Characteristics and Biodegradability of Wastewater Organic Matter in Municipal Wastewater Treatment Plants Collecting Domestic Wastewater and Industrial Discharge

Yun-Young Choi [1], Seung-Ryong Baek [1], Jae-In Kim [1], Jeong-Woo Choi [1], Jin Hur [2], Tae-U Lee [3], Cheol-Joon Park [3] and Byung Joon Lee [1,*]

[1] Department of Construction and Environmental Engineering, Kyungpook National University, 2559 Gyeongsang-daero, Sangju, Gyeongbuk 742-711, Korea; yunchoi@knu.ac.kr (Y.-Y.C.); ps5788@naver.com (S.-R.B.); jaein1224@naver.com (J.-I.K.); chjw51@knu.ac.kr (J.-W.C.)

[2] Department of Environment & Energy, Sejong University, Seoul 143-747, Korea; jinhur@sejong.ac.kr

[3] Daegu Environmental Corporation, Daegu 42720, Korea; ion094@hanmail.net (T.-U.L.); goldberg0419@hanmail.net (C.-J.P.)

[*] Correspondence: bjlee@knu.ac.kr; Tel.: +82-54-530-1444

Abstract: Municipal wastewater treatment plants (WWTPs) in Korea collect and treat not only domestic wastewater, but also discharge from industrial complexes. However, some industrial discharges contain a large amount of non-biodegradable organic matter, which cannot be treated properly in a conventional biological WWTP. This study aimed to investigate the characteristics and biodegradability of the wastewater organic matter contained in the industrial discharges and to examine the fate of the industrial discharges in a biological WWTP. In contrast to most previous studies targeting a specific group of organic compounds or traditional water quality indices, such as biological oxygen demand (BOD) and chemical oxygen demand (COD), this study was purposed to quantify and characterize the biodegradable and nonbiodegradable fractions of the wastewater organic matter. Chemical oxygen demand (COD) fractionation tests and fluorescence spectroscopy revealed that the industrial discharge from dyeing or pulp mill factories contained more non-biodegradable soluble organic matter than did the domestic wastewater. Statistical analysis on the WWTPs' monitoring data indicated that the industrial discharge containing non-biodegradable soluble organic matter was not treated effectively in a biological WWTP, but was escaping from the system. Thus, industrial discharge that contained non-biodegradable soluble organic matter was a major factor in the decrease in biodegradability of the discharge, affecting the ultimate fate of wastewater organic matter in a biological WWTP. Further application of COD fractionation and fluorescence spectroscopy to wastewaters, with various industrial discharges, will help scientists and engineers to better design and operate a biological WWTP, by understanding the fate of wastewater organic matter.

Keywords: wastewater; industrial discharge; organic matter; COD fraction; fluorescence

1. Introduction

Municipal wastewater treatment plants (WWTPs) in Korea collect and treat not only domestic wastewater, but also discharge from industrial complexes. Industrial factories have their own facilities for treating industrial wastewater and then discharge the treated water into the sewer system [1,2]. However, the treatment facilities target easily biodegradable organic matter, rather than refractory non-biodegradable organic matter, as long as the facilities can comply with traditional regulations for

biodegradable organic matter, such as for biological oxygen demand (BOD) [3]. Thus, some industrial discharges are likely to contain a large amount of refractory non-biodegradable organic matter. A conventional biological WWTP (i.e., activated sludge system) may not treat such non-biodegradable organic matter properly, and there is a risk of it escaping from the WWTP to a river or lake [4,5].

