

## Article

# Electrochemical Oxidation Treatment of Organic Matter in Wastewater from Wet Fermentation of Yunnan Arabica Coffee

Fanfan Liang<sup>1</sup>, Yan Wang<sup>1</sup>, Yungen Liu<sup>2,\*</sup>, Silin Yang<sup>1</sup>, Fajin Yin<sup>1</sup> and Liping Peng<sup>1</sup>

<sup>1</sup> College of Ecology and Environment, Southwest Forestry University, Kunming 650224, China; liangffanan@yeah.net (F.L.); wycaf@126.com (Y.W.); swfcysl@126.com (S.Y.); yinfajin98@163.com (F.Y.); 15198913793@163.com (L.P.)

<sup>2</sup> Yunnan Key Laboratory of Ecological Environment Evolution and Pollution Control in Mountainous Rural Areas, Kunming 650224, China

\* Correspondence: henryliu1008@163.com

**Abstract:** Electrochemical oxidation combined with reagents of O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> and FeCl<sub>2</sub> was conducted in this study to treat the wastewater from wet fermentation of Yunnan arabica coffee. In addition, the effect of oxidants on the efficiency of wastewater treatment, the binding capacities of the oxidants to proteins, the degradation of organic pollutants in the wastewater, and the formation of oxidized organic components were systematically investigated. The results reveal better performance of O<sub>3</sub>-combined electrochemical oxidation (63.60% COD removal efficiency) for treatment of organic species in coffee wastewater than that of the electrochemical processes with H<sub>2</sub>O<sub>2</sub> (47.70% COD removal efficiency) and FeCl<sub>2</sub> (34.48% COD removal efficiency). The synergy of the electrooxidation/O<sub>3</sub> process (0.0133 A/cm<sup>2</sup>, 20 mg/L–2 L/min) could not only raise the pH value (3.70~4.20, 5.14~5.44) of the wastewater and reduce the NaOH dosage of 2.80~3.7 g/L, but also effectively degrade the proteins, lipids, unsaturated hydrocarbons, and carbohydrates, with a total chemical oxygen demand (COD) value above 20,000 mg/L. After the oxidation treatment, some organic components remained in the wastewater, including 31.94% of S-containing organics, lignin, condensed aromatic compounds, and aromatic structural compounds, which are difficult to be utilized by microorganisms. In addition, it was found that OH<sup>-</sup> could bind to proteins and affect the required amount of NaOH addition, whereas the protein binding energy of O<sub>3</sub> is higher than that of H<sub>2</sub>O<sub>2</sub>, indicating a stronger ability of O<sub>3</sub> to oxidize proteins. Therefore, the combination of O<sub>3</sub> and electrochemical oxidation can be considered as an effective method to treat organic pollutants in the wastewater from wet fermentation of Yunnan arabica coffee.

**Keywords:** coffee wastewater; wet fermentation; electrochemical oxidation; organic components



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## 1. Introduction

Coffee is one of the most widely produced and traded agricultural products [1]. China's coffee beans are dominated by Yunnan arabica coffee because of the unique climatic conditions of its growth. To meet market demands for coffee beans with better quality, wet fermentation has been becoming the common processing method used by coffee producers. More than 80% of Yunnan arabica coffee is currently fermented using the wet processing method to degrade the mucilage, which consists of hemicelluloses, pectin substances and sugars [2]. However, for the fresh fruit fermentation of Yunnan arabica coffee, a large amount (1~2 tons) of clean water is required. Moreover, a variety of by-products, such as coffee pulp, parchment, and wastewater, are produced during the wet processing [3]. The wastewater from wet fermentation of Yunnan arabica coffee (e.g., at Yunnan Simao Beigui Coffee Co., Ltd. from 2022 to 2023) has been measured to have the following typical parameter values: chemical oxygen demand (COD) of 31,333.33–31,666.67 mg/L, five-day biochemical oxygen demand (BOD<sub>5</sub>) of 1270–1350 mg/L, total salt content of 1017–1032 mg/L,

suspended substances of 3547–3556 mg/L, total phosphorus of 116.75–116.89 mg/L, ammonia nitrogen of 143.33–147.78 mg/L, color of 200 times and pH value of 3.7–4.2. These data reveal wastewater characteristics of high acidity and color, a large concentration of various pollutants, and poor biodegradability. In cases in which the wet processing is performed at a large scale, the produced effluents will significantly exceed the self-purification capacity of the natural waterways [4].

For the treatment of coffee wastewater, efficient and cost-effective biological methods have been developed. However, they also show a number of disadvantages. For example, the combination of an anaerobic digester and an engineered wetland could remove 70% of the organic matter in coffee wastewater; however, this process requires a lengthy period of acclimatization. This is particularly problematic considering that the annual coffee harvest season is relatively short (3 to 4 months) [5–7]. Based on the observations from a field visit to a wet fermentation site of Yunnan arabica coffee, the wastewater is treated mainly by a biochemical method, such as the reserved anaerobic–anoxic–aerobic (A2O) method. In this process, the pH value of the wastewater is first adjusted with a large number of lye, followed by the addition of flocculants and coagulants into the wastewater to remove residues and pectin. To ensure that the effluent water during the biochemical process meets the secondary standard of the *Comprehensive Wastewater Discharge Standards (GB 8979-1996)*, a pool with a large area is required. Meanwhile, the generation of wastewater due to the seasonal production of coffee will result in unstable water quality and quantity, thus affecting the biochemical process.

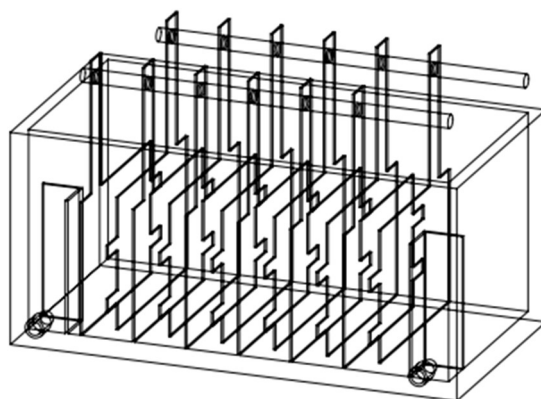
It has been found that green coffee beans are rich in unsaturated fatty acids such as oleic acid (18:1n-9), linoleic acid (18:2n-6), and linolenic acid (18:3n-3), and proteins composed of different amino acids, which are the main compounds contributing to the formation of the typical aroma during roasting [8–10]. The wet processing method allows the release of reduced sugars and free amino acids during the fermentation step [11]. Relevant studies have shown that electrochemical oxidation treatment of wastewater has the advantages of small land occupation, rapid reaction process, and oxidation capability for difficult-to-degrade organics [12,13]. For example, excellent performance of electrochemical oxidation for pretreatment of agricultural wastewater has been observed [14]. To solve the problems of high acidity and the large concentration of organic pollutants in the seasonally produced wastewater from wet fermentation of Yunnan arabica coffee, electrochemical advanced oxidation of the organic matter in the wastewater was conducted in this study. Specifically, the role of the reagents in the electrochemical treatment process of COD in wastewater was analyzed. Moreover, the effects of the electrooxidation treatment on the pH value of the wastewater and the required amount of alkali addition were also investigated, along with analysis of degraded fractions in coffee wastewater treated by electrochemical oxidation.

