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Composition Characterization and Transformation Mechanism of Dissolved Organic Matters in a Full-Scale Membrane Bioreactor Treating Co-Digestion Wastewater of Food Waste and Sewage Sludge

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Abstract: The membrane bioreactor (MBR) serves as the most widely used technology in anaerobic digestion wastewater treatment, but the composition and transformation of the dissolved organic matters (DOMs) are vague. This study focused on the composition characterization and transformation mechanism of DOMs in real co-digestion wastewater of food waste and sewage sludge from a full-scale MBR via molecular weight cut-off, 3D-EEM, FT-IR, and SPME-GC/MS. The results indicated that the co-digestion wastewater mainly comprised organics with molecular weight (MW) lower than 1 kDa and dominated by tryptophane-protein-like substances. The hydrolytic/acidogenic process improved the biodegradability with the conversion of high-MW organics into low-MW organics, while the two-stage A/O process possessed the highest contribution to the organic removal with the consumption of most DOMs. However, the deficient removal of refractory organics (MW < 5 kDa) in the ultrafiltration unit led to the residual DOMs in the effluent. The potential functional bacteria in the biological processes have also been identified and were principally affiliated with *Proteobacteria* and *Firmicutes*. These findings could help to advance the understanding of the co-digestion wastewater and provide fundamental information for the optimization and development of MBR in anaerobic digestion wastewater treatment.

Keywords: anaerobic digestion; food waste; sewage sludge; wastewater characterization; microbial community

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1. Introduction

The ever-increasing biowastes from food processing industries and water treatment facilities could lead to a host of environmental and health problems [1]. Conventional management of biowastes including transportation and disposal could help to solve part of the problems but suffers from high cost and secondary pollution [2]. Hence, the anaerobic process has been proposed to be a promising alternative to conventional biowaste management from both energy conservation and environmental protection perspectives [3]. In addition, the biogas production and treatment capacity for high-strength organics endow the anaerobic process with appreciable economic advantages [4]. However, an anaerobic process such as the co-digestion of food waste and sewage sludge would inevitably produce anaerobic digestion wastewater which requires further treatment for the sustainability of the biowaste management.

The co-digestion wastewater of food waste and sewage sludge always contains high-strength dissolved organic matters (DOMs) with complex composition and low biodegradability, making it hard to deal with [1,2]. Therefore, the composition characterization of the

DOMs in real anaerobic digestion wastewater is indispensable for biokinetic design and treatment optimization. However, there remains a large knowledge gap in the specific characteristics of the real anaerobic digestion wastewater from full-scale food waste treatment plants, especially the complex DOMs, which has hindered the development of the anaerobic digestion wastewater treatment [2–4]. Several analytical methods, including molecular weight cut-off, spectroscopic techniques (e.g., three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy, Fourier transform infrared spectroscopy (FT-IR)), and solid-phase microextraction coupled with gas chromatography–mass spectrometry (SPME-GC/MS), are commonly employed in the qualitative and/or quantitative determination and structure identification of complex DOMs [5–7], but they have rarely been applied in the systematic investigation of the co-digestion wastewater of food waste and sewage sludge. The composition characterization of DOMs in typical anaerobic co-digestion wastewater with the abovementioned approaches could advance the understanding of co-digestion wastewater and provide a fundamental reference for the investigation of the treatment technologies.

A series of advanced biochemical and ecological approaches have already been employed in the anaerobic digestion wastewater treatment in the past decades, including the constructed wetland [8], oxidation ditch [9], up-flow anaerobic sludge blanket [10], and membrane bioreactor (MBR) [11] approaches. Among these technologies, MBR has been most commonly used in practical engineering due to its high sludge conservation and convenient phase separation despite the potential membrane fouling [12]. Nevertheless, recent research has principally focused on the alleviation of membrane fouling [13] and the modification of MBR via the combination with other biochemical technologies [14,15], whereas the specific transformation mechanism of DOMs in the typical MBR has been neglected, which is essential for the optimization of the extensive in-service anaerobic digestion wastewater treatment facilities equipped with a typical MBR.

In this study, molecular weight cut-off, 3D-EEM, FT-IR, and SPME-GC/MS were applied to investigate the composition characteristics and transformation mechanism of DOMs in a full-scale MBR treating real co-digestion wastewater of food waste and sewage sludge, and the corresponding potential functional microbial communities in biological processes have also been identified. The results of this study could shed new light on co-digestion wastewater treatment and facilitate the sustainability of biowaste management.

2. Materials and Methods

2.1. Sample Collection

The wastewater samples were collected from a full-scale MBR (300 m^3 d^{-1}) in an individual anaerobic digestion wastewater treatment plant (Chongqing, China) treating co-digestion wastewater of food waste and sewage sludge, and the treatment process flow diagram and the specific sampling points are illustrated in Figure 1. The treatment process integrated pretreatment (coagulation and air floatation), hydrolytic/acidogenic process, two-stage A/O process, and ultrafiltration unit. Meanwhile, there were four sampling points, termed sampling points A, B, C, and D. The samples were collected during the steady operation state, and each sample was a mixture of 10 L wastewater collected from the completely mixed reactors. The samples collected from sampling point A were used to characterize the composition of the complex DOMs in the co-digestion wastewater, while the other samples were collected to investigate the transformation mechanism of the DOMs along the treatment processes.

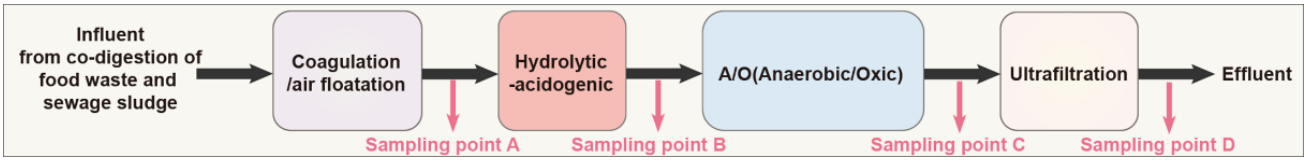

Figure 1. Process flow diagram and sampling points of the full-scale anaerobic digestion wastewater treatment facility.

2.2. Qualitative and Semi-Quantitative Analysis of DOMs

2.2.1. Conventional Water Quality Indices

The specific ultraviolet (UV) absorbance at 254 nm (UV_{254}) was used to represent complex organic matters with unsaturated bonds (e.g., aromatic contents in humic substances) in the co-digestion wastewater and determined using a spectrophotometer (DR6000, Hach, Loveland, CO, USA) with a 1 cm quartz cuvette [16]. COD (chemical oxygen demand) was measured on a spectrophotometer (DRB200, Hach, Loveland, CO, USA) following digestion, while BOD_5 (biochemical oxygen demand in five days) and TOC (total organic carbon) were determined with a BOD meter (BOD Track II, Hach) and a TOC analyzer (ANATOC, Hach), respectively. The biodegradability was described as the ratio of BOD_5 to COD. Additionally, the chromaticity (Pt-Co) was analyzed according to the standard methods [17]. The conventional water quality indices were presented as mean ± standard deviation (n = 3).