Organic matter in wastewater has been estimated as an equivalent quantity, such as chemical oxygen demand (COD) or biological oxygen demand (BOD). However, the adoption of tertiary treatment processes to remove nutrients and refractory organic matter requires a classification of the subordinate fractions of wastewater organic matter. For instance, the total COD of wastewater organic matter can be classified into the four fractions of readily biodegradable COD (RBCOD), slowly biodegradable COD (SBCOD), non-biodegradable soluble COD (NBDSCOD), and non-biodegradable particulate COD (NBDPCOD) (Figure 1) [6,7]. RBCOD, such as volatile fatty acids, is readily degraded by microbial metabolism. SBCOD, composed of particulate organic matter, is degraded slowly by a series of microbial actions, such as adsorption, hydrolysis, and metabolism. NBDSCOD, which is refractory in biodegradation, is contained in industrial discharges. Aromatic compounds are used in various industries and are typical examples of NBDSCOD. NBDPCOD is also non-biodegradable, but is removed easily by sedimentation in a conventional WWTP.
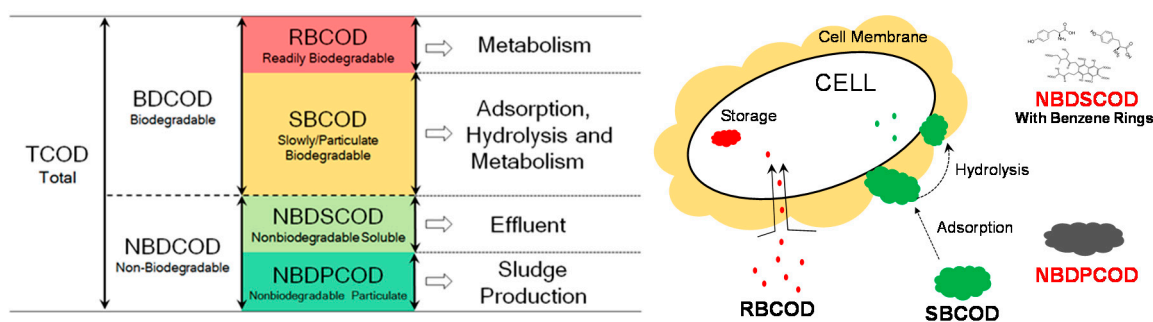

Figure 1. Schematic diagram of the chemical oxygen demand (COD) fractions and their fates in a biological wastewater treatment plant.

Depending on the type of industry, some industrial discharges are abundant in NBDSCOD, and thus, more refractory in a biological treatment process. For example, it has been reported that dyeing factories discharge a large amount of NBDSCOD [4,8,9]. Because many chemical dyes are based on aromatic or heterocyclic ring structures, which are considered non-biodegradable, industrial discharge from dyeing factories likely contains substantial amounts of NBDSCOD. In addition, industrial discharge from paper mill factories was reported to contain a large amount of lignin and lignin derivatives, which are also known to be non-biodegradable, and hence, increase the amount of NBDSCOD [10,11]. An advanced oxidation process might be required to degrade NBDSCOD containing aromatic compounds from dyeing and paper mill factories [9,12].

This study aimed to investigate how industrial discharges from dyeing and paper mill factories affect the characteristics and biodegradability of wastewater organic matter and their fate in a biological treatment process. In contrast to most previous studies targeting a specific group of organic compounds or traditional water quality indices (e.g., BOD and COD) [4,5,13], this study adopted sophisticated analytical techniques to quantify and characterize the organic matter composition of the wastewater containing industrial discharges from dyeing and pulp mill factories. A COD fractionation test, based on a respirometer technique [14], was applied to quantify the fractions of RBCOD, SBCOD, NBDSCOD, and NBDPCOD, and fluorescence spectroscopy [15] was used to characterize the chemical composition of wastewater organic matter qualitatively. Information about the COD fractions, and the fluorescence spectroscopic characteristics of wastewater organic matter, will help predict the fate of waste organic matter in a WWTP and provide the best available technologies for treating wastewater organic matter. Such quantitative and qualitative data about wastewater organic matter will be used to overcome the

design and operational difficulties of a WWTP receiving a large amount of industrial discharge from dyeing and paper mill factories.

2. Materials and Methods

2.1. Study Site and Sampling

The Jisan (JS) WWTP is located in a commercial and residential area, and the facility collects and treats mostly domestic wastewater. Thus, the JS WWTP was selected as a control system in this research. In contrast, the Dalseocheon (DS) and Hyunpoong (HP) WWTPs were selected as experimental systems, because they collect and treat large amounts of industrial discharge from dyeing and paper mill factories, respectively. The proportion of industrial discharge in influent wastewater was approximately 25% for the DS WWTP and 75% for the HP WWTP. Given that there are plans for residential complexes in the HP WWTP's catchment area, the proportion of the industrial discharge is very high for the HP WWTP.

Two sets of sampling campaigns were conducted from October to November 2015. Each sampling campaign was carried out in a WWTP, collecting 20 L of a wastewater sample and 2 L of an activated sludge sample. The wastewater sample was collected in the distribution channel between the primary sedimentation process and the biological treatment process. Thus, the wastewater sample was settled wastewater, but not raw wastewater. The activated sludge sample was collected at the end of the aeration tank of the biological treatment process, when presumably all of the soluble biodegradable organic matter would have been degraded completely. The wastewater samples were stored at 4 °C to minimize biodegradation while being transported to the laboratory. Before starting the respirometer (biodegradation) test, we warmed up the wastewater samples to 20 °C and washed the sludge sample with distilled deionized water to remove any other impurities.