## 2. Materials and Methods

### 2.1. Reagents and Instruments

The wastewater from wet processing of Yunnan arabica coffee was supplied by Yunnan Simao Beigui Coffee Co., Ltd. (Pu'er, China, production season from November 2022 to March 2023). In addition, analytical grade reagents, including H<sub>2</sub>O<sub>2</sub> (≥30% XILONG SCIENCE), polymerized aluminum chloride (PAC, Al<sub>2</sub>O<sub>3</sub> ≥ 28% Daqian Environmental Protection Technology Co., Ltd., Xinyang, China), polyacrylamide (PAM, molecular weight of 18 million Daqian Environmental Protection Technology Co., Ltd.), NaOH (≥96% Tianjin Fengboat Chemical reagent Technology Co., Ltd., Tianjin, China), Ca(OH)<sub>2</sub> (≥99.8% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), concentrated sulfuric acid (95~98% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), phthalic acid (≥99.8% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), potassium dichromate (≥99.8% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), silver sulfate (99.7% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), mercury sulfate (99% Tianjin Fengboat Chemical reagent Technology Co., Ltd.), and ferrous sulfate (FeCl<sub>2</sub> > 99% Tianjin Fengboat Chemical reagent Technology Co., Ltd.) were used in this study.

The electrochemical oxidation device is shown in Figure 1. It has a size of  $320 \times 160 \times 150$  mm (L  $\times$  W  $\times$  H) and 8 sets of pole plates ( $150 \times 100 \times 2$  mm, titanium) with a spacing of 20 mm between the plates. Water is fed in and out from the bottom of the device, and wastewater flows back and forth through the device, maintaining a liquid level of 100 mm. The device is also equipped with a high-frequency switching power supply (50 A/36 V), ozone generator (LCP-G/A-50 g/h, Chian Hebei Lantis Environmental Protection Technology Co., Ltd.), panel gas flowmeter (0.5–8.0 L/min), spectrophotometer (Hach DR-2800, HACH Company, made in China), digester (Hach DRB-200, HACH Company, made in China), pH starter (3100, Ohaus Instrument Co., Ltd., Changzhou, China), ultraviolet-visible (UV-Vis) spectrophotometer (UV-2600, Sainyu Hengping Instrument Co., Ltd., Shanghai, China), 3D fluorescence spectrometer (Perkin-Elmer-LS55, PerkinElmer, Shanghai, China), and Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS, America Bruker Daltonics, Inc., Billerica, MA, USA).



**Figure 1.** Electrochemical oxidation device.

## 2.2. Experimental Methods

The electrochemical oxidation of organic species in the coffee fermentation wastewater was conducted as follows: 7 L of the wastewater was added into the reactor together with the  $O_3$  (20 mg/L 2 L/min) or  $H_2O_2$  (30% 10 mL/L) solution. The reaction was then performed at current strength of 40 A ( $0.0133 \text{ A/cm}^2$ ) for 2, 3, 4 and 5 h. After each processing time, a sample was taken and its pH value was adjusted to 7 with the NaOH solution. Subsequently, PAC (10%, 2 mL/L) and PAM (0.1%, 2 mL/L) were added to the sample and the system was stirred for 5 min. After resting for 30 min, the supernatant was taken for analysis of the contents of organics (COD, Hach model). In another process, 7 L of the wastewater was added to the reactor together with a  $FeCl_2$  (10%, 2 mL/L),  $H_2O_2$  (30% 10 mL/L) or  $O_3$  (20 mg/L 2 L/min) solution. The electrochemical oxidation was then conducted at current intensities of 30, 40 and 50 A for 4 h. Afterwards, a sample was taken and its pH value was adjusted to 7 with the NaOH solution. Subsequently, PAM (0.1%, 2 mL/L) was added into the  $FeCl_2$ -containing sample, while PAC (10%, 2 mL/L) and PAM (0.1%, 2 mL/L) were introduced into the sample, with previously added  $O_3$ . The obtained systems were first stirred for 5 min and then rested for 30 min. Finally, the supernatants were used for determination of the organic contents.

The  $O_3$ -treated wastewater sample and the sample treated with  $FeCl_2$  at a current intensity of 40 A for 4 h were selected for analysis by fluorescence/UV-Vis spectroscopy. In addition, FTICR-MS was conducted to determine the dissolved organic matter (DOM) in the coffee wastewater after electrochemical oxidation with  $O_3$  at current of 40 A for 4 h.

## 3. Results

### 3.1. Effects of Oxidants and Current Intensity on Electrochemical Oxidation of Organics in Coffee Wastewater

Experimental results reveal that the pH value of coffee wastewater could be adjusted to neutral with 4 g/L of NaOH or 4.5 g/L of  $Ca(OH)_2$  in the wastewater. As shown in

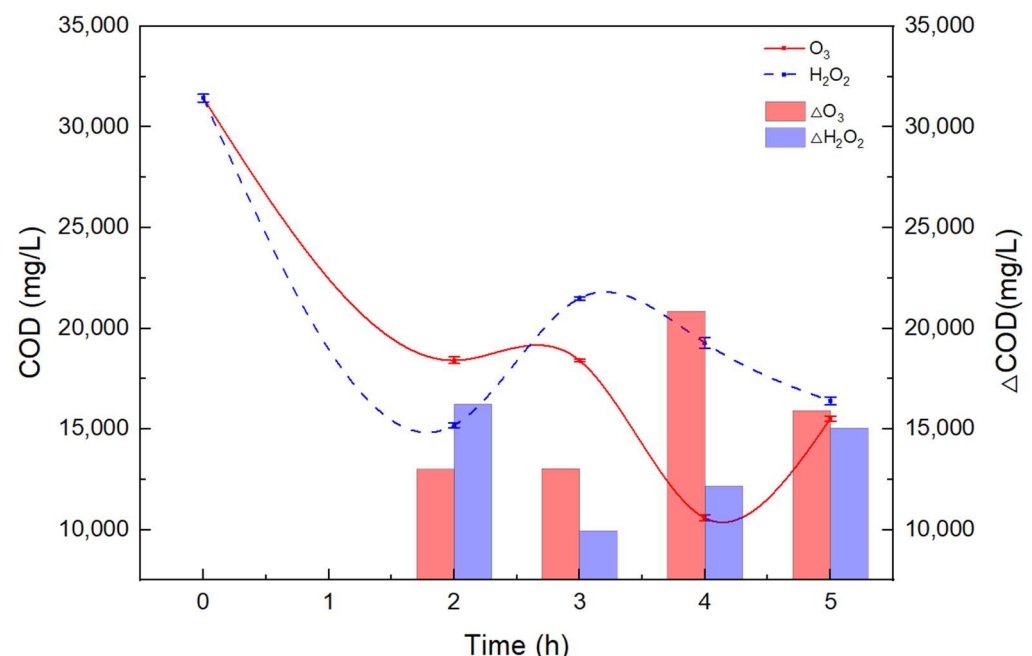
Table 1, the combination of electrochemical oxidation with oxidant of  $O_3$  or  $H_2O_2$  can reduce the consumption of NaOH. The addition of  $O_3$  and  $H_2O_2$  leads to a decrease in the required NaOH concentration of 2.8–3.76 and 0.8–1.6 g/L, respectively, indicating a more pronounced effect of  $O_3$  than that of  $H_2O_2$  on the reduction of alkali consumption during the electrochemical oxidation treatment of coffee fermentation wastewater.

**Table 1.** pH values and NaOH dosages of coffee waste at different processing times.

Oxidant	Initial pH	Processing Time h	After Treatment pH	NaOH Dosage g/L
$O_3$	3.70–4.20	2	5.14	1.20
		3	5.28	0.40
		4	5.30	0.32
		5	5.44	0.24
		2	3.33	3.20
$H_2O_2$		3	3.47	3.20
		4	3.52	2.40
		5	3.92	2.40

Notes: ozone concentration: 20 mg/L, charge density: 0.0133 A/cm<sup>2</sup>.

