Treatment of azo dye Acid Orange 52 using ozonation and completed-mixed activated sludge process

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Abstract. In this study, the characteristic of colour and COD removal of azo dye Acid Orange 52 (AO52) by ozonation, in combination with complete-mixed activated sludge process (CMAS) was evaluated. The experimentation was arranged in two phases: during the first one, only ozonation was performed, while, during the second phase, it was integrated with CMAS. The performance of colour and COD concentration of AO52 with and without CMAS treatment, is compared and evaluated. From the results, it is obvious that high decolourization from the start of CMAS was contributed from the pre-treatments. The colour removal was due to the fact that ozonation able to cleave the azo bonds that represent colour. Thus, CMAS without pre-treatment are unable to decolourize the dyes sufficiently. 59.6% COD was removed from the first-stage, while merely 9.8% COD fraction removed from the subsequence second-stage CMAS. It is suggested that the rapid COD removal without ozonation are due to activated sludge adsorption processes. The decreased of mixed liquor suspended solids (MLSS) affected the CMAS performances, as the biomass decreased due to lack of nutrient for activated sludge microorganisms to multiply. Results from pre-ozonation alone contributed more than 50% of total COD removal, which indicated that at higher ozone dosage, tend to mineralize azo dye. Thus, ozonation not oxidized the dye though complete mineralization that produce carbon dioxide and water. However, it is a potential process for enhancing colour removal and biodegradability of dye-containing wastewater, once the appropriate ozonation time is determined. Therefore, the role of ozonation seems to break down the dye molecules and created ozonation by-product that is easily biodegraded in the subsequent biological treatment.

1. Introduction

The presence of colour and its causative compounds has always been undesirable in water used for either industrial or domestic needs. Different colouring agents like dyes, inorganic pigments, tannins, lignins that usually impart colour. Amongst complex industrial wastewater with various types of colouring agents, dye wastes are predominant. Even though the concentration of dyes in wastewater is low, it often receives attention due to strong colour visible even at very low dye concentrations [1]. The azo dyes constitute the largest and the most important class of commercial dyes that is widely used in textile, plastic, leather, and paper industries as additives. The main environmental concern is related to the presence of colour and chemical load it carries, that imparts light penetration and compromises the ecosystems in the receiving water [2,3]. Without sufficient treatment, these dyes are stable and persist in the environment for an extended period of time.
Due to the complexity and diversity of the dyes employed in the industries, it has become rather difficult to find an effective and economic treatment for eliminating the wide diversity of industrial wastewater. Conventional physical and chemical methods such as coagulation, activated carbon adsorption, ion-exchange or ultra-filtration and reverse osmosis can be used efficiently to remove industrial wastewater containing dye. However, these processes are considered as non-destructive because it merely transfers the dye from liquid to solid wastes, and expensive operation is required for the post-treatment of solid wastes. Therefore, how to decolourize and degrade dyes without causing secondary pollutant has become a research emphasis nowadays. Thus, chemical method to oxidize organic materials by oxidizing agents, such as ozone, H$_2$O$_2$, UV light or combination of such oxidants that is known as Advanced Oxidation Processes (AOPs) has become a promising process. Nevertheless, it cannot satisfy the environmental discharge standard by itself alone and the cost is fairly high. The combination of ozonation and biodegradation seems to be promising unit processes to remove residual colour and COD of wastewater containing dye. The reaction between the oxidizing agent with dye in an aqueous environment lead to the decrease in the aromaticity and molecular weight, which eventually result in an increase in biodegradability and colour removal of dye. The biodegradable compound produced during ozonation would be removed by the following biodegradation. Therefore, the biodegradability enhancement is considered to be the essential factor that determines the performance of ozonation-biological treatment process.

The combined treatment processes have been suggested to treat dye-containing wastewater, including UV/O$_3$ or UV/H$_2$O$_2$ [4,5], followed by biological treatment. Several other researchers also have investigated the application of partial ozonation or AOPs as pre-treatment steps, followed by subsequence biological treatments for treating the dye-containing wastewaters [6,7]. Guelli Ulson de Souza et al. [4] stated that ozonation as a pre-treatment for combined chemical-biological treatment is a potential process for enhanced colour removal and biodegradability of wastewaters containing azo dyes Remazol Black B, once the appropriate ozonation period is determined. In addition, the measurement of combined treatment efficiency mainly depends on the purpose of treatment, but also involves the optimization of each chemical oxidation and biological steps [8].