2.2. Experimental Methods

For the respirometer test, we used a 4 L cylindrical reactor, equipped with a plastic impeller to stir the mixed liquor and an air diffuser to supply oxygen. At the beginning of the test, the wastewater and activated sludge samples were mixed rapidly in the reactor. The mixed liquor was then stirred slowly at 40 rpm to minimize surface aeration. A Styrofoam plate was placed on the surface of the mixed liquor to minimize surface aeration. The air diffuser was the only source of dissolved oxygen (DO) in the reactor. The air diffuser was powered on at DO = 2.0 mg/L, and off at DO = 5.0 mg/L, so that the DO of the mixed liquor was maintained between 2 and 5 mg/L. An LDO101 DO probe and an HQ30d DO meter (HACH Inc., Loveland, CO, USA) were used to record the DO of the mixed liquor every 10 s. Oxygen utilization rates (OUR) were calculated with the downward slopes of a DO curve (Figure 2). As shown in Equation (1) and Figure 2, RBCOD can be estimated based on the area of the plateau of the OUR graph [6,7,14,16]. The integral part of Equation (1) indicates the total amount of oxygen consumption by RBCOD degradation. Because only a part (i.e., 33%) of RBCOD is assumed to use oxygen via microbial catabolism, RBCOD is calculated by multiplying the integral part by $1/(1 - Y_{ZH})$. The integral part of Equation (1) is equivalent to the area between the OUR_{Total} and OUR_{SBCOD} plots in Figure 2b.

$$\text{RBCOD} = \frac{1}{1 - Y_{ZH}} \int_{t=0}^{t=d} (\text{OUR}_{\text{total}} - \text{OUR}_{\text{SBCOD}}) \cdot dt = \frac{1}{1 - Y_{ZH}} \int_{t=0}^{t=d} (\text{OUR}_{\text{RBCOD}}) \cdot dt \qquad (1)$$
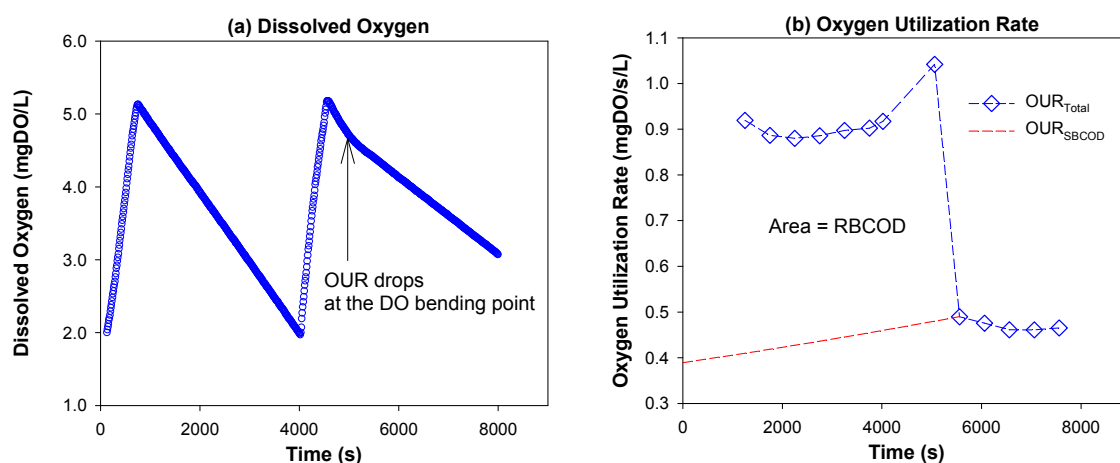
Figure 2. Results from a respirometer test, measuring the readily biodegradable COD (RBCOD) concentration in wastewater organic matter. (**a**,**b**) illustrate dissolved oxygen (DO) concentrations and oxygen utilization rate, respectively, with increasing time.

A BOD test was used to measure the biodegradable COD (i.e., BDCOD = RBCOD + SBCOD) of the wastewater organic matter, because the BDCOD was assumed to be equal to the ultimate BOD (BOD_U) [17]. A measurement of 0.3 mL of the 40 g/L allyl thiourea (ATU) stock solution was added to each BOD bottle to prevent oxygen utilization by nitrification. For each wastewater sample, a series of BOD concentrations was measured on 3, 5, 8, 12, 15, and 20 days. Subsequently, the ultimate BOD (BOD_U) was estimated with a curve-fitting analysis, adopting the first-order decaying model (Equation (2)). The curve-fitting analysis was performed with a scientific graphing program (SigmaPlotTM, Systat Inc., San Jose, CA, USA). Finally, the SBCOD of a wastewater sample was estimated by subtracting RBCOD from BDCOD.

$$BOD_U \approx BDCOD = RBCOD + SBCOD = BOD_t / \left(1 - e^{-kt} \right) \tag{2}$$

