



## Original Paper

# Molecular transformation of dissolved organic matter in refinery wastewaters: Characterized by FT-ICR MS coupled with electrospray ionization and atmospheric pressure photoionization

Chen He <sup>a</sup>, Wei-Ming Chen <sup>b</sup>, Chun-Mao Chen <sup>a, \*</sup>, Quan Shi <sup>a</sup><sup>a</sup> State Key Laboratory of Heavy Oil Processing, Beijing Key Laboratory of Oil and Gas Pollution Control, China University of Petroleum, Beijing 102249, China<sup>b</sup> Faculty of Geosciences and Environmental Engineering, Southwest Jiaotong University, Chengdu, Sichuan 611756, China

## ARTICLE INFO

## Article history:

Received 27 May 2022

Received in revised form

23 July 2022

Accepted 29 September 2022

Available online 4 October 2022

Edited by Jia-Jia Fei

## Keywords:

Refinery wastewater

DOM

Molecular transformation

ESI

APPI

FT-ICR MS

## ABSTRACT

Dissolved organic matter (DOM) in refinery wastewater is an extremely complex mixture of various organic compounds. Using mass spectrometry, it is impossible to characterize all of the DOM molecules with only one ionization source. In this study, negative-ion, electrospray ionization (ESI), positive-ion ESI, and positive-ion atmospheric pressure photoionization (APPI) were coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to analyze the molecular composition of DOM in a refinery wastewater stream during the treatment process. There were obvious differences in the heteroatom composition, number of DOM constituents, and chemical properties in refinery wastewater under the three ionization modes. Acidic CHO and CHOS compounds detected by (–)ESI, basic CHN and CHON compounds detected by (+)ESI, and hydrocarbons detected by (+)APPI were analyzed to determine the molecular transformations that occurred during treatment. In an anaerobic biological treatment process, acidic CHO and CHOS compounds with a high oxygen content were preferentially removed, and acidic CHO and CHOS compounds with a low oxygen content were produced. In an aerobic biological process, acidic CHO and CHOS compounds with a low oxygen content were preferentially removed, and acidic CHO and CHOS compounds with a high oxygen content were produced. The whole biological treatment process has a poor removal efficiency for CHN and CHON compounds, and hydrocarbons. An activated carbon (AC) adsorption process removed different heteroatom compounds mainly with a low oxygen content for acidic and basic compounds. The transformation mechanism of CHO and CHOS compounds in the biological treatment process was analyzed by the Kendrick mass defect (KMD) theory and a mass difference network analysis. In the anaerobic process, large amounts of oxygenated CHO and CHOS compounds were degraded by decarboxylation, deoxydation, demethoxylation, and dehydration reactions, and converted to lower oxygen content compounds. In the aerobic processes, these low oxygen CHO and CHOS compounds mainly underwent carboxylation and oxidation reactions. This study determined the transformation characteristics and mechanisms of different types of organic compounds in refinery wastewater during the treatment process. The results provide guidance for the design and optimization of technologies for refinery wastewater treatment.

© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Large amounts of wastewater are produced every day in the petroleum refinery production process (Wake, 2005; Wang and Chen, 2018; Ye et al., 2021). This wastewater contains dissolved organic matter (DOM), which has an extremely complex chemical

composition (Ye et al., 2020; Li et al., 2021). In recent years, pollutant emission standards in the petrochemical industry have become increasingly strict. In the Emission Standard of Pollutants for the Petroleum Refining Industry (GB 31570–2015) (China), the chemical oxygen demand (COD) emission limit has been reduced from 100 to 60 mg/L. In addition, the processing of high sulfur and high acid crude oils causes the serious emulsification of refinery wastewater (Ren et al., 2019; Samanipour et al., 2019), leading to unstable water quality and further problems, such as a low oil removal efficiency and damage to biochemical equipment (Yan

\* Corresponding author.

E-mail address: [c.chen@cup.edu.cn](mailto:c.chen@cup.edu.cn) (C.-M. Chen).

et al., 2014; Chen et al., 2015; Tong et al., 2016). Therefore, stricter discharge standards and the use of inferior heavy oil have generated new challenges in the treatment of refinery wastewater.

Indices for evaluating the quality of wastewater include total organic carbon (TOC), biochemical oxygen demand (BOD), and COD; however, it is difficult to use these traditional indices to guide the design and optimization of wastewater treatment processes (Diya'uddeen et al., 2011; Jafarinejad and Jiang, 2019). Determining the molecular composition of the DOM is the first stage in evaluating the treatment effectiveness, and will enable a better understanding of the transformation mechanism that occurs during the process (Headley et al., 2009; Maizel and Remucal, 2017; He et al., 2020b).

Negative-ion electrospray ionization (ESI) coupled with high resolution mass spectrometry (HRMS) techniques, such as time-of-flight mass spectrometer (TOF MS) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), is an effective approach for the molecular characterization of DOM, especially for naphthenic acids (NAs) in wastewater (Headley et al., 2015; Barrow et al., 2016; Morandi et al., 2015; Wang et al., 2019; Fang et al., 2020). Previous studies have investigated the transformation characteristics of DOM in refinery wastewater due to biochemical processes. Wang et al. (2015) reported that biodegradation via activated sludge was the main mechanism for removing alicyclic NAs and aromatic NAs, and the polycyclic NA congener classes were found to be relatively recalcitrant to biodegradation. Li et al. (2015a) found that weakly polar organic matter in the organic phase was liable to be degraded, and some humic-like substances in the aqueous phase were found to be refractory components in a biological process. In addition to CHO compounds, the CHOS species also had a high abundance in the wastewater and were more recalcitrant to the bio-degradation than CHO compounds (Li et al., 2020).

However, NAs are not the only organic matter present in refinery wastewater, with other hydrocarbons, and nitrogen- and sulfur-containing compounds also present (Boczka et al., 2016; Fang et al., 2017). The analysis of organic pollutants in wastewater under only the negative-ion ESI mode is not comprehensive. Li et al. (2015b) found a large amount of basic  $\text{CHON}_1$  compounds under the positive-ion ESI mode. Ajaero et al. (2019) used atmospheric pressure photoionization (APPI) FT-ICR MS to characterize the molecular composition of oil sand process-affected water in constructed wetland treatment systems and found that the use of the different ionization modes resulted in differences in the resulting molecular composition of the wastewater. He et al. (2021) applied ( $\pm$ )ESI and (+)APPI FT-ICR MS to characterize the DOM in refinery wastewater and found that the results obtained by different ionization techniques were extremely complementary. A comprehensive characterization through the combination of multiple ionization techniques will enable a better understanding of the chemical transformation in the wastewater process.

This study conducted a comprehensive characterization of DOM from a wastewater processing steam using FT-ICR MS coupled with (+)ESI, (–)ESI, and (+)APPI. We attempted to determine the molecular transformation of DOM compounds generated during the operation of biological and activated carbon (AC) adsorption processes in a practical engineering application.

## 2. Materials and methods

### 2.1. Refinery wastewater samples

Refinery wastewater samples were collected from a wastewater treatment plant operated by a petrochemical company in northern China. Five wastewater samples were collected from the effluent of

different treatment processes. The details of these treatment processes were presented in a previous study (Li et al., 2015a). Fig. 1 shows the treatment processes used for the refinery wastewater as well as the sampling points. Sample #1 was the wastewater effluent produced after preliminary oil separation and flotation. Sample #2 was the effluent from an anaerobic treatment process. Sample #3 was the effluent from a combined aerobic treatment and sludge separation process. Sample #4 was the wastewater effluent produced after biological aeration oxidation and air flotation/sand filtration. Sample #5 was the effluent from an AC adsorption process.

Wastewater samples were collected in Nalgene bottles (cleaned with ultrapure water and rinsed with sample water before use). Once the samples arrived at the laboratory, filtration was performed immediately with pre-combusted 0.45  $\mu\text{m}$  membrane filters (Millipore) to remove particles and most bacteria. The filtrates were stored at  $-4^\circ\text{C}$  prior to analysis.