At the same time, it can be deduced from Figure 2 that the reduced alkali consumption could be attributed to the removal of organic pollutants that affect the pH value of coffee wastewater by the combined electrooxidation/oxidant treatment. The lower degree of reduction in the alkali consumption caused by  $H_2O_2$  compared to that by  $O_3$  suggests that the fermentation wastewater of Yunnan arabica coffee contains refractory pollutants, which are responsible for the high difficulty encountered in biochemical treatment of coffee wastewater. The more significantly reduced alkali amount for the pH adjustment process in the combined electrooxidation/ $O_3$  treatment indicates a better performance of the strong oxidant  $O_3$  for removal of pollutants affecting the pH value of the coffee wastewater.

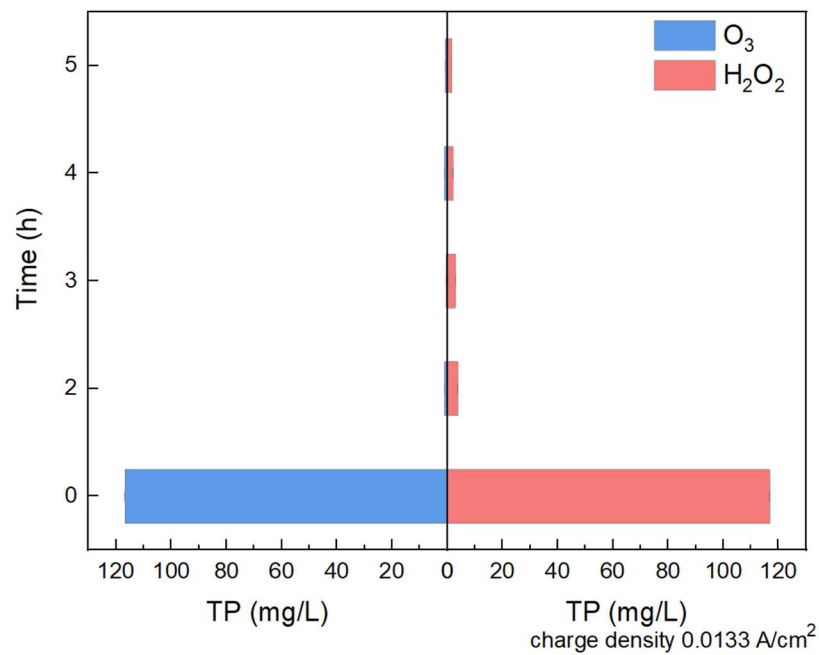


**Figure 2.** COD and reduction of coffee waste at different processing times.

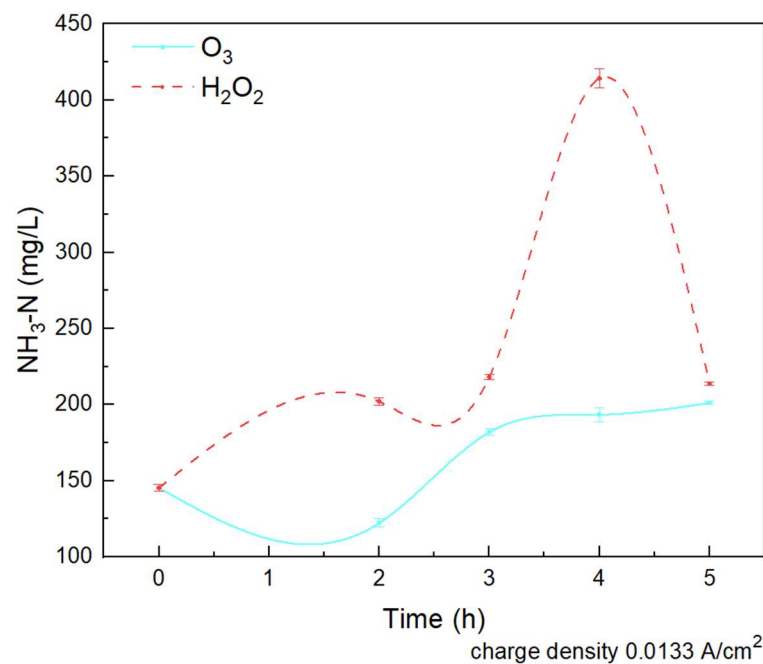
As shown in Figure 2, the synergistic effect of electrooxidation and  $H_2O_2/O_3$  could induce enhanced efficiency for removal of organic pollutants in coffee wastewater. The addition of  $H_2O_2$  in the 2-h electrochemical oxidation process obviously removed some organic pollutants. After the oxidation, the COD of the coffee fermentation wastewater was reduced from  $31,444.44 \pm 192.45$  to  $15,194.44 \pm 127.29$  mg/L, i.e., a decrease of more than

15,000 mg/L. With the increase in the treatment time to 3, 4 and 5 h, the measured CODs of the wastewater were  $21,500.00 \pm 83.33$ ,  $19,277.78 \pm 267.88$  and  $16,394.44 \pm 197.44$  mg/L, respectively, indicating that the further removal of organics with prolonged oxidation time was inhibited. In case of electrooxidation/ $O_3$  treatment with a processing time of 4 h, more significant degradation of the organics was observed. The COD of the wastewater was reduced from  $31,444.44 \pm 192.45$  to  $10,583.33 \pm 144.34$  mg/L after the treatment, revealing a COD decrease of more than 20,000 mg/L. When the oxidation time was extended to 5 h, the corresponding COD value was measured as  $15,516.67 \pm 132.29$  mg/L, showing a trend similar to that of the above electrooxidation/ $H_2O_2$  process. During the coffee fermentation stage, the mucilage layer needs to be degraded, which is correlated to acidification by lactic acid bacteria [15]. The digested mucilage is precipitated from the solution [16]. Concentrations are higher in coffee pulps than in coffee beans. For example, the content of malic acid in coffee pulps is six times higher than that in green coffee beans [11]. Sugars in wastewater are most likely released from coffee pulp and mucilage that precipitate after fermentation [17,18]. In addition, soluble proteins are present in coffee samples, with a considerable content ranging from 5% to 15%. For instance, an average protein content of 10–14% in coffee arabica has been reported [19]. Therefore, the wastewater from wet processing of Yunnan arabica coffee also contains organic acids, soluble sugars, soluble proteins and other pollutants, resulting in a complex composition and high concentration of organic pollutants, which further lead to the large difficulty in the electrochemical oxidation treatment. Adjusting the pH value of coffee wastewater to neutral consumes a large number of alkali. The addition of oxidants, especially  $O_3$ , during electrochemical oxidation of wastewater can effectively remove organic pollutants and significantly reduce alkali consumption. The more organic pollutants removed, the less NaOH required to remove organic acids, soluble sugars, soluble proteins, and other organic pollutants from the wastewater.