The NBDSCOD of a wastewater sample was measured with consecutive biodegradation and coagulation tests [18,19]. After 24-h biodegradation and 1-h sedimentation, a sample was collected from the reactor. Then 5 mL of the 1 M $ZnSO_4$ stock solution and 5 mL of the 1 M NaOH solution were added to the collected 500 mL sample, and the mixture was coagulated by stirring rapidly at 80 rpm for 1 min, and slowly at 20 rpm for 5 min. $ZnSO_4$ as a coagulant is supposed to aggregate and remove all the colloidal and particulate organic matter. Thus, the remaining organic matter in the solution phase is assumed to be soluble [19,20]. After 1-h sedimentation, a supernatant sample was taken and filtered through 0.45 μm pore size membrane filter paper (Hyundai Micro Inc., Seoul, Korea). Assuming that all the soluble biodegradable organic matter was degraded by 24-h biodegradation, and all the particulate organic matter was removed by coagulation and filtration, the COD of the final filtrate would be equal to the NBDSCOD. Finally, the NBDPCOD was estimated by subtracting the RBCOD, the SBCOD, and the NBDSCOD from the total COD.

The fluorescence emission–excitation matrices and parallel factor analysis (FEEM-PARAFAC) was applied to characterize the dissolved organic matter (DOM) composition of a wastewater sample. The wastewater samples, filtered through a 0.45 μm pore size membrane filter (Hyundai Micro Inc., Seoul, Korea) were kept in a freezer prior to the FEEM analysis. The samples were adjusted to pH 3 by adding 1 M HCl prior to absorbance and fluorescence measurements to minimize the potential interference of pH variability and metal bindings. Ultraviolet (UV) absorbance at 254 nm was measured with a Hach DR5000 UV-visible spectrophotometer (Hach, Loveland, CO, USA), with Milli-Q water as the blank. The FEEMs of the samples, diluted until UV254 < 0.05 cm^{-1}, were scanned using a Perkin-Elmer LS-55 luminescence spectrometer (Perkin-Elmer Inc., Waltham, MA, USA) over Ex/Em

wavelengths of 250–500 and 280–550 nm, with increments of 5 nm and 0.5 nm, respectively. The FEEM of each sample was subject to blank subtraction and normalized by the Raman peak of Milli-Q water excited at 350 nm. The detailed procedures of fluorescence EEMs are described elsewhere [15,21]. All of the FEEM results for the 20 collected samples were statistically analyzed by PARAFAC modeling, using MATLAB version 8.5 software (MathWorks Inc., Natick, MA, USA) with the DOMFluor toolbox [22]. The number of components was determined based on split-half analysis.

3. Results and Discussion

3.1. COD Fractionation

Results from the COD fractionation tests for the wastewater samples are summarized in Table 1. The total COD (TCOD) concentrations of the JS, DS, and HP WWTPs were relatively low at 156, 111, and 112 mg/L for the first sampling campaign, and 150, 105, and 129 mg/L for the second sampling campaign. Wastewaters in Korea usually have a low COD concentration, compared to wastewaters in other developed countries, because the wastewaters are collected in a combined sewer system [7,23]. The TCOD of the control system (i.e., the JS WWTP), collecting mostly domestic wastewater, was higher than that of the experimental systems (i.e., DS and HP WWTPs). The RBCOD for both the control and experimental systems was relatively low, less than 10 mg/L. The RBCOD of the DS and HP WWTPs, in particular, was in some cases very low, less than 1 mg/L. The combined sewer system, which often has an open channel, might reduce the fermentation from wastewater organic matter to volatile fatty acids (i.e., RBCOD) [24]. Above all, it is important to note that the DS and HP WWTP had values for NBDSCOD that were two to three times higher than those for the JS WWTP, but those values were two to three times lower than the BOD_U (i.e., BDCOD). This observation indicates that the industrial discharge from the DS and HP WWTPs changed the COD fractions of wastewater organic matter substantially.

Table 1. Summarized results of the COD fractionation tests carried out for the two sampling campaigns from October to November 2015.