2.2.2. Molecular Weight Cut-Off

To investigate the molecular weight distribution of DOMs in the co-digestion wastewater [18], the molecular weight cut-off was determined using a constant-pressure and dead-end ultrafiltration apparatus (SCM-300, China) equipped with specific plate ultrafiltration membranes (HM, China), and the employed approach can determine samples with molecular weight cut-offs of 100, 50, 30, 10, 5, 3, 1, and 0.5 kDa. The membranes were rinsed well and soaked in Milli-Q water for over 24 h before the analysis.

2.2.3. 3D-EEM

To characterize the main components of DOMs in the co-digestion wastewater, 3D-EEM was performed using a fluorescence spectrometer (F-7000, Hitachi, Tokyo, Japan) with a xenon lamp as the excitation light source. The excitation (Ex) and emission (Em) wavelengths ranged from 200 to 550 nm and from 250 to 600 nm, respectively, and the scanning speed, photomultiplier voltage, and response time were set to be 12,000 nm min^{-1}, 700 V, and automatic, respectively. The inner-filter effect was avoided by dilutions of the samples, and multiple dilutions were taken into account in the calculation of the component fluorescence intensity.

The fluorescence spectra have been resolved into seven regions (regions I–VII) according to the literature of [19]: region I (tyrosine-protein-like): Ex/Em = 200–260 nm/200–330 nm; region II (tryptophane-protein-like): Ex/Em = 200–260 nm/330–400 nm; region III (protein-like: tyrosine, tryptophan, and soluble microbial products): Ex/Em = 260–310 nm/290–400 nm; region IV (fulvic acid-like): Ex/Em = 200–260 nm/400–500 nm; region V (glycosylated protein-like): Ex/Em = 260–310 nm/400–550 nm; region VI (black sperm/lignocellulose-like): Ex/Em = 310–380 nm/330–600 nm; and region VII (humic acid-like): Ex/Em = 380–580 nm/400–600 nm. The fluorescence regional integration (FRI) was conducted to quantify the corresponding proportions of the abovementioned seven regions, and detailed methods have been described in previous research [20]. In addition, parallel factor analysis (PARAFAC) was also applied to identify the transformation mechanism of DOMs along the treatment processes and conducted using Matlab R2020a (Mathworks, Natick, MA, USA) with the DOMFluor toolbox [21].

2.2.4. FT-IR Spectroscopy

FT-IR was applied to explore the specific functional groups of DOMs in the co-digestion wastewater [22]. Homogeneous samples were freeze-dried with a vacuum freeze drier (Scientz-10N, Beijing Zhongyi Company, Beijing, China) before the FT-IR analysis. Subsequently, approximately 1 mg of each sample was thoroughly mixed with 100 mg of pre-dried KBr and pressed in a mold. The FT-IR analysis was conducted with a Fourier transform infrared spectroscopy (Nicolet iS5, Thermofisher, Waltham, MA, USA), and the blank was corrected using a clean KBr pellet with the wavelength range set to be 450–4500 cm^{-1}.

2.2.5. SPME-GC/MS

SPME-GC/MS was firstly used in the composition characterization of DOMs in the co-digestion wastewater of food waste and sewage sludge for further quantitative analysis. Solid-phase microextraction (SPME) was used as the pretreatment of the samples and performed as follows: 20 mL liquid samples were placed in a headspace vial with an effective volume of 40 mL for SPME, and the adsorption temperature, adsorption time, stirring rate, desorption temperature, and desorption time were set to be 80 °C, 40 min, 500 rpm, 250 °C, and 3 min, respectively. The samples were subsequently used for GC/MS analysis via a gas chromatography–mass spectrometry system (QP2010 ultra, Shimadzu, Kyoto, Japan) equipped with a 30 m × 0.25 mm ×25 µm chromatographic column (DB-5MS UI, Agilent, J&W Scientific, Folsom, CA, USA) to investigate the categories of the DOMs. The initial temperature of the column was set to be 40 °C for 5 min, followed by a ramp of 10 °C min^{-1} to 300 °C and kept for 2 min. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The temperatures of the ion source and transfer line were 220 and 280 °C, respectively, and the scan runs were performed with a range from m/z 45 to 450. The chromatograms were analyzed using the NIST05 library (National Institute of Standards and Technology, Gaithersburg, MD, USA, http://www.nist.gov/srd/mslist.htm, accessed on 5 March 2022), and a compound was deemed identified with a match percentage higher than 60%.

2.3. Microbial Community Analysis

The sludge samples were collected from the hydrolytic/acidogenic tank as well as the A/O process, and each sample was a mixture of six samples collected from the completely mixed reactors. The DNA was extracted with the Soil DNA Kit (GenElute, Sigma, Burlington, MA, USA) following the manufacturer's instruction, and cryopreserved at −80 °C until being sent to the Illumina MiSeq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China) for further molecular biological analysis., The detailed methods for subsequent purification, sequencing, and other analyses have been described in previous research [23]. Specifically, the amplification of the bacterial V3-V4 region in the 16S rRNA gene was performed with the universal primers 338F (ACTCCTACGG GAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), and the raw 16S rRNA reads were demultiplexed, quality-filtered by fastp (v0.20.0), and merged by FLASH (v1.2.7). Operational taxonomical units (OTUs) were then clustered using UPARSE (v7.1) with 97% similarity, and the taxonomy for each OTU was assigned using the RDP classifier against the Silva database (http://www.arb-silva.de, accessed on 5 May 2022).In addition, the downside sequencing data analyses were carried out on the platform of Majorbio (Majorbio cloud, https://cloud.majorbio.com/, accessed on 5 May 2022).

3. Results

3.1. Characterization of DOMs in the Co-Digestion Wastewater

The samples collected from the sampling point A were used for the composition characterization of DOMs in the co-digestion wastewater of sewage sludge and food waste. A high concentration of chemical oxidation demand (COD) of 9470 ± 630 mg L^{-1} was retained in the co-digestion wastewater after the coagulation and air floatation, and the

BOD_5 was 1929 ± 422 mg L^{-1}, suggesting a low biodegradability (BOD_5/COD) of around 0.2. The UV_{254} and chromaticity were also relatively high, being 6.94 and 1590 Pt-Co, respectively.

3.1.1. Molecular Weight Distribution of DOMs

The molecular weight (MW) distribution of DOMs in the co-digestion wastewater was determined through the molecular weight cut-off (Figure 2a). The DOMs with MW lower than 1 kDa were the main contributors to COD and TOC, accounting for 68.72% and 67.76%, respectively, suggesting the dominance of small organics in the co-digestion wastewater. However, the MW distributions of UV_{254} and chromaticity were more even compared with their counterparts of COD and TOC, and the MW of the contributor to UV_{254} and chromaticity was principally distributed in the range of <500 Da, 1000–500 Da, 3–1 kDa, 10–5 kDa, 50–30 kDa, 0.45–0.22 µm, showing the wider distribution of complex and unsaturated organics.