On the other hand, the addition of  $H_2O_2$  and  $O_3$  oxidants can significantly remove total phosphorus (TP) from coffee wastewater. The initial TP concentration of  $116.81 \pm 0.07$  mg/L decreased to  $1.01 \pm 0.01$  mg/L after the electrooxidation/ $O_3$  treatment and to  $3.79 \pm 0.07$  mg/L after the 2-h electrooxidation/ $H_2O_2$  process, indicating removal rates greater than 98%. Upon adding  $Fe^{2+}$  salt instead of  $O_3$  or  $H_2O_2$ , the removal rate of phosphate in wastewater began to rise at a pH value of 3 and reached 67.4% at a pH value of 8. The initial pH value of the system affects the precipitation modes of  $Fe^{2+}$  salt and phosphate, which in turn influence the removal of phosphate [20]. As shown in Figure 3, the addition of  $O_3$  in the electrochemical oxidation process is more effective than the  $H_2O_2$  addition for lowering the TP concentration in the treated wastewater.  $O_3$  shows a higher capability for removal of organic pollutants and TP because of its stronger oxidizing ability. In the process of electrochemical oxidation, hydroxyl radicals can be generated from ionized water and  $H_2O_2$  molecules. More oxidized radicals could not only oxidize and degrade the pollutants in the wastewater but also consume themselves by mutual reaction, eventually resulting in a weaker effect in water purification [21]. As shown in Figure 4, the addition of  $H_2O_2$  oxidant led to a negative effect on the removal of  $NH_3-N$  in coffee wastewater, whose concentration increased from  $145.29 \pm 2.27$  to  $414.26 \pm 6.23$  mg/L after 4-h treatment. In contrast, the 2-h electrooxidation/ $O_3$  process reduced the concentration of  $NH_3-N$  from  $145.29 \pm 2.27$  to  $122.17 \pm 2.70$  mg/L. Overall, the addition of  $O_3$  oxidant exhibits a negative effect on the removal of  $NH_3-N$  from coffee wastewater. Electrochemical oxidation of protein can produce peptides with structures similar to those of tyrosine and tryptophan, i.e., amino acids containing amino groups [22,23]. Therefore, the increase in the pollutant concentration after the combined treatment of coffee wastewater with electrochemical oxidation and oxidant is caused by  $NH_3-N$  in the degradation products.



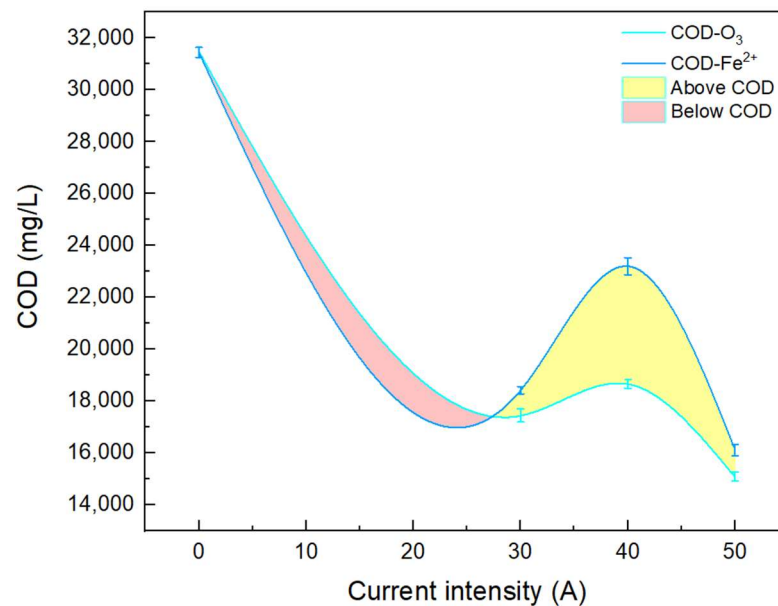
**Figure 3.** TP concentrations in coffee waste at different processing times.



**Figure 4.** Concentrations of NH<sub>3</sub>-N in coffee waste at different processing times.

After adding FeCl<sub>2</sub> to acidic wastewater containing oxidant H<sub>2</sub>O<sub>2</sub>, Fe<sup>2+</sup> ions can react with H<sub>2</sub>O<sub>2</sub> to produce additional oxidizing radicals, resulting in an increase in the number of oxidized radicals. As shown in Figure 5, the COD in the treated wastewater decreases with the rise of the current intensity. However, the simultaneous addition of FeCl<sub>2</sub> and enhancement of the current would slow down the rate of COD decrease, especially at a current intensity of 40 A. During the electrochemical oxidation process without the addition of FeCl<sub>2</sub>, the COD value of the wastewater decreased from 31,444.44 ± 192.45 to 18,666.67 ± 166.67 mg/L, corresponding to a COD decrease of more than 12,000 mg/L. In the presence of FeCl<sub>2</sub>, the relevant COD decreased only to 23,205.56 ± 327.59 mg/L, i.e., a reduction in COD of ca. 8000 mg/L. After addition of FeCl<sub>2</sub> to the acidic wastewater, the outer electrons of Fe<sup>2+</sup> ions can form carboxyl radicals with H<sub>2</sub>O<sub>2</sub> to oxidize the pollutants

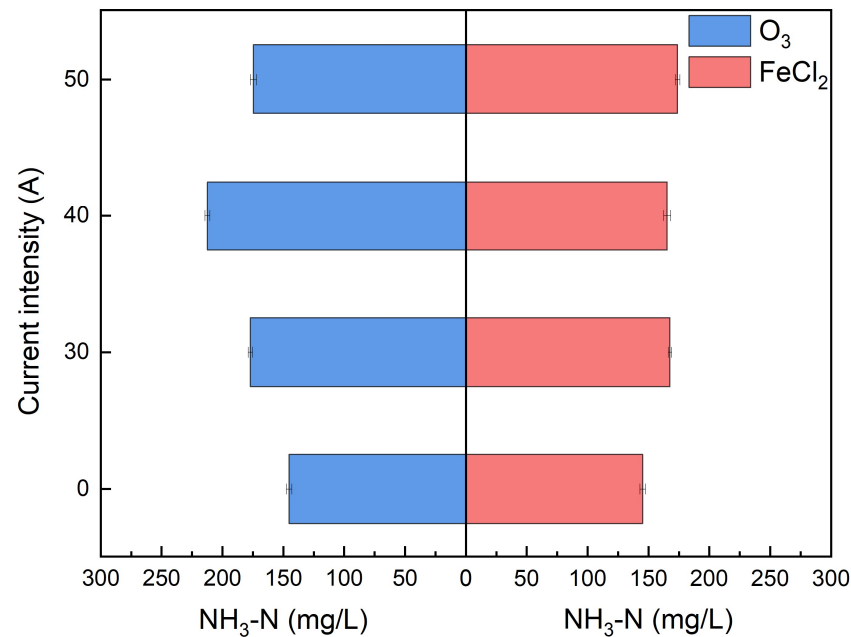
( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \cdot\text{OH}$ ). This reaction process is similar to the Fenton reaction, in which the generated hydroxyl radicals can degrade organic pollutants in the wastewater, and the degradation efficiency decreases with the increase in the current intensity [24].



**Figure 5.** COD of coffee waste after treatment at different current intensities.

It can be seen from Figure 6 that the electrochemical oxidation treatments at different current intensities show negative effects on the removal of  $\text{NH}_3\text{-N}$  in coffee wastewater. In particular, the  $\text{NH}_3\text{-N}$  concentration increased from  $145.29 \pm 2.27$  to  $212.24 \pm 2.04$  mg/L after treatment at 40 A. The addition of  $\text{Fe}^{2+}$  ions to the treatment process had a weaker effect on the elevation of  $\text{NH}_3\text{-N}$ , which was similar to that of oxidizing COD, with a high amount of  $\text{NH}_3\text{-N}$  under a high number of  $\text{O}_3$ -degraded organics and low amount of  $\text{NH}_3\text{-N}$  under a low number of  $\text{Fe}^{2+}$ -degraded organics. The concentration of  $\text{NH}_3\text{-N}$  in the wastewater treated by the combined electrooxidation/ $\text{O}_3$  process is approximately twice as high as that of the system after  $\text{Fe}^{2+}$ -combined electrochemical oxidation treatment, further indicating that electrochemical oxidation of proteins produces polypeptides with structures close to those of tyrosine and tryptophan. Therefore, the increase in the pollutant concentration after the combined electrooxidation/oxidant treatment of coffee wastewater is caused by  $\text{NH}_3\text{-N}$  in the degradation products.