### 2.2. Solid-phase extraction

The DOM was extracted from the samples by solid-phase extraction (SPE) with an HLB cartridge (Oasis HLB, 500 mg, 6 mL, Waters, Milford, MA, USA) (Dittmar et al., 2008; Li et al., 2015a). First, the SPE cartridges were activated by rinsing with 20 mL methanol and 20 mL acidified ultrapure water ( $\text{pH} = 2$ ). Approximately 100 mL of the wastewater sample was acidified to  $\text{pH} = 2$  with hydrochloric acid and then passed through the SPE cartridge at a rate of 5 mL/min. The cartridge was then rinsed with 20 mL acidified ultrapure water ( $\text{pH} = 2$ ) to remove the salt, dried with  $\text{N}_2$ , and then eluted with 10 mL of methanol to obtain the DOM. The extracted DOM was stored in the dark at  $4^\circ\text{C}$  prior to the FT-ICR MS analyses.

### 2.3. The TOC analysis

The TOC of the refinery wastewater samples was analyzed using a 5000A total dissolved organic carbon (TOC) analyzer (Shimadzu, Japan).

### 2.4. The FT-ICR MS analysis

The MS analysis of DOM was conducted using a 9.4 T Bruker Apex Ultra FT-ICR mass spectrometer (He et al., 2020a). The SPE-DOM samples were diluted in methanol and methanol/toluene (1:1) at a concentration of approximately 100 mg/L for the ESI and APPI analysis. The DOM samples were injected into the ionization source at 180  $\mu\text{L/h}$  through a syringe pump. The FT-ICR MS operating parameters under different ionization modes are presented in Table S1 (see Supporting Information). The mass spectra were recorded using 4 M word data sets. A total of 128 continuous scans were acquired for each analysis to enhance the signal-to-noise ratio ( $S/N$ ) of the mass spectra.

### 2.5. Data processing

The FT-ICR MS mass calibration, data acquisition, and processing were performed using in-house software (Shi et al., 2010; He et al., 2021). Mass peaks in the  $m/z$  range of 100–800 with an  $S/N$  greater than 6 were exported to a spreadsheet for further processing. A molecular formula calculator generated matching formulas according to the elemental combinations of  $^{12}\text{C}_{1-60}$ ,  $^{13}\text{C}_1$ ,  $^1\text{H}_{1-120}$ ,  $^{14}\text{N}_{0-3}$ ,  $^{15}\text{N}_1$ ,  $^{16}\text{O}_{0-30}$ ,  $^{18}\text{O}_1$ ,  $^{32}\text{S}_{0-2}$  and  $^{34}\text{S}_1$ . The mass accuracy window was set to 1.0 ppm in the formula assignment section. All elemental formulas had to meet the following basic chemical criteria: (1) the number of H atoms had to be at least one-third the

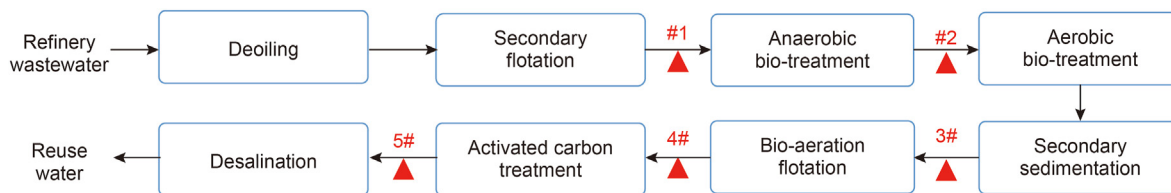


Fig. 1. Flow chart of the refinery wastewater treatment processes and the points at which samples were taken.

number of C atoms and could not exceed  $2C + N + 2$ ; (2) the sum of the H and N atoms must be even; and (3) the H/C and O/C ratios had to be less than 3 and 1.5, respectively. Each assigned class species was allocated by double bond equivalence (DBE) values (with a mass interval of 2 Da) and carbon numbers ( $\text{CH}_2$  unit, with a mass interval of 14 Da) by an automatic retrieval within a tolerance of  $\pm 0.001$  Kendrick mass defect (KMD). Through the application of these criteria, formulas were ascribed with a high level of confidence, and only formulas that were physically possible were permitted.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to analyze the similarity of the DOM constituents of refinery wastewater samples detected by (–)ESI, (+)ESI, and (+)APPI modes. A closer distribution in PCA plots or a closer branch in HCA plots indicates a higher similarity of the DOM constitution of the refinery wastewater samples. Rank abundance analysis was applied to analyze the differences in molecular numbers and relative abundances obtained by different ionization modes. The principal component analysis (PCA), hierarchical cluster analysis (HCA), and rank abundance analysis were carried out on the sum-normalized intensity. The PCA and HCA analyses based on correlation distance were conducted in OriginPro (Version, 2022; OriginLab Co., Northampton, MA, USA). The details of the KMD analysis and mass difference network analysis are provided in the Supporting Information.

### 3. Results and discussion

#### 3.1. Comparison of DOM obtained by the (+)ESI, (–)ESI, and (+)APPI modes

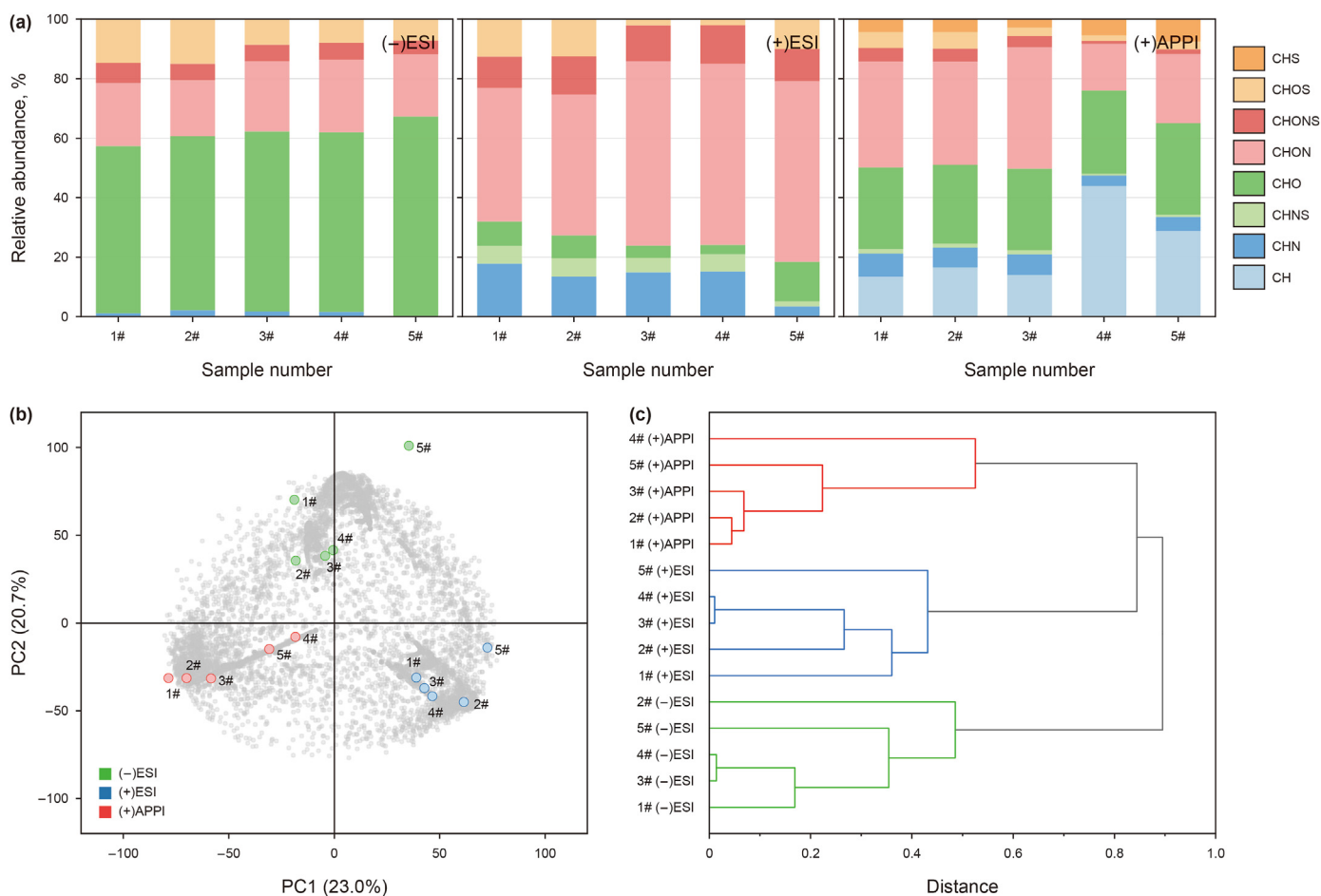
The molecular weight distribution (MWD) of DOM ranged from 100 to 450 Da (Figs. S1–S3, see Supporting Information), with the maximum relative abundance around  $m/z$  270 for all three modes of ionization. In the (–)ESI mode, a total of 6382 DOM formulas were detected, including CHO, CHN, CHON, CHOS, and CHONS compounds (Fig. 2a), which specifically included 50 classes of  $\text{O}_{1-9}$ ,  $\text{N}_{1-2}$ ,  $\text{N}_1\text{O}_{1-8}$ ,  $\text{N}_2\text{O}_{1-7}$ ,  $\text{O}_{1-9}\text{S}_1$ ,  $\text{O}_{1-7}\text{S}_2$ , and  $\text{N}_1\text{O}_{1-8}\text{S}_1$  (Fig. S4). In the (+)ESI mode, a total of 5950 DOM formulas were found, including CHN, CHNS, CHON, CHO, CHOS, and CHONS compounds (Fig. 2a), which specifically included 40 classes of  $\text{O}_{1-7}$ ,  $\text{N}_{1-2}$ ,  $\text{N}_1\text{O}_{1-7}$ ,  $\text{N}_2\text{O}_{1-6}$ ,  $\text{O}_{1-6}\text{S}_1$ ,  $\text{N}_1\text{S}_{1-2}$ ,  $\text{N}_2\text{S}_1$ ,  $\text{N}_1\text{O}_{1-5}\text{S}_1$ , and  $\text{N}_2\text{O}_{1-4}\text{S}_1$  (Fig. S5). In the (+)APPI mode, a total of 4883 DOM formulas were obtained, including CH, CHN, CHS, CHNS, CHO, CHON, CHOS, and CHONS compounds (Fig. 2a), which specifically included 38 classes of CH,  $\text{O}_{1-7}$ ,  $\text{N}_{1-2}$ ,  $\text{N}_1\text{O}_{1-7}$ ,  $\text{N}_2\text{O}_{1-5}$ ,  $\text{O}_{0-5}\text{S}_1$ ,  $\text{O}_{1-4}\text{S}_2$ ,  $\text{N}_1\text{S}_{1-2}$ ,  $\text{N}_2\text{S}_1$ , and  $\text{N}_1\text{O}_{1-3}\text{S}_1$  (Fig. S6). By comparing the analysis results under the three ionization modes, it was found that more nitrogen-containing compounds were ionized by (+)ESI than by (–)ESI, and (+)APPI preferentially ionized hydrocarbon compounds compared to the ESI modes.