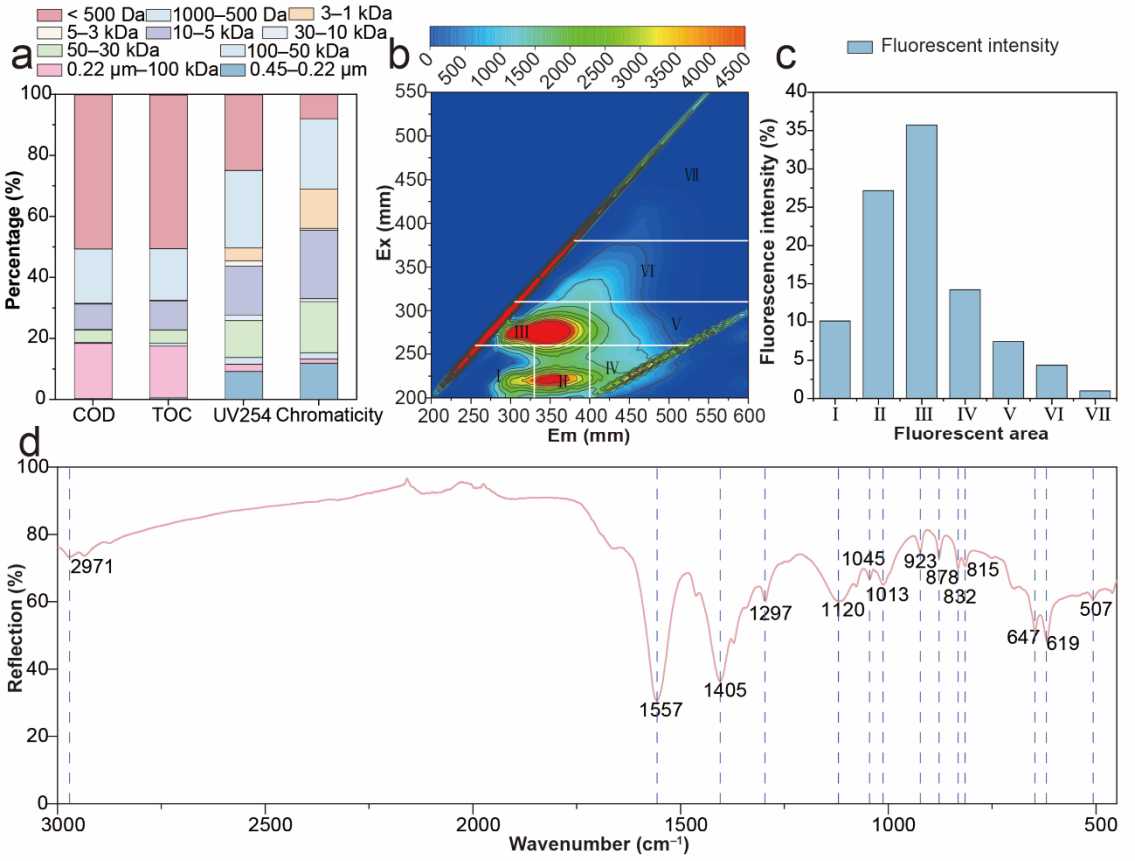

Figure 2. The characterization of DOMs in the co-digestion wastewater of sewage sludge and food waste: (**a**) molecular weight distribution; (**b**) 3D-EEM spectra; (**c**) the distribution of fluorescence regional integration; (**d**) FT-IR spectra.

3.1.2. Fluorescent Components of DOMs

Fluorescence characteristics of DOMs in the co-digestion wastewater of sewage sludge and food waste are illustrated in Figure 2b,c. The regions II–III possessed the highest proportion of the fluorescence density, 72.99%, indicating the dominance of tryptophane-protein-like substances and soluble microbial products in the DOMs [20]. Meanwhile, the proportions of regions I, IV, V, and VI were found to be 10.12, 14.20, 7.44, and 4.36%, respectively, suggesting that a considerable amount of tyrosine-protein-like, fluvic acid-like, glycosylated protein-like, and black sperm/lignocellulose-like substances existed in the DOMs. Moreover, the regions II–III were reported to represent substances with good

bioaccessibility and high biodegradability, while the regions IV–VI were reported to be the opposite, and the region I was demonstrated to represent substances with high biodegradability but poor bioaccessibility due to their hydrophobic phenol-based structure [19]. Therefore, the spectral results denoted that the DOMs in the co-digestion wastewater mainly comprised easily biodegradable tryptophane-protein-like substances, but the non-negligible existence of poorly biodegradable organics could inevitably increase the difficulty of the co-digestion wastewater treatment.

3.1.3. FT-IR Spectra of DOMs

FT-IR spectra in the region of 3000–450 cm^{-1} have been applied to provide primary information about the functional groups of DOMs in the co-digestion wastewater (Figure 2d). The FT-IR spectra exhibited 14 absorption peaks correlated with different functional groups [24,25]: 2971 cm^{-1} (aliphatic C–H stretching); 1557 cm^{-1} (N–H deformation of amide II); 1405 cm^{-1} (COO– stretching of carboxylic acids); 1297 cm^{-1} (C–O stretching of carboxylic acid and/or C–N stretching of aromatic primary and secondary amines, amide III); 1120 cm^{-1} (C–OH stretching of aliphatic OH, and/or S–O stretching of sulfonates); 1045 and 1013 cm^{-1} (C–O stretching of polysaccharide-like substances); 923 cm^{-1} (O–H out-of-plane bending); 878, 832, and 815 cm^{-1} (–NH_2 out-of-plane bending); 674 cm^{-1} (O–H out-of-plane bending of polysaccharide-like substances); 619 cm^{-1} (PO_4^{3-} bending); 507 cm^{-1} (P–Cl stretching).