In brief, the  $\text{O}_3$ -assisted electrochemical advanced oxidation could effectively remove organic pollutants and TP from coffee wastewater, while the concentration of  $\text{NH}_3\text{-N}$  in the system would increase after the treatment. The degraded organic pollutants during the oxidation process include organic acids, soluble sugars and proteins, whereas the corresponding degradation products are responsible for the elevated  $\text{NH}_3\text{-N}$  concentration in the system. On the other hand, the more organic pollutants and TP removed, the less NaOH required for adjusting the pH value of the wastewater.



**Figure 6.** Concentrations of NH<sub>3</sub>-N in coffee waste after treatments at different current intensities.

### 3.2. Fluorescence/UV–Vis Spectrophotometric Analysis of Coffee Wastewater Treated by Electrochemical Oxidation

It can be observed from Figure 7a that the peaks in the 3D fluorescence spectrum of the wastewater are located in Region II and IV, indicating that the main substances in the wastewater from wet processing of Yunnan arabica coffee are aromatic proteins, tryptophan and protein-like components. The fluorescence intensity of the wastewater was significantly reduced after the combined electrooxidation/O<sub>3</sub> treatment, suggesting that most of the aromatic proteins and protein-like components were removed by the oxidation process (Figure 7b). In contrast, the fluorescence peaks in the spectrum of the wastewater treated by the H<sub>2</sub>O<sub>2</sub>-assisted electrochemical oxidation are more obvious, which indicates an inferior capability of the treatment process for removal of aromatic proteins, tryptophan and protein-like components (Figure 7c). As discussed above, divalent iron ions could react with H<sub>2</sub>O<sub>2</sub> to form carboxyl radicals. In comparison with H<sub>2</sub>O<sub>2</sub>, O<sub>3</sub> with stronger oxidizing ability shows better performance for oxidative degradation of organic pollutants in the coffee fermentation wastewater, which is consistent with the observation in Section 3.1 that the introduction of O<sub>3</sub> oxidant to the electrochemical oxidation process of coffee wastewater can more effectively improve the degradation rate of organic pollutants compared to the addition of H<sub>2</sub>O<sub>2</sub>.

An analysis on the UV–Vis spectra shows that the absorption peaks of coffee wastewater are mainly in the wavelength range from 200 to 300 nm, and the intensities of the peaks are significantly lower after the O<sub>3</sub>-assisted electrochemical oxidation treatment of the wastewater, indicating that the contents of C=O and C=C groups, as well as the average molecular weight of the organic matter in the wastewater, were reduced by the treatment (Figure 8). In contrast, after the combined electrooxidation/FeCl<sub>2</sub> treatment of the wastewater, no obvious change in the intensity of the peaks was observed, revealing the negligible effect of this treatment process for degradation of organic substances in coffee wastewater. This could be interpreted by the mutual reaction between oxidized radicals and the consumption of partial Fe<sup>2+</sup> salts due to the removal of TP from the wastewater, both of which negatively influence the oxidative degradation of organic pollutants in the wastewater. Compared to the combined electrooxidation/FeCl<sub>2</sub> process, the H<sub>2</sub>O<sub>2</sub>-assisted electrochemical oxidation exhibited a higher capability for purification of the coffee fermentation wastewater.



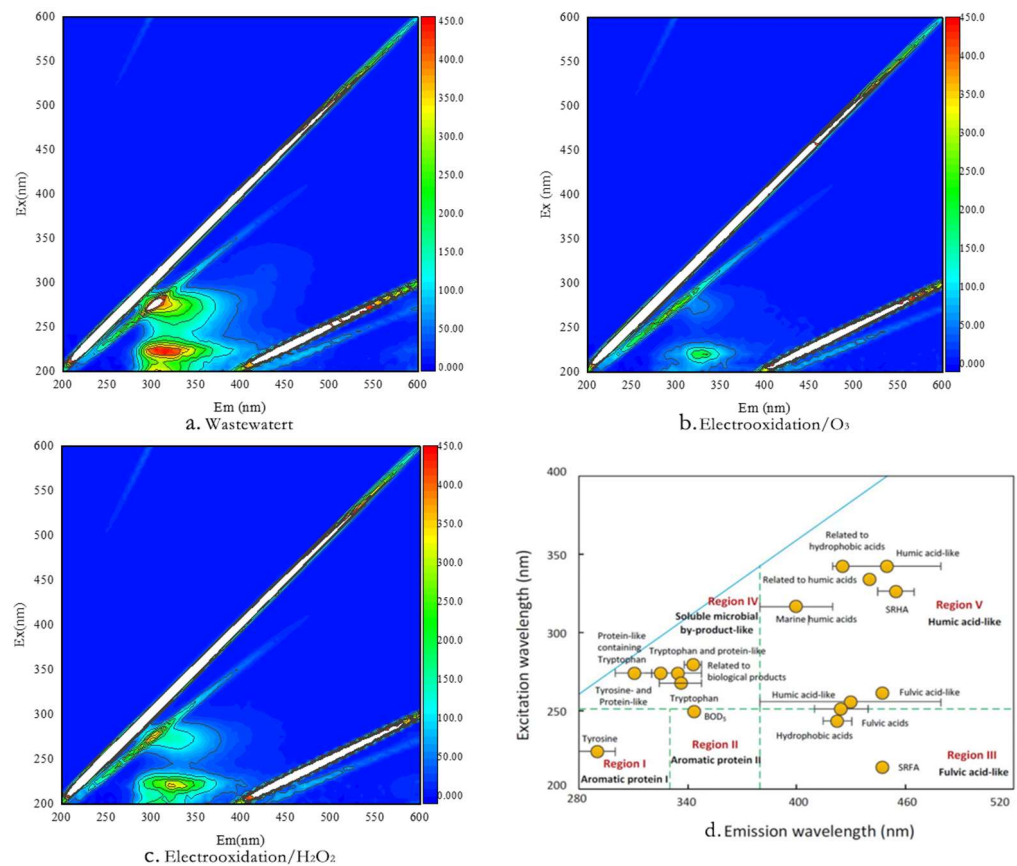


Figure 7. 3D fluorescence spectra.

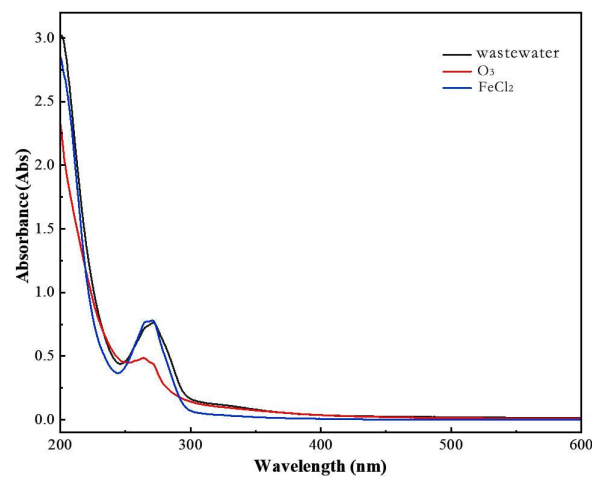


Figure 8. UV-Vis spectra.