The chemodiversity of DOM in refinery wastewater was revealed by the differences between the three ionization modes (Fig. S7). The similarities and differences in the DOM composition obtained by (±)ESI and (+)APPI were further compared based on

the PAC and HCA analyses using all the DOM obtained by the three ionization modes from the five refinery wastewater samples (15 DOM pools in total). In the PCA (Fig. 2b), PC1 and PC2 explained 23.0% and 20.7% of the variations of all samples, respectively. The DOM components identified by (+)ESI and (+)APPI were significantly different in PC1, and the DOM components identified by (–)ESI were significantly different from those identified by (+)ESI and (+)APPI in PC2. The HCA divided the ionization sources into two categories, positive ionization mode and negative ionization mode, indicating that the DOM information obtained by (–)ESI, (+)ESI, and (+)APPI differed substantially (Fig. 2c). In addition, the DOM components identified by (+)ESI and (+)APPI were grouped into one category, showing that the DOM composition obtained by the two positive modes was also significantly different. The results statistically confirmed that the three ionization modes had selective ionization characteristics for the DOM in refinery wastewater.

The chemical properties of the DOM in refinery wastewater identified by the three ionization modes were compared by calculating the weighted average value of descriptors based on the sum-normalized relative intensity (Fig. S8). The properties of DOM obtained by (–)ESI and (+)ESI were similar in terms of the H/C ratio, DBE, (DBE–O)/C ratio, and modified aromaticity index ( $\text{AI}_{\text{mod}}$ ); however, the H/C ratio of DOM obtained by (+)APPI was lower than that obtained by the ESI modes, and the DBE, (DBE–O)/C ratio, and  $\text{AI}_{\text{mod}}$  were higher than the values obtained by the ESI modes, implying that DOM with a greater unsaturation degree and a stronger aromaticity was preferentially ionized by (+)APPI. In terms of the heteroatom content (O/C, N/C, and S/C ratios), DOM rich in oxygen atoms was preferentially ionized by (–)ESI, DOM rich in nitrogen and sulfur atoms was preferentially ionized by (+)ESI, and DOM ionized by (+)APPI contained fewer heteroatoms than DOM ionized by the ESI. Considering the positive correlation between the nominal oxidation state of carbon (NOSC) and heteroatom content, the NOSC was found to be lowest in the DOM obtained by (+)APPI. In terms of  $m/z$ , the molecular weight of DOM obtained by (–)ESI was higher than that obtained by (+)ESI, while the molecular weight of DOM obtained by (+)APPI was the lowest of the three modes, indicating that there were significant differences in the molecular weight of DOM obtained by the three ionization sources.

In summary, the DOM composition of refinery wastewater obtained by (±)ESI and (+)APPI differed. By analyzing the chemical characteristics of the DOM in refinery wastewater obtained using different ionization modes, it was found that (–)ESI preferentially ionized DOM rich in oxygen and with a high molecular weight, (+)ESI preferentially ionized DOM rich in nitrogen and sulfur, and (+)APPI preferentially ionized DOM with a relatively low content of heteroatoms, and high degrees of unsaturation and aromaticity. The three ionization modes had a different ionization selectivity for DOM in refinery wastewater. Therefore, the utilization of the three ionization modes could comprehensively analyze the removal and transformation characteristics of the DOM produced in biological and AC adsorption treatment processes.



**Fig. 2.** Comparison of the DOM composition of refinery wastewater samples determined by different ionization modes: (a) heteroatom compound compositions of each sample; (b) PCA results for refinery wastewater samples; (c) HCA results based on the correlation method using all of the DOM obtained by (-)ESI, (+)ESI, and (+)APPI from five refinery wastewater samples (15 DOM pools in total).

### 3.2. Transformation characteristics of DOM in refinery wastewater

#### 3.2.1. Treatment performance of the biological and AC adsorption processes

Refinery wastewater was treated by biological treatment processes and an AC adsorption process. The biological treatment processes included an anaerobic biological (AnB) process, aerobic biological (AeB) process, and bio-aeration flotation (BAF) process. The organic matter concentration of refinery wastewater gradually decreased during treatment. After treatment, the TOC concentration decreased from 147.0 to 12.4 mg/L (Table S2), and the removal efficiency was 91.56%. The reduction of the organic matter content in refinery wastewater was highest in the aerobic biological process, with TOC reduced by 73.3 mg/L and a removal efficiency of 60.48% (Table S2), indicating that a large amount of organic matter in refinery wastewater was degraded due to aerobic processes.