ITEM	First Sampling Campaign			Second Sampling Campaign		
	JS WWTP	DS WWTP	HP WWTP	JS WWTP	DS WWTP	HP WWTP
Total COD	155.5 ± 0.5	110.7 ± 3.3	112 ± 3.2	150 ± 3.7	105 ± 3.7	129 ± 4.0
COD_{Zn24} [a]	28.5 ± 0.5	43.7 ± 2.1	67 ± 1.2	21 ± 1.0	45.5 ± 1.5	71 ± 1.7
BOD_u [b]	124	52.4	38.8	123.2	52.6	43.8
RBCOD	9.2	4.7	0.7	7.7	0.3	8
SBCOD	114.8	47.7	38.1	115.5	52.3	35.8
NBDSCOD	28.5	43.7	67.0	21.0	45.5	71.0
NBDPCOD	3.0	14.6	6.2	5.8	6.9	14.2

Notes: Total COD and COD_{Zn24} show the mean value and standard deviation of the triplicated measurements. [a] COD_{Zn24} = Measured COD after 24-h biodegradation and flocculation (Unit: mgCOD/L); [b] BOD_U = Ultimate BOD estimated by the data-fitting analysis;

The COD fractions of the JS, DS, and HP wastewaters are shown graphically in the pie charts (Figure 3). Wastewater organic matter of the JS WWTP, collecting mostly domestic wastewater, had a large BDCOD (=RBCOD + SBCOD) fraction at 81% of the TCOD. A typical BDCOD fraction was reported to be about 80% to 90% for raw and primary effluent wastewaters [7,25]. This observation, and the earlier reports, indicate that domestic wastewater consisted mostly of biodegradable organic matter. In contrast, wastewater organic matter of the DS and HP WWTPs had a small BDCOD fraction, but a large NBDSCOD fraction. The NBDSCOD of the HP WWTP was especially high at 57% of the TCOD. Because a large amount of the industrial discharge from dyeing and paper mill factories flowed to the DS and HP WWTPs, it was inevitable that the NBDSCOD fraction increased.

The COD fractionation test was also applied to the sample collected directly from the paper mill factories in the HP WWTP's catchment area. The NBDSCOD fraction of the direct industrial discharge was 80% of the TCOD, but the BDCOD fraction was only 6%. Thus, it appears that the treatment facilities in the paper mill factories were able to treat the BDCOD easily, but dumped the refractory NBDSCOD into the sewer system. Unfortunately, the current standards for industrial discharges focus on an integrated parameter, such as BOD_5 or COD_{Mn}, which do not indicate the subordinate COD fractions properly. The operators of the treatment facilities might be satisfied with their performance, but nonetheless appear to be unconcerned with the refractory NBDSCOD.
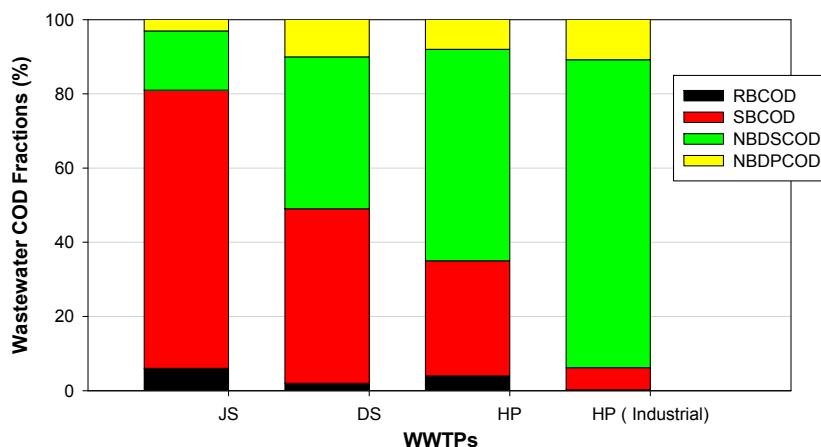

Figure 3. Estimated COD fractions of the influent wastewaters in Jisan wastewater treatment plant (JS WWTP), Dalseocheon wastewater treatment plant (DS WWTP), Hyunpoong wastewater treatment plant (HP WWTP), and the industrial discharge from pulp mill factories in the HP WWTP's catchment area. The fractions in the figures represent the average values of the two measurement campaigns.