Our previous study reported that ozonation effectively decolourized azo dye Reactive Red 120, even with lower ozone dosage [9]. However, significant COD removal was only observed within the higher range of ozone dosages. Moreover, ozonation transforms the functional groups in azo dye to produce more biodegradable byproducts, which is easily removed by biological treatment. In this study, colour and COD removal of Acid Orange 52 (AO52) were evaluated to be applying for wastewater treatment containing azo dye. The experimentation was arranged in two main phases. The first phase was aimed to evaluate the oxidation process to achieve complete colour removal of dye and partial cleavage of aromatic amines to make them easily biodegradable. Further, degradation can be achieved in the second phase by treating effluent from ozonation by biological treatment.

2. Materials and methods

2.1. Materials
The AO52 was of analytical grade obtained from Acros Organics, and used without further purification. It has a simple molecular structure, slow decomposition rate in light, relatively high stability and toxicity, complex structure and non-biodegradability is widely used in the textile, chemical and paper industries [10–12]. The chemical structures according to the manufacturer are shown in Figure 1. The molecular formula and its molecular weights are C$_{14}$H$_{14}$N$_3$NaO$_3$S, and 327.32 g/mol, respectively. The pH of aqueous solutions was adjusted using 0.1N NaOH to raise the pH or 0.1N HCl to lower the pH upon decolourization and degradation. All solutions were prepared using ultrapure water.
2.2. Phase 1: Pre-ozonation treatments
The experimental setup shown in Figure 2 consists of an oxygen cylinder, ozone generator, glass reactor and KI reactor. Ozone was generated from the A2ZZ-3G ozone generator utilizing pure oxygen of 99.5% concentration, introduced at a rate of 3 L/min. Ozone applications were semi-batch reactions carried out in a cylindrical glass reactor of the 2,000 mL working volume. The dye solution with the concentration of 100 mg/L was added into the glass reactor. Constant ozone was supplied from the bottom of the reactor through a diffuser at a dosage of 10.2 mg/min. Then, followed by 5 min aeration to remove residual ozone. All connections from the ozone generator to the reactor were made through Teflon tubing. The excess ozone, leaving the reactor was decomposed through 2,000 mL flasks filled with 2% KI solution. The experiments were conducted at room temperature, and samples were withdrawn at definite time intervals for determination of pH, colour, COD and UV-Vis spectra analysis.

2.3. Phase 2: Biological treatment
The biological treatment was performed by an aerobic complete-mix activated sludge (CMAS) reactor, consists of a 0.15 m deep cylindrical glass with an internal diameter of 0.10 m. The aerobic activated sludge as seed for CMAS reactor was collected from the biological treatment system of a glove manufacturing plant, located in Perlis, Malaysia. The bioreactor had an operating liquid volume of 1,000 mL and equipped with an air diffuser and stirrer. The air was bubbled at the bottom of the reactor by air diffuser. The agitation is maintained during the whole experiment in order to mix the medium and avoid concentration gradients within the bioreactor. The DO concentration in the medium was monitored daily to ensure oxygen concentrations between 6-8 mg/L. The overall experiments were conducted at room temperature.

The bioreactor operated in batch mode, with 24 h treatment cycles that consist of four consecutive phases: filling, reaction, settling and drawing. It was previously seeded by 500 mL of aerobic activated sludge. Then, at the beginning of the filling cycle, the reactor was filled with 500 mL of pretreated dye influent. Subsequently, aeration and agitation were switched on to begin the reaction phase to allow the biological removal of biodegradable pollutants. Once completed, both aeration and agitation were
stopped to let the biomass settle down. Finally, after 1 h of settling phase, approximately 300 mL volume of samples was withdrawn from the supernatant during the drawing phase, without disturbing the settled sludge. The bioreactor was replaced with an equal volume of pretreated dye samples, and aeration-agitation was turned on again for new treatment cycles.

The dye samples were subject to pre-ozonation, with 0, 5, 10, 15 and 20 min contact times, and left for approximately 24 h before feeding into the bioreactor. Therefore, possible interferences from any quantity of residual DO in the biological treatment were eliminated. Samples from each reactor were collected daily (until day 30) and then filtered through a 0.45 µm glass microfibre filter (Fisherbrand, 25 mm) prior to analyses to remove the biomass.