#### 3.2.2. Changing patterns of different heteroatom classes

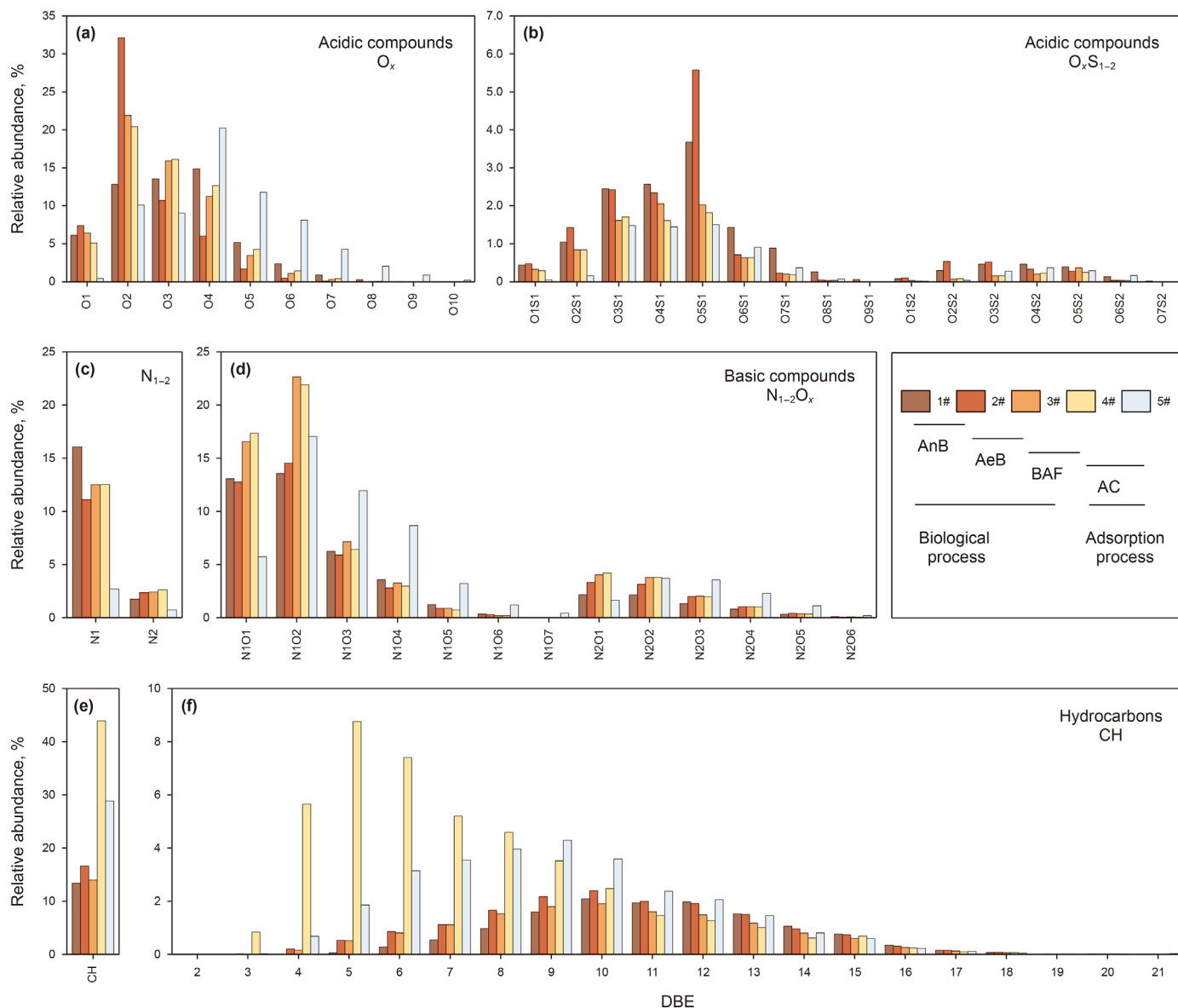
The results in section 3.1 indicate that (-)ESI could effectively ionize acidic CHO and CHOS compounds in refinery wastewater, while (+)ESI could effectively ionize basic CHN and CHON compounds, and (+)APPI could effectively ionize highly aromatic hydrocarbons. The changing patterns of these compounds during treatment with the biological and AC adsorption processes were investigated in this study. The variation in molecular changes were shown in Table S3 and Fig. S9. In addition, Fig. 3 shows the changes

in the relative abundance of acidic CHO (Fig. 3a) and CHOS compounds (Fig. 3b), basic CHN (Fig. 3c) and CHON (Fig. 3d) compounds distinguished by different heteroatom numbers (O, N, and S), and hydrocarbon compounds (Fig. 3e and f) distinguished by the DBE value during treatment with the biological and AC adsorption processes.

#### (1) Acidic compounds

During the wastewater treatment processes, as shown in Table S3, the relative content of CHO compounds increased from 56.11% to 67.17%, implying that the acidic CHO compounds were not easy to remove. The relative contents of CHOS and CHONS compounds decreased from 14.68% and 6.75% to 7.15% and 4.63%, respectively, during the treatment processes. This suggested that the acidic sulfur-containing compounds could be easily removed, especially in the aerobic bio-treatment process. The acidic  $O_x$  compounds in the refinery wastewater were mainly composed of  $O_2$  (12.81%),  $O_3$  (13.53%), and  $O_4$  (14.88%). The acidic  $O_xS_1$  compounds were mainly composed of  $O_3S_1$  (2.45%),  $O_4S_1$  (2.56%), and  $O_5S_1$  (3.67%). The acidic  $O_xS_2$  compounds were mainly composed of  $O_2S_2$  (0.30%),  $O_3S_2$  (0.46%), and  $O_4S_2$  (0.46%), among which the acidic  $O_x$  compounds had the highest relative abundance, followed by  $O_xS_1$  compounds and  $O_xS_2$  compounds.

During the AnB process, the relative abundance of the relatively high oxygen content  $O_x$  compounds ( $x \geq 3$ ) displayed a decreasing



**Fig. 3.** Relative abundance of different classes of heteroatom compounds in refinery wastewater: (a)  $O_x$  and (b)  $O_xS_{1-2}$  detected by (-)ESI; (c)  $N_{1-2}$  and (d)  $N_{1-2}O_x$  detected by (+)ESI; and (e) CH compounds, and (f) the DBE distribution detected by (+)APPI.

trend, especially the  $O_3$  and  $O_4$  compounds, which decreased from 13.53% and 14.88% to 10.71% and 6.00%, respectively. The relative abundance of relatively low oxygen content, i.e.,  $O_2$  compounds, increased significantly from 12.81% to 32.13%. Similarly, the relative abundance of  $O_xS_{1-2}$  ( $x \geq 3$ , except for  $x = 5$ ) compounds displayed a decreasing trend. For example, the relative abundance of  $O_6S_1$  compounds decreased from 1.43% to 0.71%, while the relative abundance of relatively low oxygen content  $O_xS_{1-2}$  ( $x \leq 2$ ) compounds increased from 1.04% to 1.43%. The anaerobic biological process could preferentially remove acidic  $O_x$  and  $O_xS_{1-2}$  compounds with a high number of oxygen atoms, produce compounds with a lower oxygen content, and reduce the oxygen atom content of acidic compounds in refinery wastewater.

During the AeB process, the relative abundance of relatively low oxygen content  $O_x$  compounds ( $x \leq 2$ ) decreased significantly, especially the  $O_2$  compounds, which decreased from 32.13% to 21.94%. The relative abundance of high oxygen content  $O_x$  compounds ( $x \geq 3$ ) increased; for example, the relative abundance of  $O_3$

and  $O_4$  compounds increased from 10.71% and 6.00% to 15.91% and 11.24%, respectively. Similarly, the relative abundance of  $O_xS_{1-2}$  compounds with both a high and low oxygen content decreased; for example, the relative abundance of  $O_5S_1$  compounds decreased from 5.58% to 2.03%. During the BAF process, the patterns of changes in the  $O_x$  and  $O_xS_{1-2}$  compounds were similar to those during the AeB process. The aerobic biological process could preferentially remove acidic compounds with a low oxygen content and produce acidic compounds with a high oxygen content.

During the AC adsorption process, the relative abundance of low oxygen content  $O_x$  compounds ( $x \leq 3$ ) displayed a decreasing trend; specifically, the relative abundance of  $O_2$  and  $O_3$  compounds decreased from 20.41% and 16.10% to 10.11% and 9.02%, respectively. The relative abundance of high oxygen content  $O_x$  compounds ( $x \geq 4$ ) increased significantly; for example, the relative abundance of  $O_4$  compounds increased from 12.67% to 20.23%. Similarly, the relative abundance of  $O_xS_{1-2}$  compounds with a relatively low oxygen content ( $x \leq 5$ ) decreased; for example, the relative

abundance of  $O_2S_1$  decreased from 0.84% to 0.16%. The relative abundance of  $O_xS_{1-2}$  compounds ( $x \geq 6$ ) increased significantly; for example, the relative abundance of  $O_6S_1$  increased from 0.63% to 0.91%. The AC adsorption process could preferentially remove relatively low oxygen content  $O_x$  and  $O_xS_{1-2}$  compounds, but there was little selective removal of high oxygen content compounds.

## (2) Basic compounds

The basic CHN compounds in the refinery wastewater were composed of  $N_1$  (16.06%) and  $N_2$  (1.74%), the  $N_1O_x$  compounds were mainly composed of  $N_1O_1$  (13.08%) and  $N_1O_2$  (13.57%), and the  $N_2O_x$  compounds were mainly composed of  $N_2O_1$  (2.16%),  $N_2O_2$  (2.15%), and  $N_2O_3$  (1.33%), with the relative abundance of  $N_1O_x$  compounds being higher than that of  $N_2O_x$  compounds.