3.2. FEEM-PARAFAC

FEEM-PARAFAC was used to characterize the chemical composition of wastewater dissolved organic matter (DOM) and to investigate the compositional change in biodegradation. FEEM-PARAFAC revealed that a four-component model could represent the characteristics of wastewater DOM (Figure 4). Components C1, C2, C3, and C4 were characterized with the Excitation/Emission maxima at ≤230/345, ≤220(275)/355, ≤275(220)/320, and 243/430 nm, respectively. Reviewing the references (Table 2), we could assign the four components of C1, C2, C3, and C4 to protein-like, tryptophan-like, tyrosine-like, and humic-like fluorescent DOM, respectively. The protein-like C1 component was reported to include extracellular polymeric substances (EPS) in activated sludge [26–28]. The protein-like C1 component increased in a substrate utilization phase (i.e., growth phase), but decreased in an endogenous phase (i.e., death phase). The C1 component was thus considered a by-product associated with microbial growth. The tryptophan-like C2 component was reported to be resistant to filtration, but susceptible to biodegradation, therefore it should be soluble and biodegradable [27,29,30]. The tyrosine-like C3 component was reported to be a soluble microbial product (SMP) commonly present in recycled wastewater [27,31–33]. It could be removed by physico-chemical treatments, such as coagulation, but not entirely with a biological treatment system. The fulvic-like C4 component was reported to be recalcitrant in a conventional activated sludge system, because it is considered non-biodegradable [11,29,30].
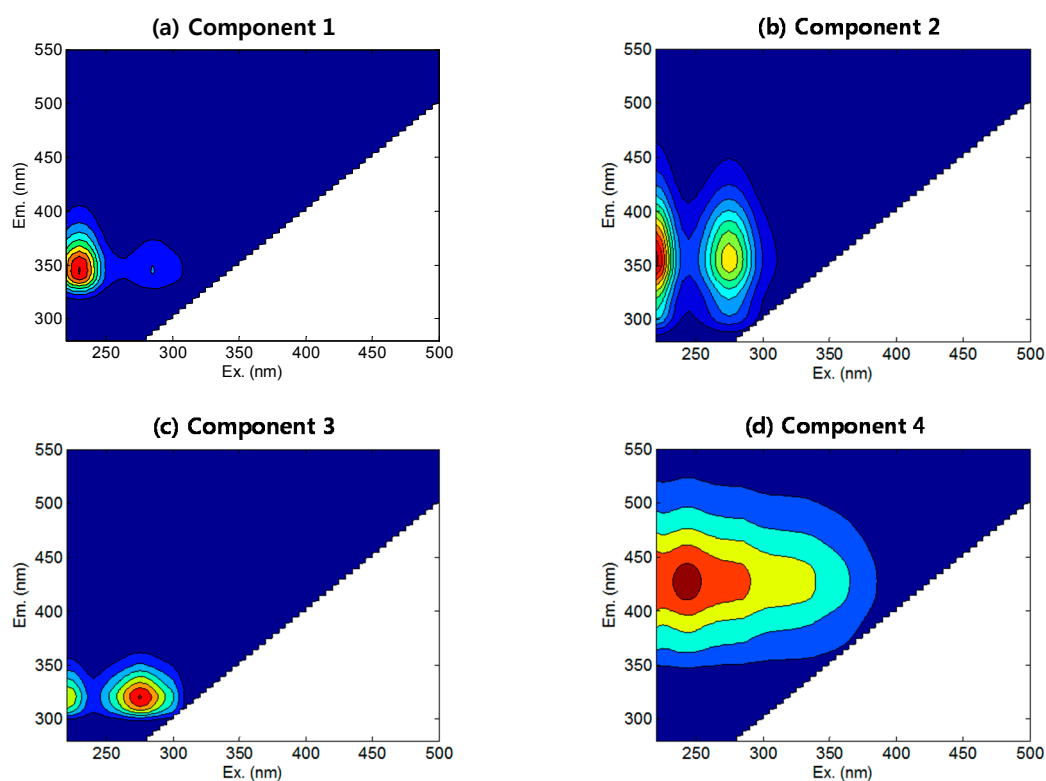
Figure 4. Typical excitation–emission matrices (EEM) for the wastewater samples. (**a**) protein-like C1 component; (**b**) tryptophan-like C2 component; (**c**) tyrosine-like C3 component; (**d**) fulvic-like C4 component.

Table 2. Characteristics of the fluorescence excitation–emission matrices (FEEM) peaks in this study, and commonly observed FEEM peaks from previous studies. Values in brackets represent secondary peaks.

This Study		Previous Study		
Comp.	$\lambda_{ex}/\lambda_{em}$	$\lambda_{ex}/\lambda_{em}$	Substance	Reference
C1	**230/345**	230/350	Protein-like	Shen et al. (2012) [27]
		280/340	Protein-like	Ni et al. (2009) [26]
		250–280/<380	Protein-like	Fan et al. (2014) [28]
C2	**220(275)/355**	220(280)/350	Tryptophan-like, SMP	Yu et al. (2013) [29]
		275(240)/346	Tryptophan-like	Cohen et al. (2014) [30]
		220(275)/343	Tryptophan-like	Shen et al. (2012) [27]
C3	**275(220)/320**	270/300	Tyrosine-like	Murphy et al. (2011) [31]
		279/315	Protein-like	Shen et al. (2012) [27]
		280(230)/310	Tyrosine-like	Li et al. (2014) [32]
		280/320	Phenol-like, protein-like	Ou et al. (2014) [33]
C4	**243/430**	230–245/425–430	Fulvic-like	Yu et al. (2013) [29]
		270(350)/432	Humic-like	Cohen et al. (2014) [30]
		230–275/400–520	Fulvic-like, Lignins	Carstea et al. (2016) [11]

