flow. Ozone capacity was used to find the mass of ozone dissolved in the ozonation process with various ozone contact time.

2.3.4. Ozonation process with various contact time. The ozonation process was performed using 50 mL methylene blue 10 mg/L solution. Then the solution was ozonized with various contact times of of 5, 7, 9, 11 and 13 min at pH 7, then the treated methylene blue concentration was measured using UV-Vis spectrophotometer.

2.3.5. Ozonation process with various pH. The process was performed with 50 mL methylene blue 10 mg/L sample solution. The sample was ozonized with the pH of 3, 5, 7, 9 and 11 for 5 min, then the absorbance of the treated methylene blue was measured with UV-Vis spectrophotometer.

2.3.6. Ozonation process with various temperatures. The ozonation process was performed with 50 mL methylene blue 10 mg/L sample solution. The solution was ozonized with temperature of 15, 20, 25, 30 and 35 °C for 5 min. at pH 7, then the treated methylene blue absorbance was measured with UV-Vis spectrophotometer.

2.3.7. LC-MS/MS evaluation. The ozonation process is performed with 50 mL methylene blue 10 mg/L sample solution. The solution was ozonized at pH 7 for 90 min until it became transparent (colorless). The UPLC condition used was the ACQUITY UPLC @ BEH C18 column of 1.7 µm with eluent A = Aquabidest THF 0.1% and B = CH3CN (50:50), the flow rate used was 0.4 mL/min, M/z : star = 100 end = 1200 ES +, injection volume: 2 µL. The type of MS used is Waters Xevo TQD Triple Quadrupole. The results of ozonation were identified by LC-MS/MS to predict the onset of structural degradation.

3. Results and discussion

### *3.1. Determination of maximum wavelength of methylene blue*
Maximum wavelength of methylene blue dye sample solution was performed using UV-Vis spectrophotometer. The maximum wavelength is determined by measuring the absorbance of the sample at different wavelengths in the visible range, between 400 and 700 nm, due to a colored solution. Fig. 1 shows maximum absorbance of the methylene blue sample that occurs at a wavelength of 665 nm.
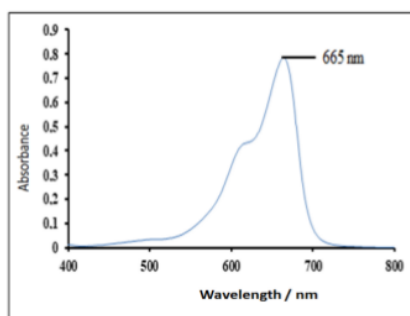
Figure 1. Spectrum of methylene blue (MB) measured in visible range.

3.2. Calibration curve of methylene blue solution
The calibration curve was established by preparing standard solutions of methylene blue at different concentrations, those are 1, 2, 3, 4 and 5 mg/L. Absorbance data measured then were plotted as a curve of absorbance vs concentration. Fig. 2 can seen the graph is linear with $y = 0.1672x - 0.0246$ and $r^2 = 0.9929$.
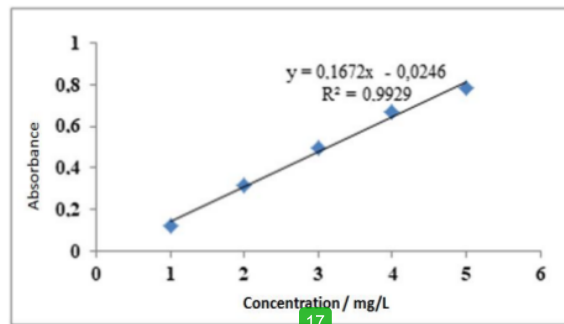
Figure 2. Calibration graph of methylene blue solution

3.3. Effect of dissolved ozone concentration

Fig. 3 represents the destructed methylene blue with dissolved ozone. The results show that the greater the mass of dissolved ozone, the higher the percentage of methylene blue destructed. From Fig. 3, it is known that the highest dissolved ozone mass is 1.81 grams, giving a percentage of methylene blue reduced by 73.01 %. The lowest mass of dissolved ozone is 0.69 grams to give a percentage of methylene blue reduced by 3.28 %.
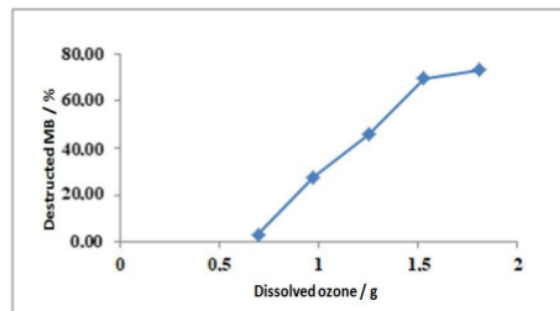
Figure 3. Destructed methylene blue (MB) as a function of dissolved ozone