During the AnB process, the relative abundance of  $N_1$  decreased from 16.06% to 11.10%, while the relative abundance of other basic nitrogen-containing compounds, such as  $N_2$  and  $N_{1-2}O_x$ , did not change significantly. The anaerobic process could preferentially remove  $N_1$  compounds, but its removal efficiency for other basic nitrogen-containing compounds was limited.

During the AeB process, the relative abundance of each basic nitrogen-containing compound ( $N_{1-2}$  and  $N_{1-2}O_x$ ) increased substantially; for example, the relative abundance of  $N_1O_2$  compounds increased from 14.54% to 22.66%. The relative abundance of some  $N_{1-2}O_x$  compounds decreased during the BAF process, although the relative abundance of  $N_{1-2}O_x$  compounds during the aerobic process was generally higher than that during the anaerobic process, indicating that the aerobic process could not remove most of the basic nitrogen-containing compounds in refinery wastewater.

During the AC adsorption process, the relative abundance of  $N_{1-2}$  and  $N_1O_x$  compounds with a low oxygen content ( $x \leq 2$ ) decreased substantially; for example, the relative abundance of  $N_1$  and  $N_1O_1$  compounds decreased from 12.52% and 17.34% to 2.70% and 5.71%, respectively. However, compounds with a high oxygen content increased ( $x \geq 3$ ); for example, the relative abundance of  $N_1O_3$  and  $N_2O_3$  increased from 6.41% and 1.97% to 11.96% and 3.55%, respectively, indicating that the AC adsorption process could preferentially remove  $N_{1-2}$  and low oxygen content  $N_{1-2}O_x$  compounds, while high oxygen content  $N_{1-2}O_x$  compounds could not be selectively removed.

## (3) Hydrocarbon compounds

The relative abundance of hydrocarbon compounds in the refinery wastewater was 13.40%, with their DBEs mainly distributed in the range of 9–14 (Fig. 3e and f). During the AnB process, the relative abundance of aromatic hydrocarbons with a relatively high DBE ( $\geq 12$ ) decreased slightly; for example, the relative abundance of hydrocarbons with a DBE of 12 decreasing from 1.97% to 1.91%. Despite this, the relative abundance of hydrocarbons overall increased from 13.40% to 16.60%. The anaerobic biological treatment process could not preferentially remove hydrocarbon compounds in refinery wastewater.

During the AeB process, the relative abundance of hydrocarbons with different DBEs decreased to different degrees. The relative abundance of hydrocarbons with DBEs of 8 and 11 decreased from 1.66% and 1.99% to 1.52% and 1.60%, respectively. Overall, the relative abundance of hydrocarbons decreased from 16.60% to 13.97%, indicating that the AeB process had a strong removal effect on aromatic hydrocarbons with different DBEs. During the BAF process, the relative abundance of hydrocarbons increased substantially from 13.97% to 43.91%. The relative abundance of hydrocarbons with a relatively low DBE ( $\leq 10$ ) increased significantly, particularly the relative abundance of hydrocarbons with a DBE of 5, which

increased from 0.51% to 8.76%. The relative abundance of hydrocarbons with a relatively high DBE ( $\geq 11$ ) decreased; for example, the relative abundance of hydrocarbons with a DBE of 11 decreased from 1.60% to 1.46%. These results indicated that hydrocarbons with a relatively low DBE were not preferentially removed by the BAF process. Although the AeB process was effective for the removal of hydrocarbons with different DBEs and the BAF process could remove hydrocarbons with relatively high DBEs, the overall relative abundance of hydrocarbons in refinery wastewater increased significantly during the aerobic process, especially hydrocarbons with DBEs lower than 10. It was therefore concluded that aerobic processes cannot preferentially remove hydrocarbons from refinery wastewater.

During the AC adsorption process, the relative abundance of hydrocarbons decreased substantially from 43.91% to 28.83%, with a particularly notable decrease in the relative abundance of hydrocarbons with a relatively low DBE ( $\leq 8$ ). The relative abundance of hydrocarbons with a DBE of 5 decreased from 8.76% to 1.86%, while the relative abundance of hydrocarbons with a DBE of 10 increased from 2.48% to 3.59%. The AC adsorption process could therefore preferentially remove hydrocarbons with a relatively low DBE.

By analyzing the changing patterns of acidic compounds (CHO and CHOS), basic nitrogen-containing compounds (CHN and CHON), and hydrocarbons (CH) in the biological and AC adsorption processes, it was found that the anaerobic process removed acidic CHO and CHOS compounds with a relatively high oxygen content and produced acidic compounds with a low oxygen content. The aerobic process removed the acidic CHO and CHOS compounds with a low oxygen content produced by the anaerobic process, while the whole biological process could not preferentially remove basic compounds (CHN and CHON) and hydrocarbons. The AC adsorption process further removed CHO, CHOS, and CHON compounds with a low oxygen content and hydrocarbons with a low DBE.

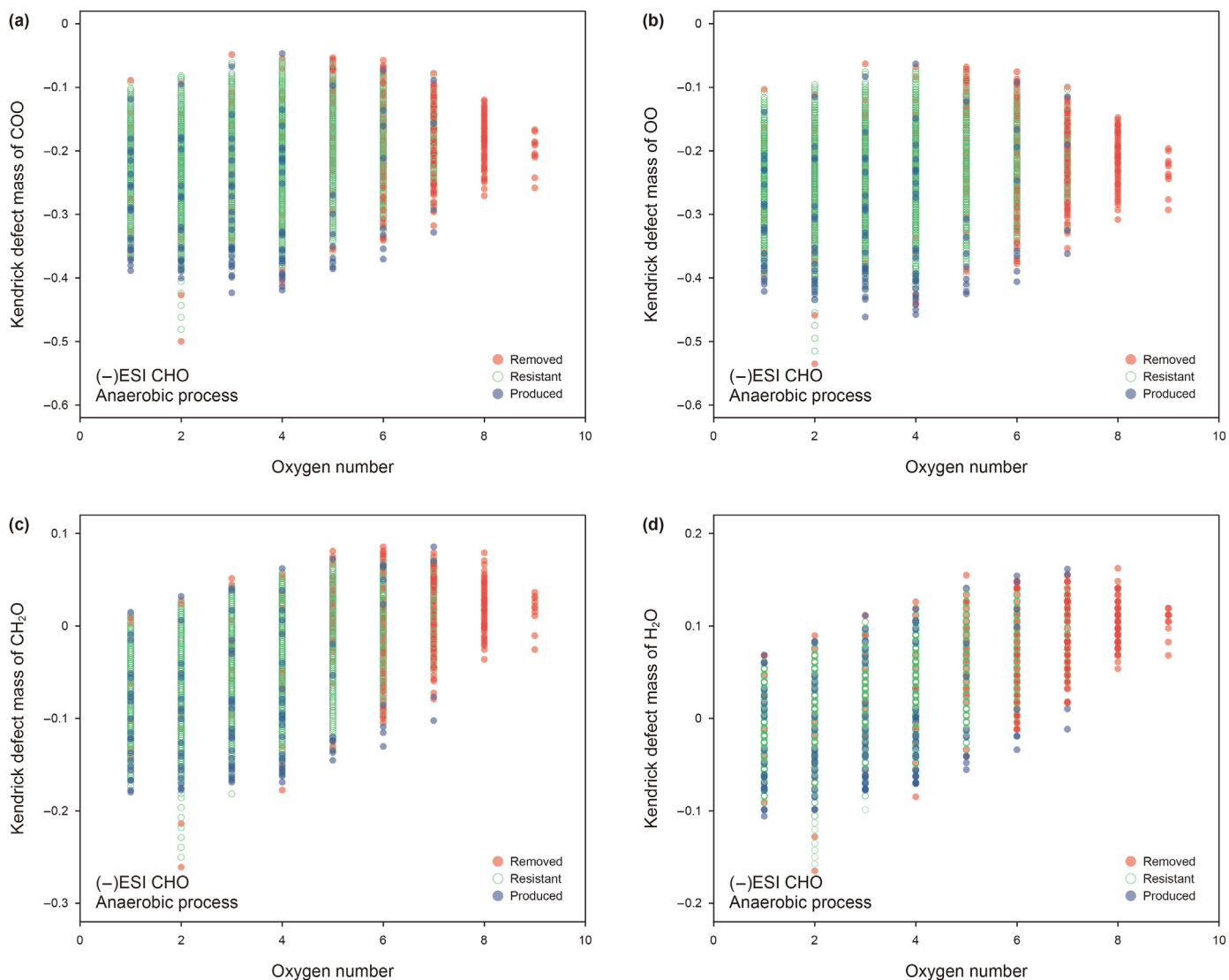
## 3.3. Transformation mechanisms of DOM in the combined treatment process