Figure 5a–c illustrate the relative fractions of the C1, C2, C3, and C4 components in the JS, DS, and HP wastewaters, respectively. The blak bars indicate the relative fractions of the influent wastewater, whereas the red bars show the fractions of the treated wastewater based on the 24-h biodegradation test. The dominant component in the JS influent wastewater was found to be the tryptophan-like C2 component, which had a relative fraction of 75% (Figure 5a). The dominant components in the DS and HP influent wastewaters were the protein-like C1 and tyrosine-like C3

components (Figure 5b,c). The HP influent wastewaters also contained the tryptophan-like C2 and the fulvic-like C4 components, which had relative fractions of 8% and 15%, respectively (Figure 5c).
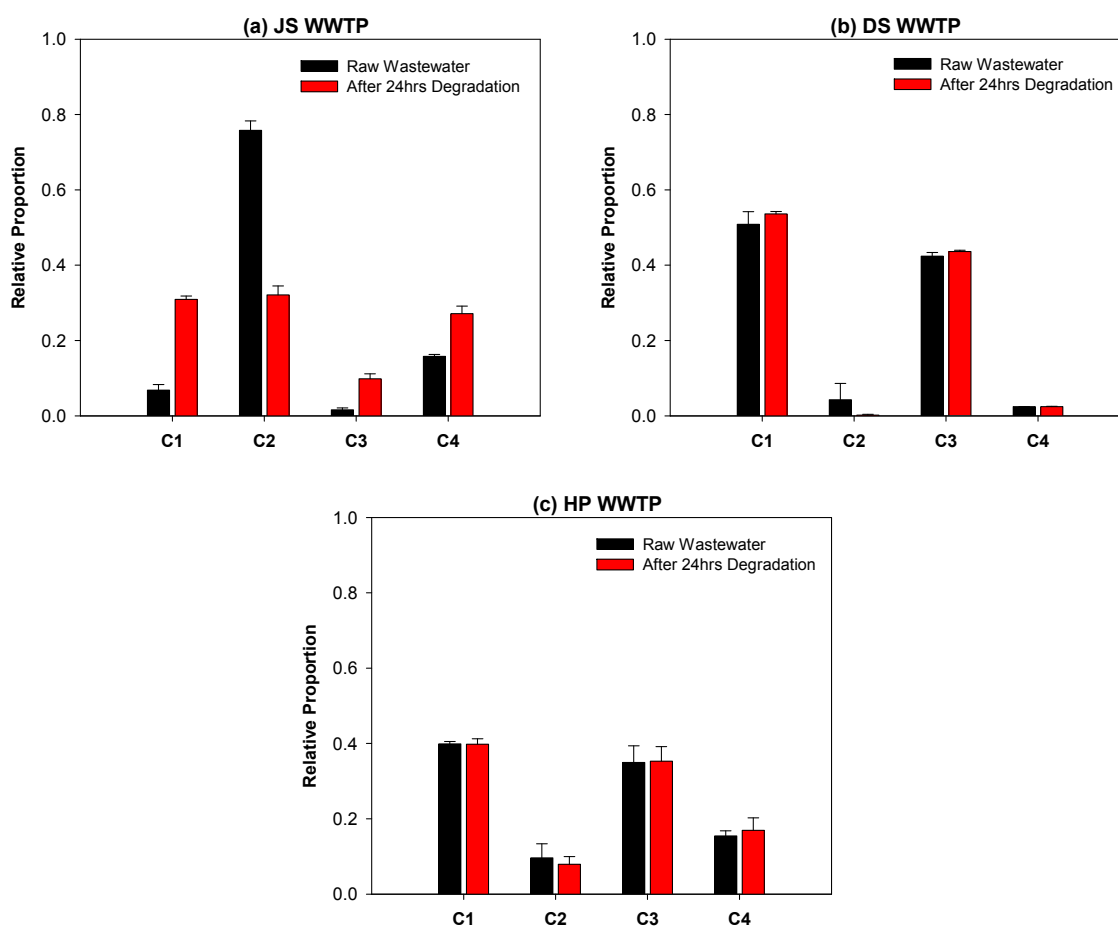

Figure 5. Relative fractions of the protein-like (C1), tryptophan-like (C2), tyrosine-like (C3), and fulvic-like (C4) components of the wastewater dissolved organic matter in (**a**) JS; (**b**) DS; and (**c**) HP WWTPs. The black and red bars indicate the relative fractions before and after the 24-h biodegradation test, respectively.