3.1.4. SPME-GC/MS Results of DOMs

SPME-GC/MS was conducted for further quantitative investigation of the DOMs in the co-digestion wastewater, and there were 59 kinds of organics detected in the co-digestion wastewater (Table S1). The DOMs could be divided into two categories—cyclic organics and non-cyclic organics—according to different structures. The peak area of cyclic organics accounted for 85.01%, while its counterpart of non-cyclic organics only had a proportion of 14.99%. This result indicated that the cyclic organics were the dominant DOMs in the co-digestion wastewater. In addition, the cyclic organics mainly comprised sulfur ring-containing, monocyclic naphthene-containing, heterocyclic ring-containing, bridged hydrocarbon-containing, benzene ring-containing, and cyclene-containing organics, and their proportions were determined to be 1.37, 5.85, 10.05, 11.14, 24.24, and 32.36%, respectively. The cyclene-containing and benzene ring-containing organics were the dominant cyclic organics, whereas the non-cyclic organics principally consisted of sulfur-containing organics, aliphatics, ethers, aldehydes, alcohols, and ketones, and their contents accounted for 0.39, 0.54, 1.19, 1.91, 2.88, and 8.07%, respectively. The ketones dominated in the non-cyclic organics. Notably, the cyclic organics in the co-digestion wastewater also contained other functional groups such as carbon–carbon double bonds, carboxyls, carbonyls, oxhydryls, ether bonds and ester groups besides the cyclic structures. The organics with proportions higher than 2% included (-)-terpinen-4-ol (16.83%), α-terpineol (9.91%), 4-methylphenol (7.84%), eucalyptol (7.07%), 2-propylphenol (4.49%), indole (3.79%), [1S-(1a,3a,5a)]-4-methylene-1-(1-methylethyl)-bicyclo [3.1.0] hexan-3-ol (3.35%), naphthalene (3.31%), levulinic camphor (3.28%), 2-ethyl hexanol (2.69%), 3-methylindole (2.41%), isomenthone (2.35%), 2,2-dimethyl-5-(1-methyl-1-propenyl) tetrahydrofuran (2.15%), and 4-(2-methoxypropan-2-yl)-1-methylcyclohex-1-ene (2%), and these organics accounted for over 71.47% of the total DOMs in the co-digestion wastewater.

3.2. Transformation of DOMs in the Co-Digestion Wastewater

The samples collected from the sampling points B, C, and D were analyzed to investigate the transformation mechanism of DOMs in the co-digestion wastewater. The variation in COD, BOD_5, UV_{254}, and chromaticity along the treatment process is shown in Figure 3. The contributions of the hydrolytic/acidogenic tank, two-stage A/O process, and ultrafiltration units to COD removal were 17.95%, 62.37%, and 12.90%, respectively. However, the biodegradability of the co-digestion wastewater after the pretreat-

ment was only 0.2, making it recalcitrant for the later biological removal; thus, the hydrolytic/acidogenic process was indispensable due to its contribution to the elevation of BOD_5 from 1929 ± 422 mg L^{-1} to 3324 ± 764 mg L^{-1} and the improvement of biodegradability from 0.2 to 0.43. The UV_{254} and chromaticity increased by 15.85% and 28.93%, respectively, after the hydrolytic/acidogenic process, differing from the changes in COD. The effluent of the two-stage A/O process still contained a high concentration of COD (1864 ± 209 mg L^{-1}) with extremely low biodegradability (0.04), and the removal of these refractory organics was also limited in the ultrafiltration units, resulting in considerable organic residual in the effluent.

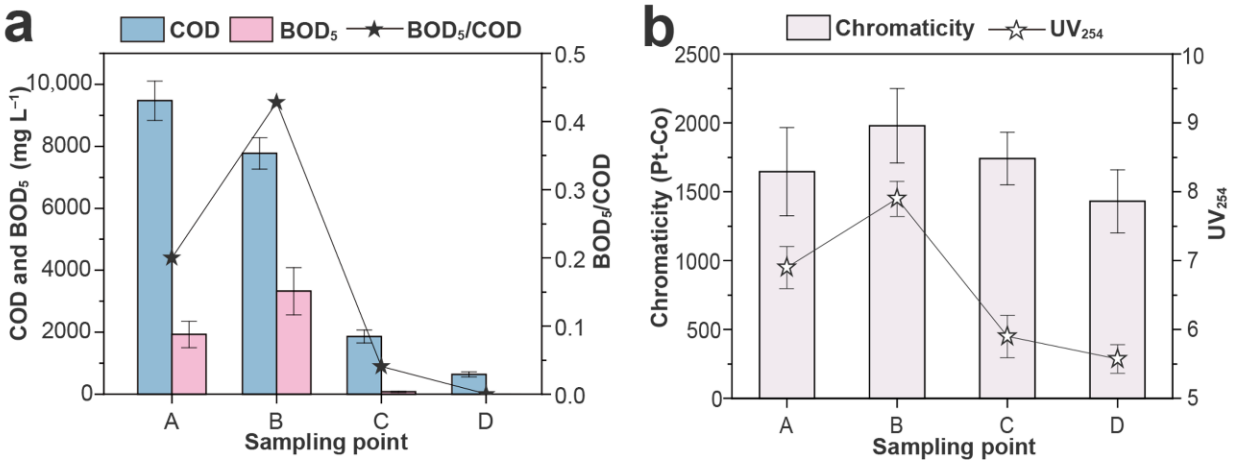

Figure 3. The variation in (**a**) COD, BOD_5, (**b**) UV_{254}, and chromaticity of the co-digestion wastewater along the treatment process.

3.2.1. Transformation of Molecular Weight Distribution

The transformation of molecular weight distribution of DOMs is exhibited in Figure 4a–d. The influent co-digestion wastewater mainly comprised organics with MW lower than 1 kDa from the perspectives of COD and TOC. However, the composition of DOMs has shifted to even smaller organics in the hydrolytic/acidogenic process, resulting in an obvious increase in organics with MW of 5–3 kDa, 3–1 kDa, and <500 Da. Subsequently, most of the small organics with MW lower than 1 kDa were consumed in the two-stage A/O process and there was a significant increase in organics with MW larger than 1 kDa, and the organics with MW lower than 5 kDa accounted for around 90% of the DOMs after the ultrafiltration units. Concerning the UV_{254} and chromaticity, the hydrolytic/acidogenic process principally degraded large organics with unsaturated bonds and/or colored functional groups into small ones, and the two-stage A/O process mainly reduced the UV_{254} and chromaticity through the removal of large organics with MW of 0.45–0.22 μm. Since small organics were preferred in the biological process, the chromaticity removal in the two-stage A/O process was limited, and the effluent of the ultrafiltration units still contained relatively high chromaticity due to its deficient removal of refractory organics with MW larger than 5 kDa.