### 3.3.1. Biological processes

The biological processes include anaerobic and aerobic processes, and the removal effects of the two processes differed. The acidic compounds (CHO and CHOS) with a high oxygen content were removed by the anaerobic process, and low oxygen content compounds were preferentially removed by the aerobic process, which implies that oxygen-related bioreactions may occur in biological processes, including (de)carboxylation, (de)methoxylation (sugar chain prolongation/lessening), water addition/lessening, and (de)oxydation (Qi et al., 2016, 2022; Zhang et al., 2019). Therefore, COO, OO,  $H_2O$ , and  $CH_2O$  were selected as the repeating structural units for a KMD analysis of CHO and CHOS compounds. According to the definition of the KMD, DOM with the same KMD value belongs to the same class of homologs. The condensation is mainly related to KMD(CH<sub>2</sub>), and fewer carboxyl structures are mainly related to KMD(COO). For example, in the KMD(COO) diagram, DOM on the same horizontal line was homologous with the same repeated carboxyl structure, and the higher the number of oxygen atoms the more COO structures there were in the molecule.

#### (1) The anaerobic process

The changes in KMD(COO), KMD(OO), KMD( $CH_2O$ ), and KMD( $H_2O$ ) of the acid CHO compounds in refinery wastewater during the anaerobic process was shown in Fig. 4. At the same KMD



**Fig. 4.** The results of a KMD analysis of CHO acidic compounds in refinery wastewater following treatment by an anaerobic process based on the (a) COO; (b) OO; (c) CH<sub>2</sub>O; and (d) H<sub>2</sub>O functional groups.

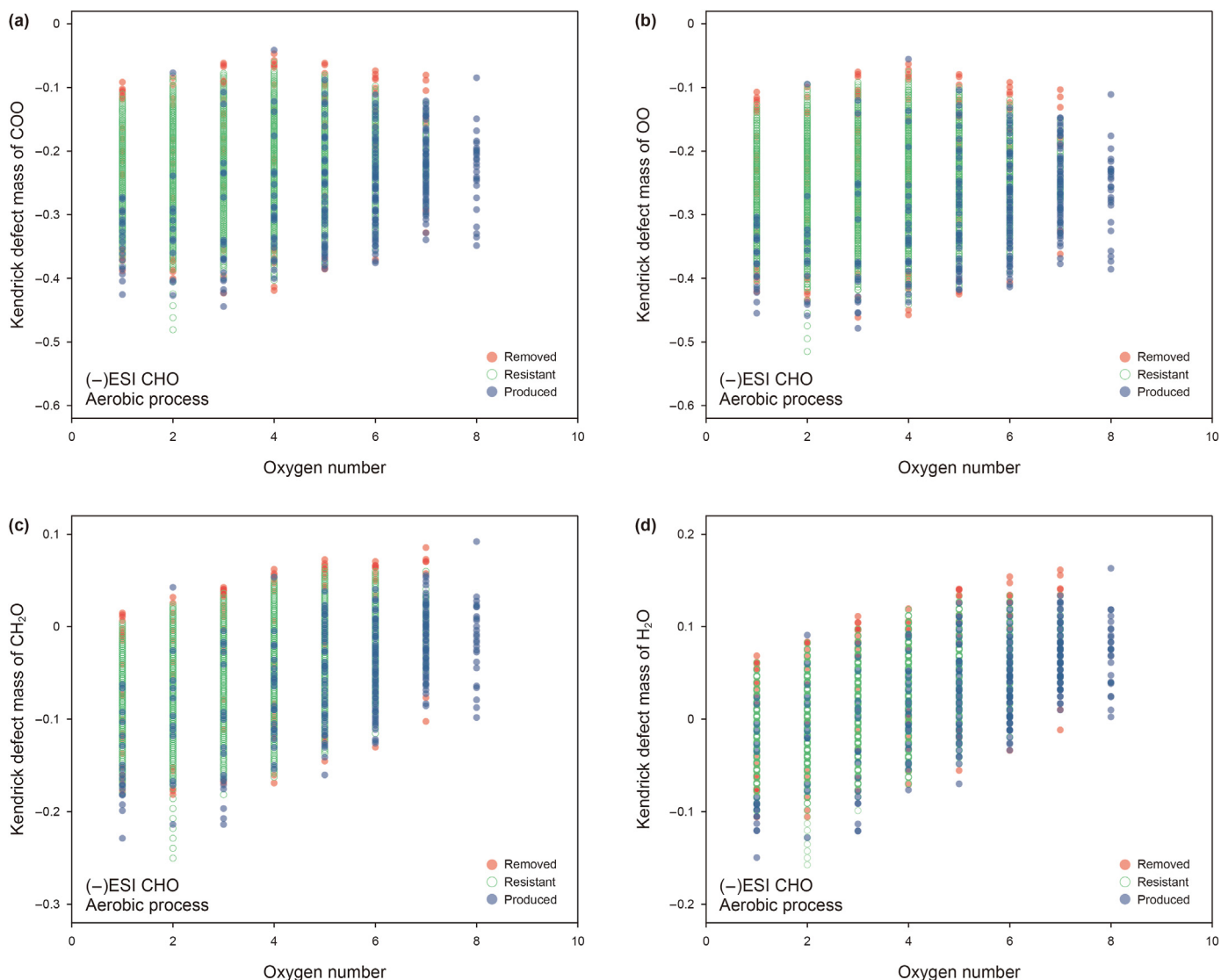
value, a large number of CHO acid compounds, with oxygen numbers higher than 6, were removed by the anaerobic process, resulting in CHO acid compounds with lower oxygen numbers ( $O < 6$ ) (Fig. 4). Likewise, on the same KMD diagram, CHOS acidic compounds with a high number of oxygen atoms were removed by the anaerobic process, resulting in CHOS acidic compounds with a lower oxygen content (Fig. S10). Therefore, in the anaerobic process, the CHO and CHOS acidic compounds with a high oxygen content were removed via decarboxylation, deoxydation, demethoxylation, and water lessening reactions and transformed into compounds with a lower oxygen content.

## (2) The aerobic process

The changes in the KMD of acidic CHO compounds in refinery wastewater during the aerobic process was shown in Fig. 5. The aerobic process removed the CHO acidic compounds with a low oxygen content and produced CHO acidic compounds with a high oxygen content (mainly concentrated in the part with oxygen numbers higher than 5). After the aerobic process, the low oxygen content CHOS compounds at the same KMD value were removed,

and high oxygen content compounds were produced (Fig. S11). The results indicated that CHO and CHOS acidic compounds mainly underwent carboxylation, oxidation, methoxylation, and water addition reactions during their transformation and removal.

The mass difference of the DOM in refinery wastewater following the anaerobic and aerobic processes was analyzed, and the four oxygen-related reactions referred to above were matched (Fig. S12). In the anaerobic process, the number of “deoxygenation” reactions (8,080) was much larger than the number of “oxygenation” reactions (6,512), and decarboxylation and deoxygenation were the main reactions that occurred during this process. During the aerobic process, the number of “oxygenation” reactions was higher (Table S4–S5), but the main reaction pathways of the acidic CHO and CHOS compounds were different. The carboxylation and oxygenation reactions occurred mainly in the CHO and CHOS<sub>1</sub> compounds, while CHOS<sub>2</sub> mainly underwent methoxylation and water addition reactions. Therefore, in the biological processes, the anaerobic process degraded and transformed DOM through various deoxygenation reactions and reduced the oxygen content in the molecules. In the aerobic process, DOM was degraded via various oxygenation reactions, increasing the intramolecular oxygen



**Fig. 5.** The results of a KMD analysis of CHO acidic compounds in refinery wastewater following treatment by an aerobic process based on the (a) COO; (b) OO; (c) CH<sub>2</sub>O; and (d) H<sub>2</sub>O functional groups.

content, while (de)carboxylation and (de)oxygenation were the main oxygen-containing reaction pathways in the two biological processes.