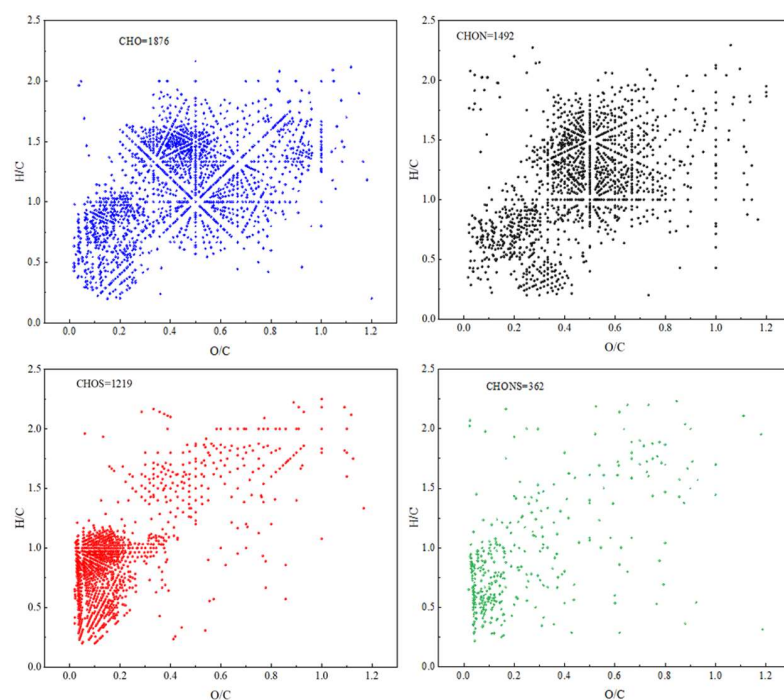
As shown in Section 3.1, the introduction of Fe<sup>2+</sup> and O<sub>3</sub> during electrochemical oxidation of coffee wastewater can promote the degradation of organic pollutants, and this promotion effect is more pronounced in the case of O<sub>3</sub> addition than that of Fe<sup>2+</sup> salt. The contents and molecular weights of aromatic proteins, tryptophan, and proteins in coffee wastewater were significantly reduced after the combined electrooxidation/O<sub>3</sub> treatment, indicating the high effectiveness of electrochemical advanced oxidation for removal of the organic matter in the wastewater. In the electrochemical oxidation process with added Fe<sup>2+</sup> salt instead of O<sub>3</sub>, however, the organic pollutants in the wastewater were only slightly removed. These results suggest that, in comparison with the addition of Fe<sup>2+</sup> salt, the

introduction of  $O_3$  can more efficiently promote the electrooxidation of organic pollutants (including refractory organics) in coffee fermentation wastewater.

### 3.3. Analysis of Degraded Fractions in Coffee Wastewater Treated by Electrochemical Oxidation

The results in Section 3.1 show that the addition of ferrous sulfate during electrochemical oxidation of the coffee fermentation wastewater has a negative effect on the removal of organics. To determine the DOM in the wastewater treated by the combined electrooxidation/ $O_3$  process, FTICR-MS measurements were conducted and the results were analyzed with the Kendrick mass defect (KMD) method due to its unique advantage in the characterization of organics containing the same series of molecular fragments. In this method,  $CH_2$  is the most commonly used group, and other functional groups, such as  $OCH_2$ ,  $COO$ ,  $O$ ,  $H_2O$ , and  $H_2$  can also be used [25]. In addition, the van Krevelen diagram (VK diagram with H/C as the vertical coordinate and O/C as the horizontal coordinate) was applied to the DOM resolution. Based on the values of O/C and H/C for each molecular formula, DOM molecules in the wastewater fall into different regions of the VK diagram, including lipids, proteins/amino sugars, saccharides, unsaturated hydrocarbons, polycyclic aromatic hydrocarbons, lignans, and tannins [26].

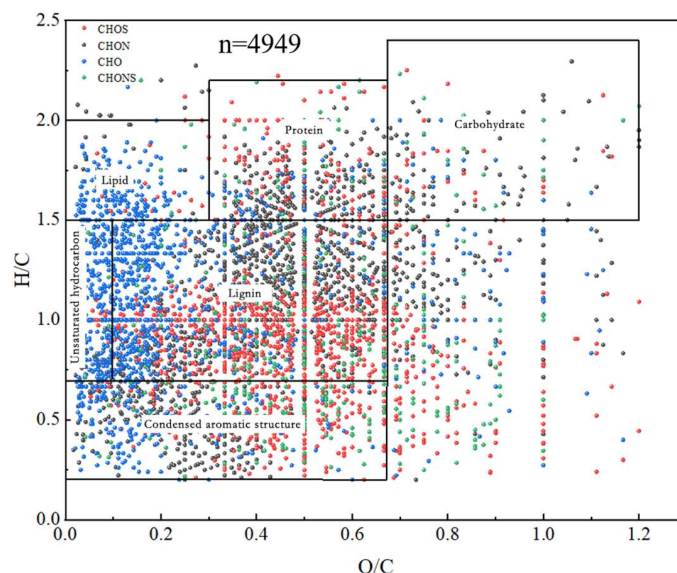
As shown in Figure 9, a total of 4949 compounds were detected in the coffee wastewater after the  $O_3$ -assisted electrochemical oxidation treatment. Among these species, CHO exhibits the highest content (37.91%), followed by CHON (30.15%), CHOS (24.63%) and CHONS (7.31%), with a decreasing trend in concentration. The S-containing fractions, including CHOS and CHONS, have a total content of 31.94% in the treated wastewater, and biological degradation is difficult.



**Figure 9.** VK diagram of DOM in electrooxidized coffee wastewater.

It can be seen from Figure 10 that the coffee wastewater after the combined electrooxidation/ $O_3$  treatment contains lignin and condensed aromatic structures as the main refractory components, as well as some proteins, lipids, carbohydrates, unsaturated hydrocarbons, and other compounds degraded by microorganisms. As discussed in Section 3.2, the proteins, lipids, unsaturated hydrocarbons and carbohydrates in the wastewater from wet processing of Yunnan arabica coffee can be degraded and removed by the combined treatment with electrochemical oxidation and  $O_3$ , leading to a decrease in the molecular weights and an increase in the molecular diversity of DOM in the system. To further reduce the contents of

lignin and condensed aromatic compounds, the degree of electrochemical oxidation was increased, while the content of labile DOM components in the wastewater was reduced to ca. 30% of the original value. For the subsequent treatment of this wastewater system, biochemical methods are not suggested.