2.4. Analytical methods
The ozone concentration in feed gas was determined by the KI-starch titration method [13]. The decolourization and degradation of azo dye were determined by measurements of absorbance at wavelengths ranging from 200 to 800 nm by UV-Vis spectrophotometer (Hitachi U-2810). The maximum visible region (\(\lambda_{\text{max}}\) 465 nm) was employed as a base for characterization of decolourization for AO52, using a 10 mm quartz cell. The pH was measured by Hanna Instruments HI223 pH meter, and COD determined in accordance with the closed reflux, colorimetric method [13].

3. Results and discussions

3.1. Effect of pH
The reaction pH is an important parameter that influences the formation of hydroxyl radicals during ozonation. Figure 3 showed the pH of AO52 as a function of contact time. Dye solution decreases rapidly as the early stage of the experiment, dropping from the initial solution of pH 7.0 to about 3.5 after 5 min (when almost decolourization occurred). Then, the decrease continued until a steady state was reached. The drop of pH showed the formation of acidic by-products, such as \(\text{H}_2\text{SO}_4\) originating from the sulfonate groups and lesser extent, from the formation of aliphatic acids and \(\text{NH}_4^+\) [14].

![Figure 3. pH evolution in relation to ozonation contact time.](image-url)

3.2. Effect of pre-ozonation on colour and COD removal
The effect of pre-ozonation on the UV-vis spectra of AO52 after 20 min of treatment was shown in Fig. 3. The peak was slowly collapsed with the increase of contact time, and finally no peak was observed during 20 min of ozonation. The spectra of initial solution represent the UV band characteristics of \(-\text{N}=\text{N}-\) groups (465 nm) and related it to the benzene rings (240 nm) bonded to the \(-\text{N}=\text{N}-\) group [15–17]. During the experiment, the intensity of absorption at 465 nm declined extremely rapidly in comparison to benzene rings, as reaction time proceeded. This resulted in the decolourization of the dye sample. The colour removal percentage reached 100% during 10 min of contact times.
Figure 4. UV-Vis absorbance spectra in relation to ozonation time (100 mg/L, pH 7).

Figure 5 showed the COD concentration for AO52 after ozonation treatment. For a given time, an increase in contact time resulted in an increase in COD removal efficiency. Initial COD value is 128 mg/L, and decrease to 43 mg/L, which indicates partial mineralization of dyes. Continuous increase of reaction time had not increased the COD removal quickly. As the oxidation continued, recalcitrant organic compounds that could be oxidized became less available and that the remaining inert organic compounds after ozonation were difficult to break down.

Figure 5. COD concentration in relation to ozonation time (100 mg/L, pH 7).

3.3. Effect of pre-ozonation on CMAS treatment and for colour and COD removal

Figure 6 shows the wavelength of AO52 indicated by UV-Vis Spectrophotometer, submitted to different pre-ozonation contact time and CMAS treatment (between day 0 and 30). It is obvious that 0 min (without ozonation pre-treatment) curves of day 0 and 30 almost overlapped suggesting that the CMAS treatment alone hardly affects the decolourization and degradation. However, the specified wavelength peaks of dyes with ozonation (5, 10, 15 and 20 min ozonation) pre-treatment was disappeared, which indicated that the azo group of the synthetic dyes were transformed by the ozonation-CMAS treatment. Nevertheless, it seemed possible to obtain bio-treatable solutions once the colour had disappeared. The biological process is more efficient at mineralizing the by-products of the ozonation, but less efficient in colour reduction than the ozonation process itself [4].
From the data in Figure 7, it is apparent that concentrations gradually decrease from the initial concentration of 84, 62, 52 and 44 mg/L for 5, 10, 15 and 20 min ozonation, respectively. However, after day 30 of biological treatment, merely pre-ozonation of 10, 15 and 20 min shows a significant trend of reduction. Even though 20 min seems to have higher removal compared to a 15 min, it is suggested that 15 min is economically desirable due to the low ozonation required for adequate mineralization of AO52. These results imply that subsequent biological treatment after ozonation for 15 min was effective enough for the reduction of COD concentration.