### 3.3.2. The AC adsorption process

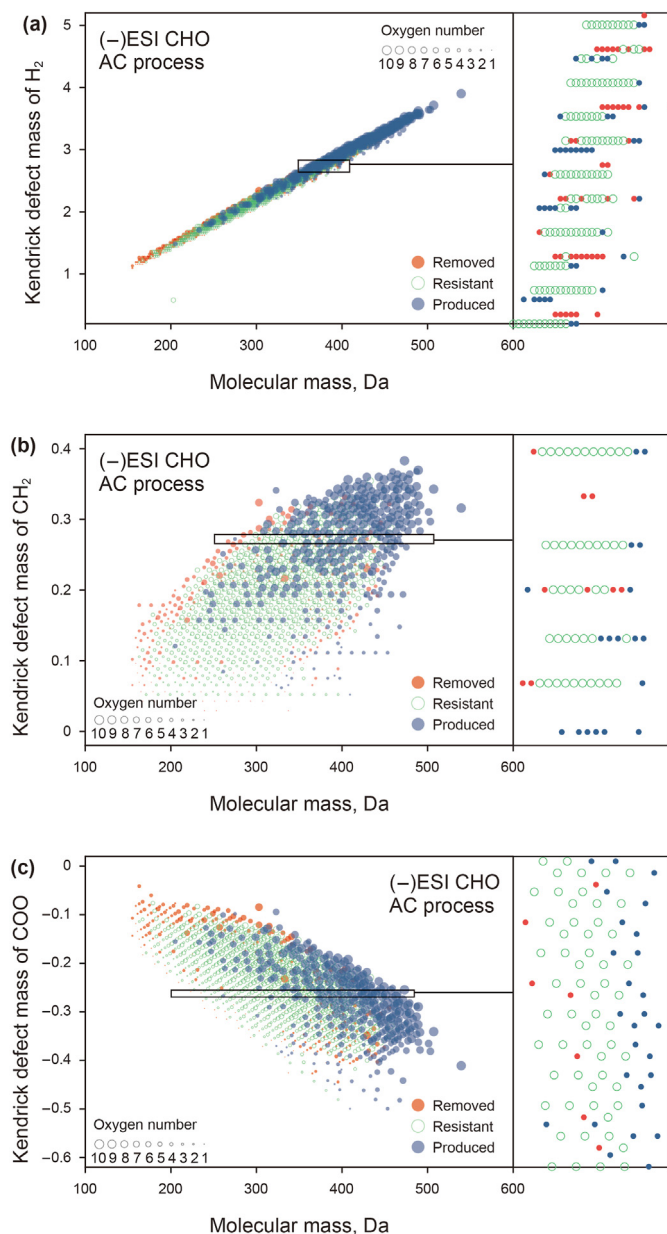
The AC adsorption process removed CHO, CHOS, and CHON compounds with a low oxygen content as well as some basic CHN compounds and hydrocarbons from the effluent of biological processes. To analyze the characteristics of the DOM that was removed, a KMD analysis was conducted based on the H<sub>2</sub>, CH<sub>2</sub>, and COO functional groups (Fig. 6 and S13–S16).

Fig. 6 shows the distributions of KMD(H<sub>2</sub>), KMD(CH<sub>2</sub>), and KMD(COO) for the acidic CHO compounds that were removed and produced. After the AC adsorption process, the acidic CHO compounds were mainly distributed on the left side of the KMD(H<sub>2</sub>) diagram, although some of the acidic CHO compounds that could not be removed and produced were distributed on the right side of the KMD(H<sub>2</sub>) diagram, indicating that the AC adsorption process preferentially removed saturated CHO acidic compounds. Likewise, Fig. 6b and c shows that the CHO acidic compounds removed by the

AC adsorption process had shorter side chain structures and fewer carboxyl group structures than those removed by the other processes.

For the other heteroatom compounds, the acidic CHOS compounds that can be removed by the AC adsorption process did not show any obvious pattern in terms of KMD(H<sub>2</sub>) and KMD(CH<sub>2</sub>), but in the KMD(COO) diagram, the acidic CHOS compounds that were removed were mainly distributed in the part with the lowest molecular weight, indicating that the acidic CHOS compounds that were removed had few carboxyl structures (Fig. S13). There was no obvious regularity in the selected repetitive structure for basic CHN compounds (Fig. S14). Basic CHON compounds that could be removed by the AC adsorption process were mainly distributed on the right side of KMD(H<sub>2</sub>), KMD(CH<sub>2</sub>), and KMD(COO), indicating that CHON basic compounds with a low degree of condensation, fewer side chains, and fewer carboxyl structures were easily removed by the AC adsorption process (Fig. S15). Similarly, the CH compounds that could be removed by the AC adsorption process were mainly distributed on the right side of the KMD(H<sub>2</sub>) and KMD(CH<sub>2</sub>) diagrams (Fig. S16). This indicated that hydrocarbons





**Fig. 6.** The results of a KMD analysis of CHO acidic compounds in refinery wastewater following treatment by an AC process based on the functional groups of (a)  $H_2$ ; (b)  $CH_2$ ; and (c)  $COO$ .

with a low degree of condensation were more easily removed by the AC adsorption process.

#### 4. Conclusions

In this study, ( $\pm$ )ESI/(+)APPI coupled with FT-ICR MS was used to investigate the molecular composition and transformation characteristics of DOM in refinery wastewater during a combined treatment method using biological and AC adsorption processes. The use of three different ionization modes resulted in significant differences in the molecular composition of the DOM detected in the refinery wastewater. The (–)ESI effectively ionized acidic CHO and CHOS compounds, (+)ESI effectively ionized basic CHN and CHON compounds, and (+)APPI effectively ionized hydrocarbons. The results showed that the removal characteristics and

mechanisms of the different heteroatom compounds in the combined biological and AC adsorption process were different. The whole biological process could remove acidic CHO and CHOS compounds, but had a limited removal efficiency for basic CHN, CHON, and CH compounds. During the anaerobic process, CHO and CHOS acidic compounds with relatively high oxygen contents were removed mainly via decarboxylation and deoxygenation reactions, while in the aerobic process, acidic CHO and CHOS compounds with relatively low oxygen contents were removed mainly via carboxylation and oxygenation reactions. Although the removal characteristics of the different compounds varied in the AC adsorption process, overall, it could effectively remove the different types of compounds in refinery wastewater.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

This work was supported by the National Key Research and Development Program of China (2018YFA0605800 and 2020YFA0607600), the National Natural Science Foundation of China (42003059), and Science Foundation of China University of Petroleum, Beijing (No. 2462021XKBH005).

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.petsci.2022.09.035>.

#### References

- Ajaero, C., Peru, K.M., Hughes, S.A., et al., 2019. Atmospheric pressure photoionization fourier transform ion cyclotron resonance mass spectrometry characterization of oil sand process-affected water in constructed wetland treatment. *Energy Fuel* 33 (5), 4420–4431. <https://doi.org/10.1021/acs.energyfuels.9b00469>.
- Barrow, M.P., Peru, K.M., McMartin, D.W., et al., 2016. Effects of extraction pH on the fourier transform ion cyclotron resonance mass spectrometry profiles of athabasca oil sands process water. *Energy Fuel* 30 (5), 3615–3621. <https://doi.org/10.1021/acs.energyfuels.5b02086>.
- Boczkaj, G., Makos, P., Przyjazny, A., 2016. Application of dynamic headspace and gas chromatography coupled to mass spectrometry (DHS-GC-MS) for the determination of oxygenated volatile organic compounds in refinery effluents. *Anal. Methods* 8 (17), 3570–3577. <https://doi.org/10.1039/c5ay03043a>.
- Chen, C., Yoza, B.A., Chen, H., et al., 2015. Manganese sand ore is an economical and effective catalyst for ozonation of organic contaminants in petrochemical wastewater. *Water, Air, Soil Pollut.* 226 (6), 182. <https://doi.org/10.1007/s11270-015-2446-y>.
- Dittmar, T., Koch, B., Hertkorn, N., et al., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol Oceanogr. Methods* 6 (6), 230–235. <https://doi.org/10.4319/lom.2008.6.230>.
- Diya'uddeen, B.H., Daud, W.M.A.W., Abdul Aziz, A.R., 2011. Treatment technologies for petroleum refinery effluents: a review. *Process Saf. Environ. Protect.* 89 (2), 95–105. <https://doi.org/10.1016/j.psep.2010.11.003>.
- Fang, Z., He, C., Li, Y., et al., 2017. Fractionation and characterization of dissolved organic matter (DOM) in refinery wastewater by revised phase retention and ion-exchange adsorption solid phase extraction followed by ESI FT-ICR MS. *Talanta* 162 (1), 466–473. <https://doi.org/10.1016/j.talanta.2016.10.064>.
- Fang, Z., Huang, R., How, Z.T.T., et al., 2020. Molecular transformation of dissolved organic matter in process water from oil and gas operation during UV/H<sub>2</sub>O<sub>2</sub>, UV/chlorine, and UV/persulfate processes. *Sci. Total Environ.* 730, 139072. <https://doi.org/10.1016/j.scitotenv.2020.139072>.
- He, C., Fang, Z., Li, Y., et al., 2021. Ionization selectivity of electrospray and atmospheric pressure photoionization FT-ICR MS for petroleum refinery wastewater dissolved organic matter. *Environ. Sci. J. Integr. Environ. Res.: Process. Impacts* 23 (10), 1466–1475. <https://doi.org/10.1039/d1em00248a>.
- He, C., Zhang, Y., Li, Y., et al., 2020a. In-house standard method for molecular characterization of dissolved organic matter by FT-ICR mass spectrometry. *ACS Omega* 5 (20), 11730–11736. <https://doi.org/10.1021/acsomega.0c01055>.