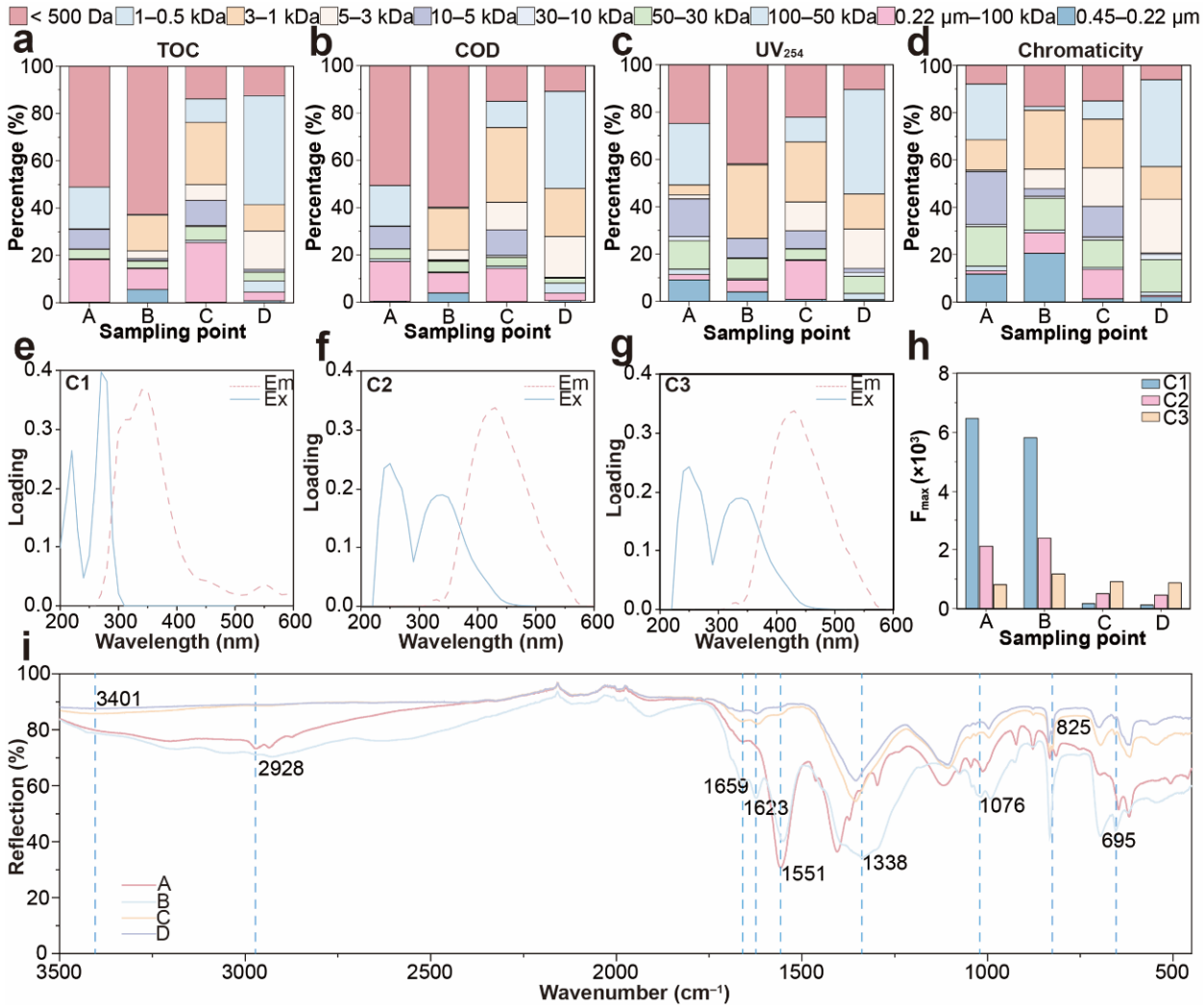

Figure 4. The transformation of DOMs in the co-digestion wastewater revealed by (**a**–**d**) molecular weight cut-off, (**e**–**h**) 3D-EEM, and (**i**) FT-IR.

3.2.2. Transformation of Fluorescent Components

The fluorescent spectra have been resolved into three effective fluorescent components according to PARAFAC (Figure 4d–f). The three fluorescent components were named C1, C2, and C3 with the maximum Ex/Em of 220(275)/340 nm, 225(290)/370 nm, and 250(340)/430 nm, respectively [26]. In addition, C1, C2, and C3 have been assigned to tryptophane-protein-like substances, small-molecular humic acid-like substances in the ultraviolet area A (UVA), and large-molecular aromatic humic acid-like substances in the ultraviolet area C (UVC), respectively [22]. The fluorescent results showed that the tryptophane-protein-like substances represented by C1 dominated in the influent co-digestion wastewater after pretreatment, in line with the results of the fluorescent regional integration (see Section 3.1.3). In addition, the Fmax(C1) only decreased by 10.13% after the hydrolytic/acidogenic tank, but the reduction in Fmax(C1) reached 96.97% in the two-stage A/O process. The removal of C2 reached 78.86% and 9.21% in the two-stage A/O process and ultrafiltration, respectively, consistent with the variation in UV_{254} and chromaticity. However, C3 could not be efficiently removed by both the A/O process and ultrafiltration despite its analogous changes with C2 in the hydrolytic/acidogenic process, suggesting its contribution to the refractory DOMs in the effluent.

3.2.3. Transformation of FT-IR Spectra

The transformation of the main functional groups in DOMs of the co-digestion wastewater is illustrated in Figure 4i. The absorption peaks with wavenumbers of 1405, 1297, 1120, 923, 878, and 619 cm^{-1} disappeared after the hydrolytic/acidogenic process, and these peaks corresponded to the COO– stretching of carboxylic acids; C–O stretching of carboxylic acid and/or C–N stretching of aromatic primary and secondary amines, amide III; C–OH stretching of aliphatic OH and/or S–O stretching of sulfonates; O–H out-of-plane bending; $–NH_2$ out-of-plane bending; and PO_4^{3-} bending, respectively. Several absorption peaks with wavenumbers of 1659, 1623, 1338, 1076, and 695 cm^{-1} were introduced in the spectra by the hydrolytic/acidogenic process, and these added peaks were correlated with stretching of amide I, stretching of COO–, O–H out-of-plane bending of polysaccharide-like substances, O–C–C stretching of aliphatic esters, and carboxylic acid dimer or amide, respectively [24,25].

In addition, significant changes in the FT-IR spectra were also observed after the two-stage A/O process. The adsorption peaks of 2928, 1659, 1551, and 1018 cm^{-1} disappeared after the A/O process, compared with the effluent of the hydrolytic/acidogenic tank. These peaks corresponded to aliphatic C–H stretching, stretching of amide I, N–H bending of amides, and C–O stretching of polysaccharide-like substances, respectively. In addition, newly added peaks were located at wavenumbers of 3401 and 825 cm^{-1} and were related to N–H stretching and $–NH_2$ out-of-plane bending, respectively.

3.2.4. Transformation of SPME-GC/MS Results

SPME-GC/MS was applied for further quantitative analysis of the transformation of DOMs in the co-digestion wastewater along the treatment process (Tables S1–S5). There were 59 kinds of organic compounds in both the influent and the effluent of the hydrolytic/acidogenic tank, and no obvious reduction in the total peak areas was observed, indicating that the species and concentrations of DOMs were not reduced by the hydrolytic/acidogenic process. The cyclic organics still dominated in the effluent of the hydrolytic/acidogenic tank with a high proportion of 83.24% and mainly comprised bridged hydrocarbon-containing, monocyclic hydrocarbon-containing, heterocyclic ring-containing, benzene ring-containing, and cyclene-containing organics with proportions of 9.42, 10.37, 11.01, 14.03, and 38.42%, respectively. Compared with the influent of the hydrolytic/acidogenic tank, no sulfur ring-containing organics were detected in the effluent. The monocyclic hydrocarbon-containing organics increased by 177%, while the benzene ring-containing organics decreased by 42%. In addition, no significant change was observed concerning the other cyclic organics and non-cyclic organics. These results indicated that the hydrolytic/acidogenic process could convert the sulfur ring-containing and benzene ring-containing organics into monocyclic hydrocarbon-containing organics. In addition, the shared organic components in the influent and effluent of the hydrolytic/acidogenic tank as well as their changes (%) are exhibited in Table S3. There were 31 kinds of shared organic components, among which 14 kinds of organics increased and 17 kinds of organics decreased after the hydrolytic/acidogenic process. The organic components increasing by over 20% included hexanal dimethyl acetal, 3-octanol, (1α,2α,5α)-2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hexan-2-ol, DL-menthol, α-pinoresinol, 2-methyl-5-(1-methylethenyl)cyclohexanone, 2-propylphenol, 6-ethyl-7-hydroxy-4-octen-3-one, 3-methylindole, and elemiol, while the organic components decreasing by over 10% included 2-heptanone, 6-methyl-2-heptanone, 1,4-eudesmol, eucalyptol, 2,2-dimethyl-5-(1-methyl-1-propenyl)tetrahydrofuran, 3-nonen-2-one, [1S-(1a,3a,5a)]-4-methylene-1-(1-methylethyl)-bicyclo [3.1.0] hexan-3-ol, levocamphor, and 7-methoxy-3,7-dimethyl-octanal. These results suggested that the hydrolytic/acidogenic process tended to transform the carbonyl-containing and heterocyclic-containing organics into hydroxyl-containing organics.