The products obtained from the cleavage of the azo bonds are aromatic amines, which further mineralization of these compounds has been reported under aerobic conditions by Fongsatitkul et al. [19]. Since no external carbon source was added into the treatment systems, all of the COD (organic matter) was contributed by the dyes. In general, the results could be attributed to adsorption, biodegradation, or simultaneous adsorption and bio-degradation processes. The basis of adsorption phenomenon and the extent can be co-related to increasing adsorption sites to increase in reaction time. The removals are gradually declining due to the exhaustion of the adsorbent site. Moreover, the biomass decreased affected the CMAS treatment for COD removals.

Results from pre-ozonation alone contributed more than 50% of total COD removal, which indicated that at higher ozone dose, tend to mineralize azo dye. On the other hand, at a medium ozone dose (5 to 10 min), the role of ozonation seems to breakdown the azo dye molecule and created ozonation product that is easily biodegraded in biological treatment. Therefore, ozone was not oxidized azo dye though complete mineralization to produce CO$_2$ and H$_2$O. Khadhraoui et al. [14] mentioned that ozonation of dyes usually leads to small organic molecular fragments, such as acetic
acids, epoxides, aldehydes, and ketones that leads to a residual COD. Therefore, COD resulting from these small new formed molecules can be further degraded by a biological process.

4. Conclusion
In this study, the characteristic of colour and COD removal of AO52 azo dye by pre-ozonation followed by CMAS treatment was evaluated for applying in azo dye industrial effluent treatment. The drop of pH indicated the formation of acidic by-products. Based on the results, the biological treatment is more efficient at mineralizing the by-products of the ozonation, but less efficient in colour reduction than the ozonation process itself. Even though the combine treatment was not efficient enough to completely oxidize the contaminants, it contributes to higher dye mineralization in comparison to ozonation by itself. Ozonation of dyes usually leads to small organic molecular fragments that lead to a residual COD. Therefore, COD resulting from these small new formed molecules can be further degraded by biological treatment. Therefore, it is recommended that ozonation should be applied at medium ozone dosages as a pre-treatment for combined ozonation-biological treatment for azo dye removal.

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References
[1] Abidin C Z A, Muhammad Ridwan F, Ong S A, Mohd Makhtar S N N and Rahmat N R 2015 Sci. Asia 41 49–54
[2] Tomei M C, Pascual J S and Angelucci D M 2016, J. Clean. Prod. 129 468–77
[3] Franca R D G, Vieira A, Mata A M T, Carvalho G S, Pinheiro H M and Lourenco N D 2015 Water Res. 85 327–36
[4] Guelli Ulson de Souza S M A, Santos Bonilla K A and Ulson de Souza A A 2010 J. Hazard. Mater. 179 35–42
[5] Ledakowicz S, Solecka M and Zylla R 2001 J Biotechnol 89 175–84
[6] Qi L, Wang X and Xu Q 2011 Desalination 270 264–68
[7] Gokcen F and Ozbelge T A 2006 Chem. Eng. J 123 109–15
[8] Oller I, Malato S and Sanchez-Perez J A 2011 Sci. Total Environ. 409 4141–66
[9] Fahmi, Abidin C Z A and Rahmat N R 2010 Int. J. Environ. Sci. Dev. 1 2 193–8
[10] Liu J, Ma S and Zang L 2013 Appl. Surf. Sci. 265 393–8
[11] Zhao Y, Chu J, Li S H, Chen Y, Sheng G P, Chen Y P, Li W W, Liu G, Tian Y C, Xiong Y and Yu H Q 2011 Chem. Eng. J. 170 440–4
[12] Chen L Y 2000 Water Res. 34 3 974–82
[13] APHA-AWWA-WEF 2005 Standard Methods for the Examination of Water and Wastewater 21st ed. (Washington DC: APHA)
[14] Khadhraoui M, Trabelsi H, Ksibi M, Bouguerra S and Elleuch B 2009 J. Hazard. Mater. 161 2 3 974–81
[15] Wang A, Qu J, Liu H and Ge J Chemosphere 55 1189–96
[16] Feng W, Nansheng D and Helin H 2000 Chemosphere 41 1233–38
[17] Solozhenko E G, Soboleva N M and Goncharuk V V 1995 Water Res. 29 9 2206–10
[18] Fongsatitkul P, Elefisiotis P, Yamasmit A and Yamasmit N 2004 Biochem. Eng. J. 21 213–20