3.4. Effect of ozonation time

Fig. 4 shows that the highest percentage of methylene blue is reduced after 13 min which corresponds to 73.01 %, while the lowest percentage of methylene blue corresponds to a contact time of 5 min, at 3.28%. The duration of contact time may affect the ozonation process. To determine the effect, the contact time may be related to the percentage of destructed methylene blue. The longer the contact time, the more the percentage of methylene blue is destructed. A significant increase occurred within 7 min, but after 11 min the percentage of destruction of methylene blue tends to be stable. The concentration results also decrease as the duration increases and the lowest concentration is 2.67 mg/L during the 13 min of contact time at pH 7.
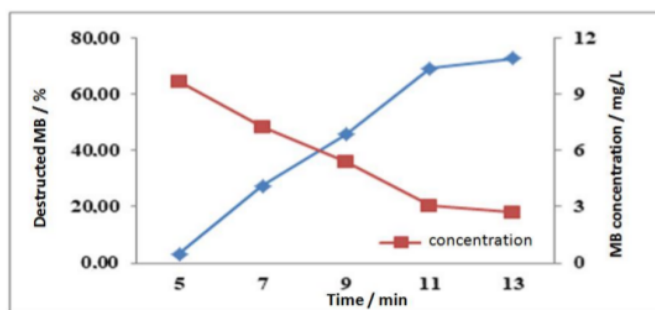
Figure 4. The correlation of destructed methylene blue (MB) and concentration against contact time of the ozone.

3.5. Effect of ozonation pH

This variation in pH was performed to determine the optimal pH conditions that could destroy methylene blue. In this work, it is known that the higher the pH of the methylene blue solution, the greater the percentage of methylene blue affected by the ozone.
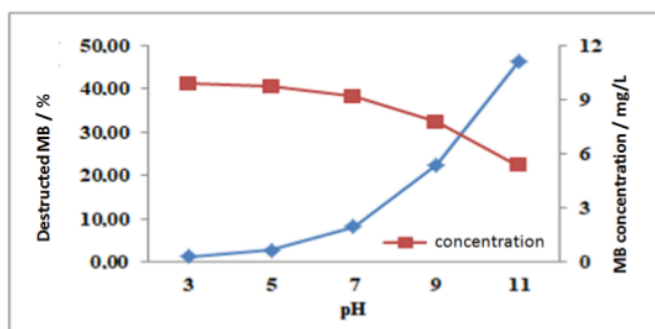

Figure 5. The destruction percentage of methylene blue (MB) and its concentration decrease as a function of pH.

Fig. 5 describes the highest percentage of decreasing methylene blue concentration that is at pH 11 and equal to 46.34 %, while the lowest percentage of decreasing methylene blue concentration at pH 3 is equal to 1.36 %. Concentration also decreases when experiencing an increasing in pH with lowest concentration was found at pH 11 of 5.37 mg / L. It is known that at basic solution i.e. pH 11 the optimum pH was obtained. The chromophore group of reactive dyes as artificial sample is much easier to be destructed by ozonation reaction, most of which experience interference at higher pH and result in significant conversion of ozone molecules to OH radicals [12]. The higher the pH used in this study, the higher the percentage of the methylene blue destruction. This is due to the methylene blue is a cationic dye (positively charged). In addition, the increased $^-$OH will increase the amount of radical OH produced and a direct reaction of ozonation is dominant if there are inhibitors of radical components. In addition, high pH water contains hydroxide ions act as initiators in ozone decomposition [13]. Ozone reactivity in organic matter is influenced by pH. At low pH, ozone will react exclusively with compounds that have specific groups through selective reactions such as electrophilic, nucleophilic, or dipolar additional reactions (direct ozonation). Under normal conditions, ozone will decompose to produce OH radicals which are strong oxidizing agents and react with various organic and inorganic compounds in water (indirect ozonation). In general, direct ozonation dominates at low pH (pH <4), direct and indirect ozonation occurs at pH 4-9, and indirect ozonation dominates at pH> 9.