3.3. Insight into Microbial Community

3.3.1. Microbial Community in the Hydrolytic/Acidogenic Process

The coverage score of over 0.999 indicated an adequate sequencing depth for the description of the bacteria microbiome from the hydrolytic/acidogenic process, and the microbial community structures at the phylum level are illustrated in Figure 5a. There were 13 phyla with a relative abundance higher than 0.1%, including *Proteobacteria* (26.74%), *Firmicutes* (26.24%), *Chloroflexi* (12.75%), *Synergistetes* (12.71%), *Actinobacteria* (6.06%), *Bacteroidetes* (4.49%), *Coprothermobacteraeota* (3.97%), *Atribacteria* (3.8%), *Armatimonadetes* (1.03%), *unclassified_k_norank_d_Bacteria* (0.96%), *Deinococcus-Thermus* (0.41%), *Patescibacteria* (0.23%), and *WPS-2* (0.23%). Among these phyla, *Proteobacteria* and *Firmicutes* were the most dominant phyla, and both were reported to play a key role in the hydrolytic/acidogenic process [27,28]. *Proteobacteria* was proven to be widely distributed in the fermentation of food waste and sewage sludge and capable of converting organic matters into volatile fatty acids (VFAs) [29,30], while *Firmicutes* was reported to dominate in the anaerobic digestion process with potential for the degradation of organic matters such as aliphatic-like, protein-like, and polymeric carbohydrates [31,32]. In addition, *Chloroflexi*, Synergistetes, *Actinobacteria*, *Bacteroidetes*, and *Coprothermobacteraeota* were also reported to participate in the hydrolytic and acidogenic processes with the capacity of improving the biodegradability of wastewater via the transformation of complex DOMs into small-molecular organics [32–36].

The microbial community at the genus level is depicted in Figure 5b for further investigation of the functional microbial community in the hydrolytic/acidogenic process. The genera affiliated with *Proteobacteria* mainly included *Desulfobulbus* (10.58%), *Comamonas* (8.88%), and *Advenella* (1.1%). Among these genera, *Desulfobulbus* was the most dominant genus in the hydrolytic/acidogenic process and was widely reported to play a vital role in the mesophilic anaerobic digestion of sewage sludge [37]. *Comamonas* was proven to be able to degrade many kinds of aromatic compounds [38], and *Advenella* was also widespread in mesophilic fermentation and related to organic removal [39]. The genera affiliated with *Firmicutes* principally included *Fastidiosipila* (4.02%), *Eubacterium* (2.67%), *Sedimentibacter* (1.97%), *Syntrophomonas* (1.39%), *norank_f_Syntrophomonadaceae* (1.21%), *Caldicoprobacter* (1.14%), and *Proteiniclasticum* (1.03%). Among these genera, *Fastidiosipila* was reported to be a strict anaerobe and capable of degrading proteins and amino acids [40], and *Eubacterium* could ferment monosaccharides into lactate and/or acetate [41]. *Sedimentibacter* was proven to be able to convert alcohol to acetate or butyrate [42], while *Syntrophomonas* and *norank_f_Syntrophomonadaceae* could utilize long-chain fatty acids with 4–18 carbon atoms to produce acetate or propionate [4]. In addition, *Caldicoprobacter* and *Proteiniclasticum* were also reported to be capable of degrading xylanase and proteins, respectively [43,44]. These functional bacteria affiliated with *Proteobacteria* and *Firmicutes* could have played an indispensable role in the hydrolytic/acidogenic process, and there were also several functional bacteria affiliated with other phyla with non-negligible contribution, such as *Thermovirga* (5.30%), *norank_f__Synergistaceae* (4.01%), *Aminobacterium* (1.47%), and *Acetomicrobium* (1.26%) belonging to *Synergistetes*. *Thermovirga* and *norank_f__Synergistaceae* were both reported to be capable of fermenting proteins, amino acids, and some other small-molecular organic acids [4], while *Aminobacterium* could be able to ferment amino acids and organic acids into VFAs [39]. *Acetomicrobium* was reported to possess the ability to secrete thermophilic esterases for the hydrolysis of esters into small-molecular organic acids [45]. Additionally, *Coprothermobacter* (3.97%), affiliated with *Coprothermobacteraeota*, and *Corynebacterium* (1.21%), affiliated with *Actinobacteria*, were also reported to be capable of degrading proteins and complex organic matters to small-molecular organics.