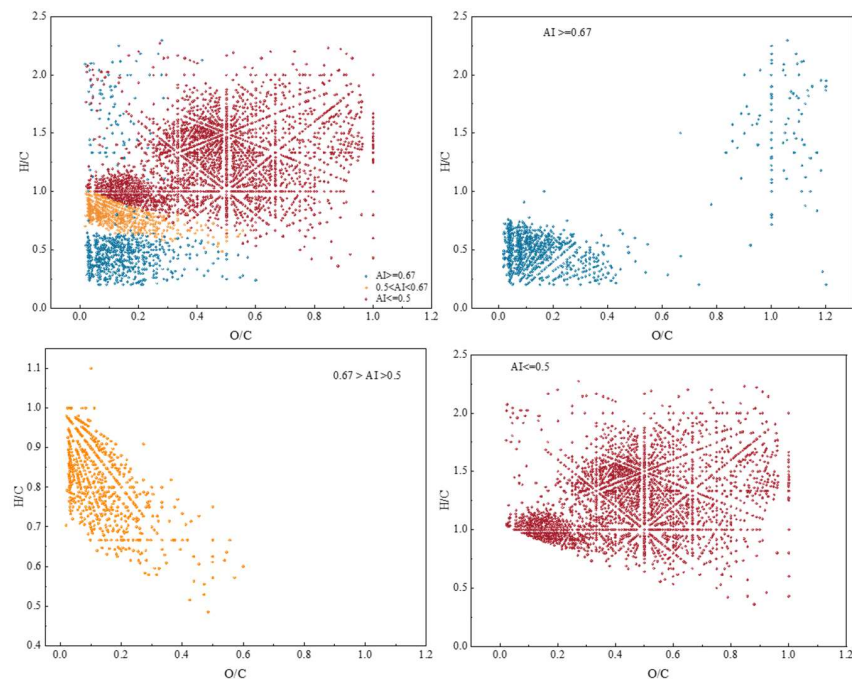


**Figure 10.** VK diagram and molecular composition of DOM in electrooxidized coffee wastewater. Lipid: (H:C = 1.5–2.0, O:C = 0–0.3); Protein: (H:C = 1.5–2.2, O:C = 0.3–0.67); Lignin: (H:C = 0.7–1.5, O:C=0.1–0.67); Carbohydrate: (H:C=1.5–2.4, O:C=0.67–1.2); Unsat. hydrocarbon: (H:C = 0.7–1.5, O:C = 0–0.1); Condensed aromatic structure: (H:C = 0.2–0.7, O:C = 0–0.67).

The condensation degree of molecules is usually expressed by the double bond equivalent (DBE), which is numerically equal to the total number of rings and double bonds in the molecule. Considering the contribution of oxygen atoms to the double bonds in the DOM molecules, Koch and Dittmar proposed the aromaticity index (AI) according to the data from the negative ions of electrospray ionization (ESI) FTICR–MS to indicate the unsaturation degree of compounds [27,28]. Based on the magnitude of AI and the ratios of O/C and H/C, DOM molecules can be classified into the following five groups: saturated compounds, unsaturated aliphatic compounds, highly unsaturated compounds, polyphenolic compounds, and polycyclic aromatic hydrocarbons, which exhibit an increasing trend in biological inertia [29]. As shown in Figure 11, an analysis of the aromaticity of DOM fractions in the electrochemically treated wastewater reveals the largest proportion (61.27%) of non-aromatic compounds, mainly including hypoxic phenolic compounds ( $AI \leq 0.5$ ,  $H/C < 1.5$ , and  $O/C < 0.5$ ), hyperoxic phenolic compounds ( $AI \leq 0.5$ ,  $H/C < 1.5$ ), and aliphatic compounds ( $2.0 \geq H/C \geq 1.5$ ). The contents of polycyclic aromatic hydrocarbons (18.39%) and aromatic compounds (14.35%) rank second and third, respectively. Compared to non-aromatic compounds, species containing aromatic ring structures are more difficult to be degraded by microorganisms.

Studies of the molecular fractions of DOM in municipal sewage treated with different flocculants show that conventional flocculants and coagulants could only remove organic compounds with low H/C ratios and high O/C ratios. For efficient removal of aliphatic compounds, highly unsaturated compounds, phenolic compounds, and sulfur-containing organics, some novel flocculants have been developed and excellent performance has been observed [30,31]. In this work, the PAM and PAC added during the electrochemical oxidation treatment of coffee fermentation wastewater mainly play a role in the removal of suspended solids (SS), rather than organics. Therefore, the combination of electrochemical oxidation with  $O_3$  contributes significantly to the removal of organic pollutants from coffee wastewater. The electrochemical advanced oxidation can be used as an effective

method to treat industrial wastewater, in which the sulfur-containing compounds are difficult to be degraded biologically by microorganisms. In this process, the increase in the amount of oxidant could lead to reduced content of sulfur-containing compounds such as CHOS. Therefore, it is not recommended to apply biological methods for the subsequent purification of coffee wastewater after electrochemical oxidation treatment.



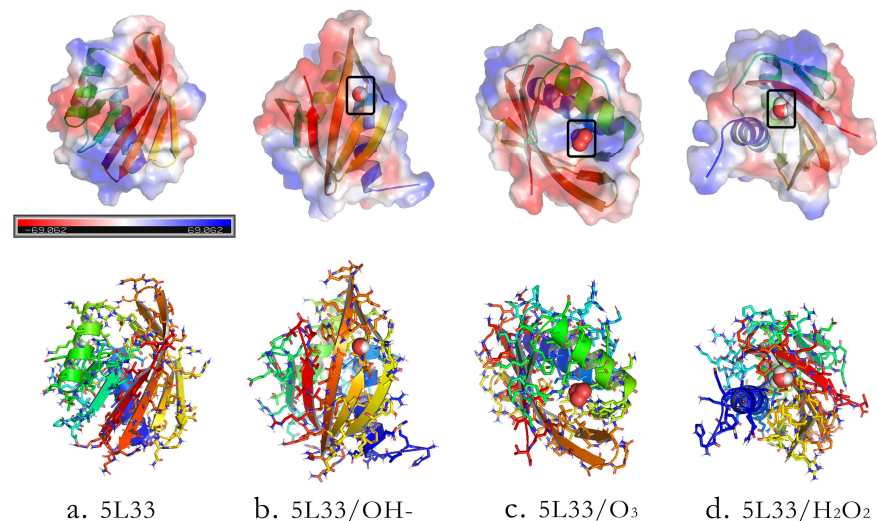
**Figure 11.** VK diagram and molecular composition of DOM in electrooxidized coffee wastewater. Aromatic compounds ( $0.67 > AI > 0.5$ ); polycyclic aromatic hydrocarbon compounds ( $AI \geq 0.67$ ); non-aromatic compounds ( $AI \leq 0.5$ ).

### 3.4. Simulation Experiments of Binding $\text{OH}^-$ , $\text{H}_2\text{O}_2$ and $\text{O}_3$ to Proteins

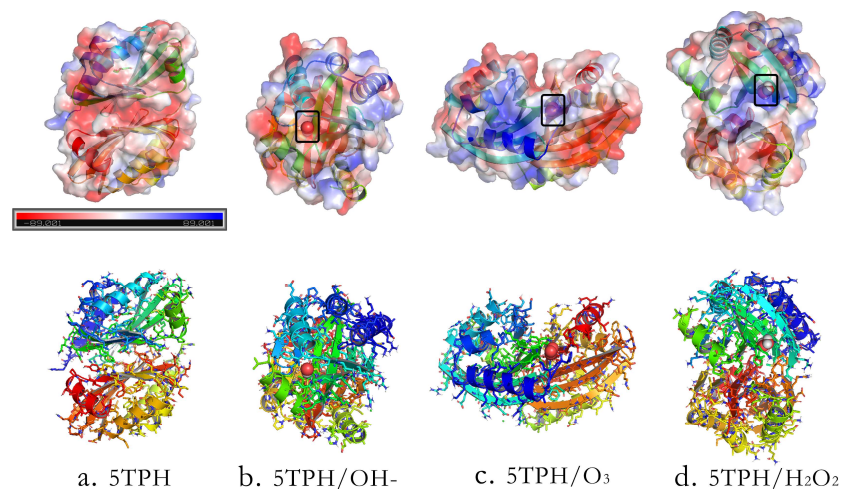
As discussed above, the wastewater from wet fermentation of Yunnan arabica coffee contains a large amount of proteinaceous organic matter, and thereby a large quantity of NaOH will be consumed in adjusting the pH value of the wastewater to neutral. It is inferred that the content of hydroxy ions in the wastewater is affected by the proteinaceous organic matter. On the other hand, it was concluded that the electrooxidation process with  $\text{O}_3$  could more effectively remove the organic matter in coffee wastewater than the electrochemical treatment with  $\text{H}_2\text{O}_2$ . To verify the assumption that hydroxide ions were consumed by proteinaceous organic matter in the wastewater and to compare the ability of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  to oxidize proteinaceous organic matter, simulation experiments were conducted with open-source software Autodock\_vina and AutoDockTools-1.5.7. Active sites and ligand-binding cavities in native proteins are often formed by curved  $\beta$  sheets, and the control of  $\beta$ -sheet curvature would allow the design of proteins with cavities customized to bind with specific ligands. Toward this end, Marcos et al. investigated the mechanisms of controlling  $\beta$ -sheet curvature by folding simulations based on the geometry of  $\beta$  sheets in natural protein structures [32]. Two proteins with curved  $\beta$ -folded layers (RCSB PDB: 5L33, 5TPH) studied by Marcos et al. were selected in this study for docking  $\text{OH}^-$ ,  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  in the simulation experiments.