3.6. Effect of ozonation temperature

Temperature variations were carried out to determine the effect of temperature on the destruction of methylene blue dyes with ozone. In this work, it is known that the greater the temperature, the greater the percentage of ozone-destructed methylene blue.
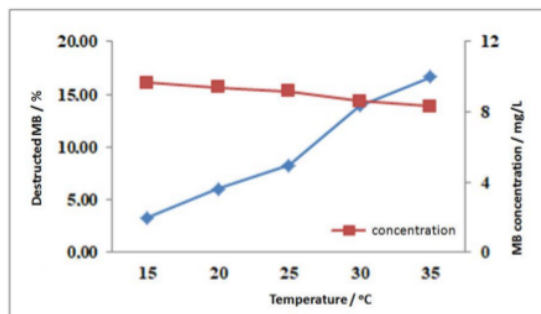
Figure 6. Percentages and concentration of methylene blue destructed varied with temperature

Fig. 6 can be seen that the highest percentage of reduced methylene blue is at temperature of 35 °C which is equal to 16.67 %. Meanwhile, the lowest percentage is at temperature of 15 °C that is equal to 3.28 %. In concentration point of view, it also decreases when experiencing an increase in temperature. The lowest concentration of 8.33 mg/L at a temperature of 35 °C and pH 7. Based on the graph, it can also be seen that temperature affects the presence of ozone in water. At high temperatures, the presence of ozone in the water diminished but the concentration decreases. The concentration decreases due to increasing temperature which causes the frequency of collisions interparticles higher. The greater the temperature, the greater the percentages of methylene blue are destructed.

3.7. Evaluation of methylene blue ozonation results with LC-MS/MS.

Characterization with LC-MS/MS was conducted to evaluate the compound breakdown product after ozonation of methylene blue by molecular weight of resulting molecules. Separation of compounds use reverse phase liquid chromatography with ACQUITY UPLC @ BEH C18 1.7 μm stationary phase and mobile phase mixture of acetonitrile and THF 0.1% aquabidest.
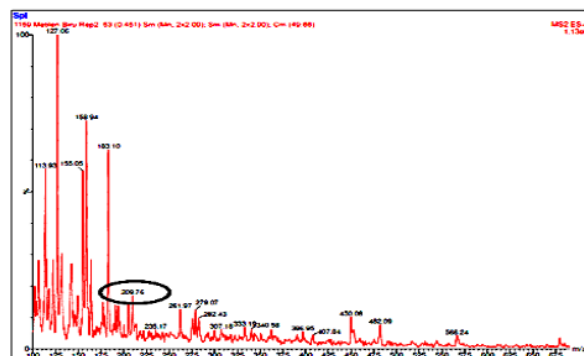
Reverse phase liquid chromatography is liquid chromatography with reverse phase, stationary phase, in the form of hydrophobic (non-polar) molecules and mobile phase in the form of hydrophilic (polar) molecules. Hydrophobic molecules tend to be adsorbed in the stationary phase and hydrophilic molecules in the mobile phase will pass through the column and elute first. Hydrophobic molecules can be eluted from the column by reducing the polarity of the mobile (non-polar) phase which can reduce hydrophobic interactions. The more hydrophobic a molecule is, the stronger it is bound to the stationary phase and the higher the concentration to elute the molecule. Column C18 is a resin dedicated specifically to the inverted phase which is an octadecyl carbon chain that is bonded to silica. Chromatography principally is distribution level of particles in stationary and mobile phases. Total ion chromatogram resulting from ozonation in Fig. 7.
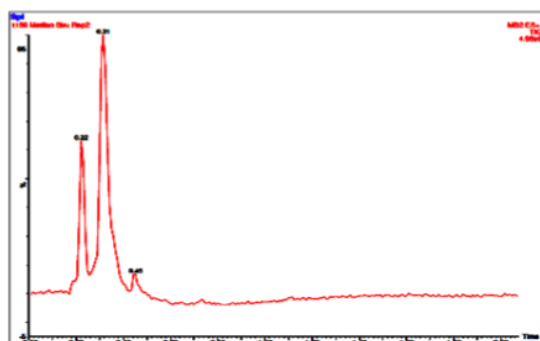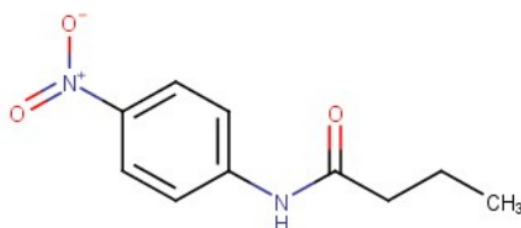
**Figure 7.** Ion chromatogram of total destruction products