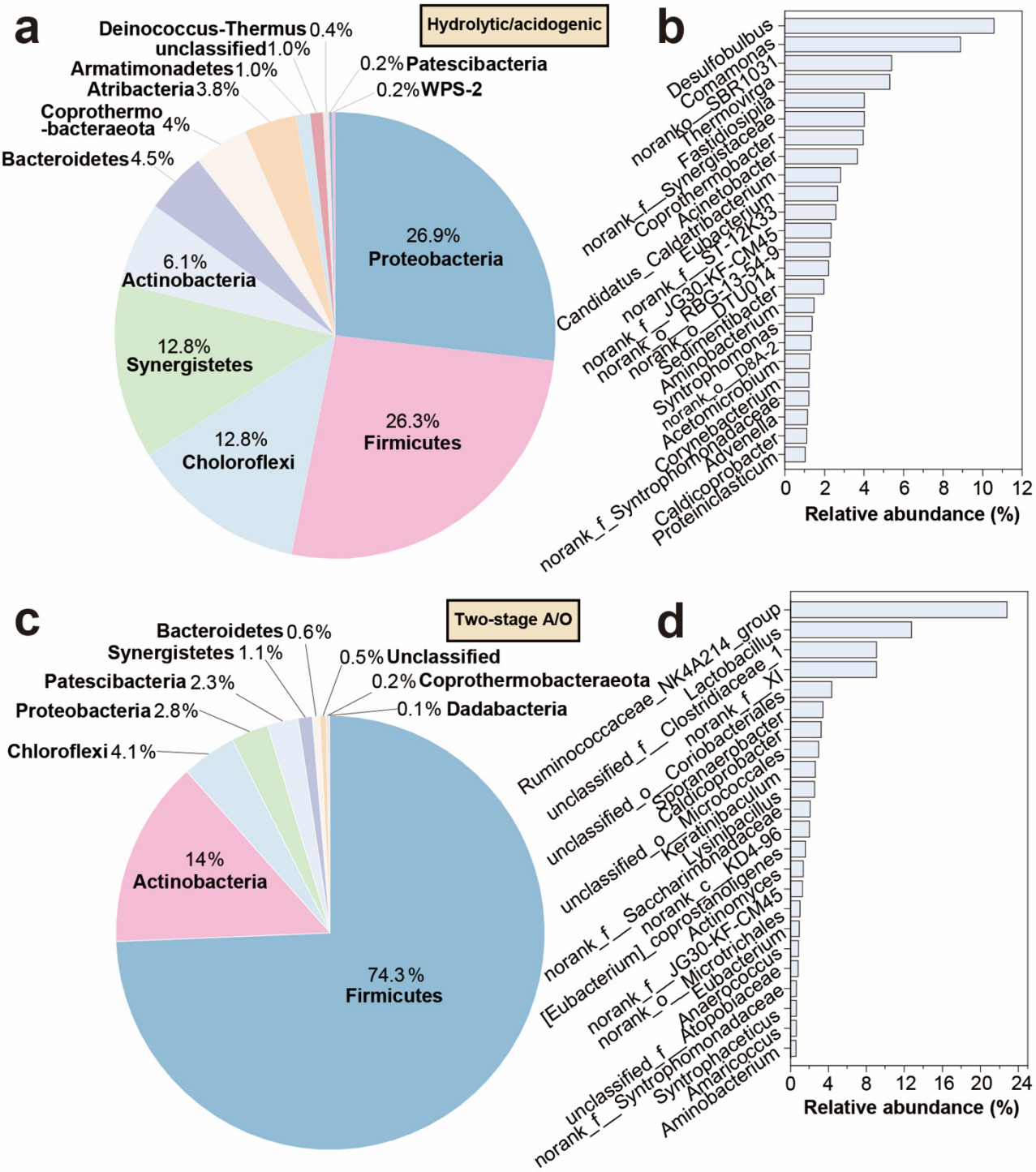

Figure 5. Microbial communities at the phylum and genus levels of the hydrolytic/acidogenic process (**a**,**b**) and the two-stage A/O process (**c**,**d**) revealed by 16S rRNA amplicon sequencing.

3.3.2. Microbial Community in the Two-Stage A/O Process

The coverage score determined to be 0.997 denoted an adequate sequencing depth, and the microbial community of the two-stage A/O process at the phylum level is shown in Figure 5c. There were 10 phyla with a relative abundance higher than 0.1%, namely *Firmicutes* (74.12%), *Actinobacteria* (13.93%), *Chloroflexi* (4.10%), *Proteobacteria* (2.80%), *Patescibacteria* (2.33%), *Synergistetes* (1.05%), *Bacteroidetes* (0.56%), *unclassified_k__norank_d__Bacteria* (0.50%), *Coprothermobacteraeota* (0.19%), and *Dadabacteria* (0.10%). *Firmicutes* with the capac-

ity of biodegrading many kinds of complex organics was the most dominant phylum in the two-stage A/O process, and the other phyla were all related to the removal of organic matters in many biological processes [29–32].

In regard to the microbial community of the two-stage A/O process at the genus level, most of the functional genera related to the degradation of organic matters were affiliated with *Firmicutes* (Figure 5d), including *Ruminococcaceae_NK4A214_group* (22.83%), *Lactobacillus* (12.74%), *unclassified_f_Clostridiaceae_1* (9.08%), *Sporanaerobacter* (3.42%), *Caldicoprobacter* (3.21%), *Keratinibaculum* (2.61%), *Lysinibacillus* (2.53%), *Eubacterium_coprostanoligenes_group* (1.57%), *Eubacterium* (0.91%), *Anaerococcus* (0.85%), and *Syntrophaceticus* (0.63%). The genera belonging to the family *Ruminococcaceae* were reported to be capable of degrading proteins and polysaccharides [46], and *Lactobacillus* was proven to be widespread in all kinds of food waste fermentation with the capacity of utilizing polysaccharides [47]. *Clostridiaceae* was reported to participate in the degradation of lignocellulose with the secretion of endonuclease gluconase and glucoside hydrolase [48], while *Sporanaerobacter* and *Keratinibaculum* were both correlated to the removal of peptide chains and proteins [49,50]. *Lysinibacillus* was demonstrated to exhibit high chemotactic activity against triglycerides and play a key role in the degradation of hydrophobic compounds (e.g., olive oil, glycerol, and polycyclic aromatic hydrocarbons) [51], and *Syntrophaceticus* was related to the removal of acetate [52].

4. Discussion

4.1. Composition Characterization of DOMs

Extensive residual organic matters were retained after the pretreatment and dominated by DOMs, since large amounts of organics were transformed from the solid biowaste into the liquid phase [53] The biodegradability was relatively low, around 0.2, suggesting a considerable content of refractory organics due to the utilization of most easily biodegradable organics for biogas production in the co-digestion process [4]. In addition, the high UV_{254} and chromaticity implied the existence of complex organics with unsaturated bonds and colored functional groups, while the molecular weight distribution denoted that the DOMs in the co-digestion wastewater mainly comprised organics with MW lower than 1 kDa, and the unsaturated organics contributing to UV_{254} and chromaticity possessed a wider MW distribution. These results could provide fundamental information concerning the molecular weight distribution of DOMs in co-digestion wastewater. The 3D-EEM spectral results indicated that tryptophane-protein-like substances dominated in the DOMs, and the non-negligible existence of poorly biodegradable organics increased the difficulty of the co-digestion wastewater treatment. Additionally, the FT-IR suggested that the DOMs in the co-digestion wastewater contained aliphatic compounds, amine-containing organics (e.g., proteins, nucleic acids, etc.), carboxylic acid-containing organics, aromatic compounds, alcohols, sulfonyl-containing organics, polysaccharide-like substances, and phosphorous-containing organics, and these DOMs were further quantitatively analyzed by SPME-GC/MS (Table S1), which provided the first systematic quantitative analysis of the complex DOMs in the real co-digestion wastewater of food waste and sewage sludge.