Macromolecule content-5TPH: total structure weight of 28.97 kDa, atom count of 1850, medelled residue count of 241, deposited residue count of 246, unique protein chains of 1; Macromolecule content-5TPH: total structure weight of 12.85 kDa, atom count of 987, medelled residue count of 106, deposited residue count of 108, unique protein chains of 1. Figures 12a and 13a illustrate the electrostatic potential diagrams of proteins 5L33 and 5TPH, respectively. It can be seen that the electrostatic potential of 5TPH is higher than

that of 5L33. A total of nine binding sites and binding energies of the proteins with  $\text{OH}^-$  and  $\text{O}_3$  were calculated during the simulation experiments. The binding site diagrams in Figure 12b–d show that  $\text{OH}^-$ ,  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  bind at the electropositive sites of 5L33 with the corresponding binding energies of  $-1.8$ ,  $-2.9$  and  $-2.8$  kcal/mol, respectively. The absolute values of the binding energies of these species with 5L33 increase in the order of  $\text{OH}^- < \text{H}_2\text{O}_2 < \text{O}_3$  (Table 2). Figure 13b–d present the binding site diagrams of 5L33 with  $\text{OH}^-$ ,  $\text{O}_3$  and  $\text{H}_2\text{O}_2$ , respectively. It can be seen that  $\text{OH}^-$  ions bind at the electronegative sites of 5TPH, while  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  bind at the electropositive sites. As shown in Table 2, the binding energies of 5TPH with  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$  and  $\text{O}_3$  rise from less than 2 kcal/mol to nearly 3 kcal/mol in absolute values. The binding energies of 5L33 with  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$  and  $\text{O}_3$  are higher than the corresponding values of 5TPH with the species, which is caused by the structural differences between 5L33 and 5LPH. These results indicate that  $\text{OH}^-$  can be bound to the proteins, confirming the assumption that the presence of protein pollutants in the wastewater from wet fermentation of Yunnan arabica coffee is responsible for the high NaOH consumption in the process of adjusting the pH value of the wastewater.  $\text{O}_3$  is easier to bind to the electropositive sites of proteins than  $\text{H}_2\text{O}_2$  because of its stronger oxidation capacity, which is the reason why the binding energies of  $\text{O}_3$  with 5L33 and 5TPH are higher than those of  $\text{H}_2\text{O}_2$ . The excellent performance of the combined electrooxidation/ $\text{O}_3$  process for removal of the organic matter in the coffee fermentation wastewater could be attributed to the strong oxidizing ability of  $\text{O}_3$  and the high binding energy of  $\text{O}_3$  with proteins, which promote the degradation of protein-like organics in the wastewater.



**Figure 12.**  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$  and  $\text{O}_3$  binding 5L33.



**Figure 13.**  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$  and  $\text{O}_3$  binding 5TPH.

**Table 2.** Binding energies of proteins with OH<sup>−</sup>, O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>.

Mode	5L33 kcal/mol			5TPH kcal/mol		
	OH <sup>−</sup>	O <sub>3</sub>	H <sub>2</sub> O <sub>2</sub>	OH <sup>−</sup>	O <sub>3</sub>	H <sub>2</sub> O <sub>2</sub>
1	−1.8	−2.9	−2.8	−1.5	−2.8	−2.4
2	−1.6	−2.9	−2.7	−1.4	−2.4	−2.4
3	−1.5	−2.5	−2.6	−1.2	−2.3	−2.3
4	−1.5	−2.5	−2.4	−1.2	−2.3	−2.3
5	−1.5	−2.3	−2.3	−1.2	−2.3	−2.2
6	−1.4	−2.3	−2.3	−1.2	−2.3	−2.1
7	−1.4	−2.3	−2.1	−1.1	−2.2	−2.1
8	−1.3	−2.2	−2.1	−1.1	−2.1	−2.1
9	−1.2	−2.1	−2.0	−1.1	−2.0	−2.0

#### 4. Conclusions

Due to the proteinaceous organic pollutants in the wastewater from the wet fermentation of Yunnan arabica coffee, a large amount of NaOH is consumed in the pH adjustment process. O<sub>3</sub>-combined electrochemical oxidation (63.60%) for treatment of organic species in coffee wastewater is better than that of electrochemical processes with H<sub>2</sub>O<sub>2</sub> (47.70%) and FeCl<sub>2</sub> (34.48%). The synergy of electrooxidation/O<sub>3</sub> process (40A, 20mg/L-2L/min) could not only raise the pH value (3.70~4.20, 5.14~5.44) of the wastewater and reduce the NaOH dosage of 2.80~3.7 g/L, but also effectively degrade the proteins, lipids, unsaturated hydrocarbons, and carbohydrates, with a total chemical oxygen demand (COD) value above 20,000 mg/L. The electrochemical oxidation in the presence of oxidant O<sub>3</sub> or H<sub>2</sub>O<sub>2</sub> can not only effectively degrade the proteins, lipids, unsaturated hydrocarbons, and carbohydrates in the wastewater, but also significantly remove TP from the system. Compared to the H<sub>2</sub>O<sub>2</sub>-assisted and FeCl<sub>2</sub>-assisted electrochemical process, the combined electrooxidation/O<sub>3</sub> treatment of the coffee fermentation wastewater shows better performance for removal of the organic pollutants. Fluorescence/UV-Vis spectrophotometric analysis of the treated wastewater reveals that the degradation fractions consist of S-containing organics, lignin, aromatic structures, and condensed aromatic compounds, which are all difficult to be degraded by microorganisms. Therefore, the treatment process of coffee fermentation wastewater should be based on the electrochemical advanced oxidation technique rather than the biological methods. In addition, results from the simulation experiments show that the binding energies of proteins 5L33 and 5TPH with O<sub>3</sub> are higher than those with H<sub>2</sub>O<sub>2</sub>, and the binding of OH<sup>−</sup> ions with protein organic pollutants leads to an increase in the consumption of NaOH for adjusting the pH value of the wastewater. In sum, the results from this study demonstrate that O<sub>3</sub>-assisted electrochemical oxidation can be applied as an effective method for degradation of the organic matter in the wastewater from wet fermentation of Yunnan arabica coffee, and could simultaneously reduce the consumption of NaOH.

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