One peak at spectrogram with a retention time of 0.415 min as shown in Fig. 7 is molecular ions [M + H] + 209 m / z (M + 1). It is possible that the compound appeared at the retention time has a molecular weight of 208 g / mol.

Figure 8. Spectrograms of peak at retention time of 0.451 min

It is estimated that an ion with m/z = 127 is a fraction of a compound that appears at one of the peaks with a retention time of 0.451 min (Fig. 8). The compound may be less stable so it splits into one stable fraction that has m/z 127. Therefore, spectrogram with m/z of 127 is as the base peak.

Based on the results of LC-MS/MS analysis, destruction of methylene blue compounds by ozonation is inferred to produce compound with mass of 208 g/mol as N-(4-nitrophenyl) butanamide ($C_{10}H_{12}N_2O_3$) compound. From the spectrogram there is a similarity between molecular weight of ozonated methylene blue compound with N-(4-nitrophenyl) butanamide compound. The structure of the N-(4-nitrophenyl)butanamide compound is shown in Fig 9.

Figure 9. Molecular structure of N-(4-nitrophenyl)butanamide

The possible breakdown patterns of compounds that produce m/z of 183 and 127 are shown in Fig. 10.
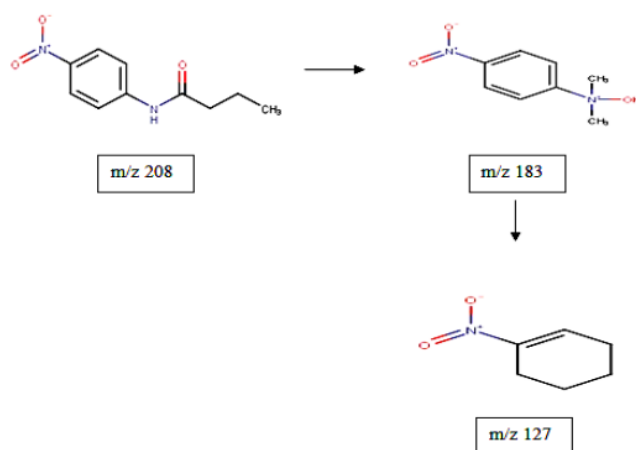
Figure 10. Possible breakdown of N-(4-nitrophenyl)butanamide

The expected result of a stable fraction of the N-(4-nitrophenyl) butanamide compound is 1-nitrocyclohexene, which is much safer and can reduce the hazard or impact of methylene blue waste. According to Pubchem, it is known that the compound 1-nitrocyclohexene ($C_6H_9NO_2$) is dangerous only if it is exposed to eyes that cause eye irritation. Structure of 1-nitrocyclohexene is shown in Fig. 11.
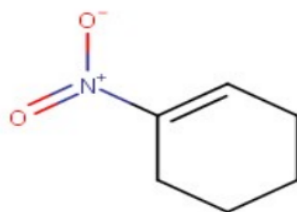
Figure 11. Molecular structure of 1- nitrocyclohexene

4. Conclusion

Methylene blue can be decolorized by ozonation process with the decrease in methylene blue concentration up to 73.01 % during contact time of 13 minutes at pH 7. pH affects the ozonation of methylene blue destruction ca. 46.34 % at pH 11. At pH 7 the influence of temperature on methylene blue destruction gave the best result up to 16.67 % at 35 °C. Furthermore, LC-MS/MS evaluation shows the destruction product of methylene blue compound, producing N-(4-nitrophenyl) butanamide ($C_{10}H_{12}N_2O_3$). The expected result of stable fraction is 1-nitrosyclohexene ($C_6H_9NO_2$).

Acknowledgments

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