4.2. Transformation Mechanism of DOMs

The influent co-digestion wastewater contained a high content of refractory DOMs, making it hard to deal with [2]. Thus, the hydrolytic/acidogenic process was indispensable for its contribution to the improvement of the biodegradability. The hydrolytic/acidogenic process mainly executes the conversion of high-MW organics into low-MW organics, including the transformation of higher aliphatic carboxylic acids into lower ones (thus forming carboxylic acid dimers), the amidation of primary and secondary amines, the degradation of aliphatic alcohols and sulfonyl compounds, and the formation of aliphatic esters and polysaccharide-like substances. These reactions significantly improved the biodegradability of the co-digestion wastewater without destroying the intrinsic easily biodegradable organics. Notably, the increase in the UV_{254} and the chromaticity after the hydrolytic/acidogenic process could be attributed to the intermediates with unsaturated bonds and colored func-

tional groups generated from the irreversible intramolecular polymerization reaction [54], in accord with the findings in FT-IR. In addition, the two-stage A/O process serves as the principal contributor to the DOM removal with the elimination of the most easily biodegradable tryptophane-protein-like substances and small-molecular humic acid-like substances in UVC (amide-like, aliphatic-like, and polysaccharide-like substances), but failed in efficient removal of large-molecular aromatic humic acid-like substances in UVA. The ultrafiltration also exhibited poor removal of the large-molecular aromatic humic acid-like substances in UVA (MW < 5 kDa), making them the main residual in the effluent. These implications were further verified by SPME-GC/MS. Overall, the hydrolytic/acidogenic process improved the biodegradability of the DOMs, and the two-stage A/O process removed most biodegradable organics. However, the effluent of the two-stage A/O process still contained considerable refractory organics, and the deficient removal of the refractory organics with MW lower than 5 kDa in the ultrafiltration unit resulted in the residual DOMs in the effluent, which requires further optimization for more efficient removal.

Regarding the microbial community analysis, a host of potential hydrolytic/acidogenic functional bacteria affiliated with *Proteobacteria* and *Firmicutes* were detected in the hydrolytic/acidogenic tank, in line with the findings of previous research [27,28]. These functional bacteria mainly included *Desulfobulbus* [37], *Comamonas* [38], and *Advenella* [39] affiliated with the *Proteobacteria* phylum and *Fastidiosipila* [40], *Eubacterium* [41], *Sedimentibacter* [42], *Syntrophomonas* [4], *norank_f_Syntrophomonadaceae* [4], *Caldicoprobacter* [43], and *Proteiniclasticum* [44] affiliated with the *Firmicutes* phylum. These genera could improve the biodegradability of the co-digestion wastewater with the potential for the conversion of large-molecular and complex organics (e.g., proteins, polysaccharides, aromatic components, heterocyclic organics, and long-chain fatty acids) into small-molecular organic acids (e.g., acetic acid, lactic acid, propionic acid, and butyric acid). The two-stage A/O process enriched potential functional populations with the capacity of degrading many kinds of organic matters in the effluent of the hydrolytic/acidogenic process (e.g., proteins, polysaccharides, lignocellulose, and many small-molecular organics), such as *Ruminococcaceae_NK4A214_group* [46], *Lactobacillus* [47], *unclassified_f_Clostridiaceae_1* [48], *Sporanaerobacter* [49], *Caldicoprobacter* [43], *Keratinibaculum* [50], and *Lysinibacillus* [51] from *Firmicutes* phylum. These functional genera could guarantee the biological transformation of DOMs in the co-digestion wastewater. The abovementioned microbial populations could cooperate in the biological process for the treatment of the co-digestion wastewater of food waste and sewage sludge and achieve the removal of most DOMs with only extremely refractory organics residual in the effluent.

4.3. Environmental Implications

Anaerobic digestion is regarded as the most promising approach for the management of biowaste including food waste and sewage sludge due to its advantages in energy recycling, environmental protection, and economic conservation. However, the ineluctable by-product of the anaerobic process—the anaerobic digestion wastewater—could bring about severe environmental and health problems [15]. The limited knowledge concerning the complex DOMs in anaerobic digestion wastewaters such as the co-digestion wastewater of food waste and sewage sludge could cause adverse effects in the biokinetic design and treatment optimization [1,2,16]. Given that the previous research mainly focused on the general water quality indices such as COD, BOD_5, TOC, and chromaticity [3,42,53], and most of the MBR designs rely solely on the empirical parameters, this study provided the first systematic characterization of the complex DOMs in real co-digestion wastewater of food waste and sewage sludge with multiple scales (molecular weight distribution, 3D-EEM, FT-IR, and SPME-GC/MS), which could advance the understanding of co-digestion wastewater and provide a fundamental reference for the investigation of co-digestion wastewater treatment.

In addition, MBR has been widely applied in the treatment of co-digestion wastewater, but the specific transformation mechanism of DOMs in the full-scale MBR has rarely been

explored. This study investigated the transformation of DOMs in the hydrolytic/acidogenic process, two-stage A/O process, and ultrafiltration units, and the results showed that the biological processes contribute the strongest treatment efficiency in the typical MBR, including the improvement of biodegradability in the hydrolytic/acidogenic process and the consumption of most DOMs in the two-stage A/O process, and the poor DOM removal performance was principally attributed to the deficient removal of refractory organics with MW lower than 5 kDa in the ultrafiltration unit. Thus, the major improvement of the MBR treating efficiency lies in the enhanced removal of the residual refractory organics in the effluent of the biological processes. On the one hand, the optimization of the membrane in the ultrafiltration units could solve the problems, since most of the in-service MBRs depend on the membrane to remove the residual refractory organics and the rapid development of the membrane technology has provided more alternatives for contaminant interception [55]. On the other hand, the specific composition of these residual organics has been characterized by the SPME-GC/MS in this study, and the main components included nonyl aldehyde (31.39%), 1,1,3-trimethoxypropane (23.97%), chloropicrin (6.90%), and 5,5-dimethyl-3-(3-methyl-cyclooxiran-2-yl)-cyclohex-2-enone (6.78%). Targeting these specific refractory organics, some physicochemical methods, such as advanced oxidation [56] and enhanced coagulation, could also be feasible for the improvement of the treating efficiency.

5. Conclusions

The influent co-digestion wastewater of food waste and sewage sludge in a full-scale MBR mainly contained organics with MW < 1 kDa and was dominated by tryptophane-protein-like substances. The hydrolytic/acidogenic process and two-stage A/O contributed to the improvement of biodegradability and the removal of most DOMs, respectively, but the poor removal of refractory organics (MW < 5 kDa) in the ultrafiltration unit resulted in the effluent organic residue, requiring further optimization. In addition, the enrichment of microbial communities with the potential for hydrolytic/acidogenic and organic biodegradation has ensured the biological transformation of DOMs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14116556/s1, Table S1: Description of DOMs in the co-digestion wastewater of sewage sludge and food waste revealed by SPME-GC/MS. Table S2: Description of DOMs in the effluent of the hydrolytic/acidogenic tank revealed by SPME-GC/MS. Table S3: Shared DOMs between the influent and the effluent of the hydrolytic/acidogenic tank revealed by SPME-GC/MS. Table S4: Description of DOMs in the effluent of the two-stage A/O process revealed by SPME-GC/MS. Table S5: Description of DOMs in the effluent of the ultrafiltration units revealed by SPME-GC/MS.

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