- He, C., Zhong, H., Zhang, Y., et al., 2020b. Organic matter in delayed coking wastewater: molecular composition and its effect on emulsification. *Fuel* 279, 118432. <https://doi.org/10.1016/j.fuel.2020.118432>.
- Headley, J.V., Kumar, P., Dalai, A., et al., 2015. Fourier transform ion cyclotron resonance mass spectrometry characterization of treated athabasca oil sands processed waters. *Energy Fuel*. 29 (5), 2768–2773. <https://doi.org/10.1021/ef502007b>.
- Headley, J.V., Peru, K.M., Barrow, M.P., 2009. Mass spectrometric characterization of naphthenic acids in environmental samples: a review. *Mass Spectrom. Rev.* 28 (1), 121–134. <https://doi.org/10.1002/mas.20185>.
- Jafarinejad, S., Jiang, S.C., 2019. Current technologies and future directions for treating petroleum refineries and petrochemical plants (PRPP) wastewaters. *J. Environ. Chem. Eng.* 7 (5), 103326. <https://doi.org/10.1016/j.jece.2019.103326>.
- Li, Y., Fang, Z., He, C., et al., 2015a. Molecular characterization and transformation of dissolved organic matter in refinery wastewater from water treatment processes: characterization by fourier transform ion cyclotron resonance mass spectrometry. *Energy Fuel*. 29 (11), 6956–6963. <https://doi.org/10.1021/acs.energyfuels.5b01446>.
- Li, Y., He, C., Li, Z., et al., 2020. Molecular transformation of dissolved organic matter in refinery wastewater. *Water Sci. Technol.* 82 (1), 107–119. <https://doi.org/10.2166/wst.2020.334>.
- Li, Y., Wu, B., He, C., et al., 2021. Comprehensive chemical characterization of dissolved organic matter in typical point-source refinery wastewaters. *Chemosphere* 286 (Pt 1), 131617. <https://doi.org/10.1016/j.chemosphere.2021.131617>.
- Li, Y., Xu, C., Chung, K.H., et al., 2015b. Molecular characterization of dissolved organic matter and its subfractions in refinery process water by Fourier transform ion cyclotron resonance mass spectrometry. *Energy Fuel*. 29 (5), 2923–2930. <https://doi.org/10.1021/acs.energyfuels.5b00333>.
- Maizel, A.C., Remucal, C.K., 2017. The effect of advanced secondary municipal wastewater treatment on the molecular composition of dissolved organic matter. *Water Res.* 122, 42–52. <https://doi.org/10.1016/j.watres.2017.05.055>.
- Morandi, G.D., Wiseman, S.B., Pereira, A., et al., 2015. Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. *Environ. Sci. Technol.* 49 (20), 12395–12404. <https://doi.org/10.1021/acs.est.5b02586>.
- Qi, Y., Fu, P., Volmer, D.A., 2022. Analysis of natural organic matter via fourier transform ion cyclotron resonance mass spectrometry: an overview of recent non-petroleum applications. *Mass Spectrom.* 41 (5), 647–661. <https://doi.org/10.1002/mas.21634>.
- Qi, Y., Hempelmann, R., Volmer, D.A., 2016. Two-dimensional mass defect matrix plots for mapping genealogical links in mixtures of lignin depolymerisation products. *Anal. Bioanal. Chem.* 408 (18), 4835–4843. <https://doi.org/10.1007/s00216-016-9598-5>.
- Ren, L., Wu, J., Qian, Q., et al., 2019. Separation and characterization of sulfoxides in crude oils. *Energy Fuel*. 33 (2), 796–804. <https://doi.org/10.1021/acs.energyfuels.8b03494>.
- Samanipour, S., Hooshyari, M., Baz-Lomba, J.A., et al., 2019. The effect of extraction methodology on the recovery and distribution of naphthenic acids of oilfield produced water. *Sci. Total Environ.* 652, 1416–1423. <https://doi.org/10.1016/j.scitotenv.2018.10.264>.
- Shi, Q., Hou, D., Chung, K.H., et al., 2010. Characterization of heteroatom compounds in a crude oil and its saturates, aromatics, resins, and asphaltenes (SARA) and non-basic nitrogen fractions analyzed by negative-ion electrospray ionization fourier transform ion cyclotron resonance mass spectrometry. *Energy Fuel*. 24 (4), 2545–2553. <https://doi.org/10.1021/ef901564e>.
- Tong, K., Lin, A., Ji, G., et al., 2016. The effects of adsorbing organic pollutants from super heavy oil wastewater by lignite activated coke. *J. Hazard Mater.* 308, 113–119. <https://doi.org/10.1016/j.jhazmat.2016.01.014>.
- Wake, H., 2005. Oil refineries: a review of their ecological impacts on the aquatic environment. *Estuar. Coast Shelf Sci.* 62 (1), 131–140. <https://doi.org/10.1016/j.ecss.2004.08.013>.
- Wang, B., Cui, H., Liu, H., et al., 2019. Derivatization for nontargeted screening of acids in oilfield refinery wastewater: identification and behaviors of recalcitrant chlorinated naphthenic acids. *Environ. Sci. Technol.* 53 (2), 1022–1030. <https://doi.org/10.1021/acs.est.8b05310>.
- Wang, B., Wan, Y., Gao, Y., et al., 2015. Occurrences and behaviors of naphthenic acids in a petroleum refinery wastewater treatment plant. *Environ. Sci. Technol.* 49 (9), 5796–5804. <https://doi.org/10.1021/es505809g>.
- Wang, M., Chen, Y., 2018. Generation and characterization of DOM in wastewater treatment processes. *Chemosphere* 201, 96–109. <https://doi.org/10.1016/j.chemosphere.2018.02.124>.
- Yan, L., Wang, Y., Li, J., et al., 2014. Comparative study of different electrochemical methods for petroleum refinery wastewater treatment. *Desalination* 341 (1), 87–93. <https://doi.org/10.1016/j.desal.2014.02.037>.
- Ye, H., Chen, L., Kou, Y., et al., 2021. Influences of coagulation pretreatment on the characteristics of crude oil electric desalting wastewaters. *Chemosphere* 264, 128531. <https://doi.org/10.1016/j.chemosphere.2020.128531>.
- Ye, H., Liu, B., Wang, Q., et al., 2020. Comprehensive chemical analysis and characterization of heavy oil electric desalting wastewaters in petroleum refineries. *Sci. Total Environ.* 724, 138117. <https://doi.org/10.1016/j.scitotenv.2020.138117>.
- Zhang, B., Shan, C., Hao, Z., et al., 2019. Transformation of dissolved organic matter during full-scale treatment of integrated chemical wastewater: molecular composition correlated with spectral indexes and acute toxicity. *Water Res.* 157, 472–482. <https://doi.org/10.1016/j.watres.2019.04.002>.