The changes from the black bars to the red bars indicate the compositional changes of the wastewater DOM in biodegradation (Figure 5). The dominant tryptophan-like C2 component in the JS wastewater decreased substantially from 75% to 31% in the 24-h biodegradation test, whereas the C1, C3, and C4 components increased (Figure 5a). These results indicate that only the C2 component is biodegradable, but that the other components are non-biodegradable, and are produced by microbial metabolism (i.e., biodegradation). The dominant protein-like C1 and tyrosine-like C3 components in the DS and HP wastewater exhibited little change in biodegradation, and were thus characterized as non-biodegradable (Figure 5b,c). In the authors' opinion, aromatic compounds discharged from dyeing and paper mill factories might be the major source of the C1 and C3 components [11,28]. The fulvic-like C4 component, which was evident in the HP wastewater at a relative fraction of 15% (Figure 5c), might be caused by lignins and lignin derivatives discharged from the paper mill factories [11]. Overall, the JS wastewater contained a large amount of biodegradable components (i.e., the tryptophan-like C2 component) and exhibited an apparent compositional change of wastewater organic matter during the biodegradation process, whereas the DS and HP wastewaters contained mostly non-biodegradable components. Thus, the results from FEEM-PARAFAC also supported the

findings from the COD fractionation tests, in that the JS wastewater consisted mostly of BDCOD, but the DS and HP wastewaters consisted mostly of NBDSCOD.

3.3. Fate of Wastewater Organic Matter in a Biological WWTP

The box plots in Figure 6a illustrate the statistical analysis of the effluent COD concentrations, which had been monitored from the discharge of the WWTPs' biological treatment process over the last three years (from 2013 to 2015). The average effluent COD concentrations of the DS and HP WWTPs were 21.0 and 25.5 mg/L, respectively, which is much higher than the values for the JS WWTP (7.5 mg/L). In addition, the difference in the effluent COD concentrations between the control (i.e., the JS WWTP) and experimental systems (i.e., the DS and HP WWTP) was statistically significant. Indeed, industrial discharge with a substantial amount of NBDSCOD increased the effluent COD concentrations of the biological treatment process. The box plots of the normalized COD removal efficiency (Figure 6b) also illustrate the apparent differences between the control and experimental systems. The averages of the normalized COD removal efficiencies were 0.85, 0.69, and 0.64 for the JS, DS, and HP WWTPs, respectively, indicating that industrial discharge with a substantial amount of NBDSCOD decreased the COD removal efficiency of the biological treatment process. We thus concluded that, based on our determination of the effluent COD concentration and the COD removal efficiency, industrial discharge with a substantial amount of NBDSCOD affected the fate of wastewater organic matter in a WWTP.
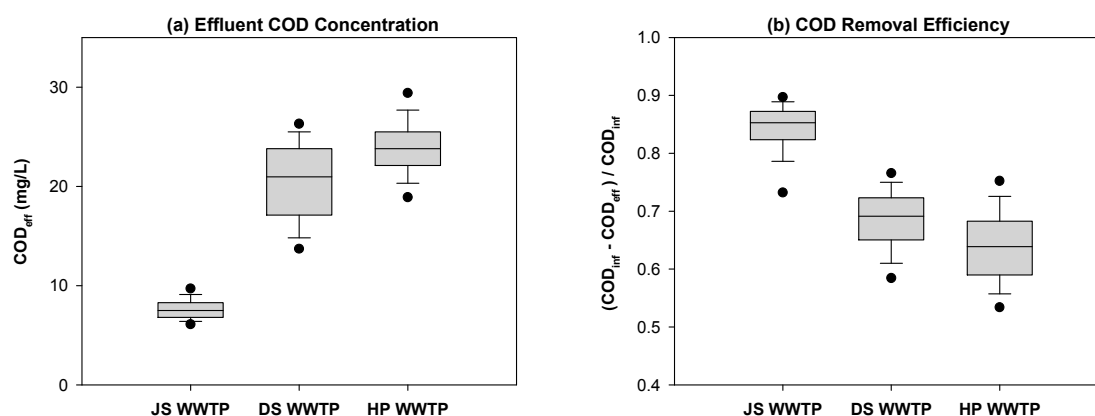

Figure 6. Box plots of (**a**) the effluent COD concentrations and (**b**) the normalized COD removal efficiency of the biological treatment processes of the JS, DS, and HP WWTPs. The WWTPs' monitoring data from recent years (2013–2015) were used for the statistical analysis.

Wastewater from dyeing and paper mill factories has been reported to contain a large amount of non-biodegradable organic compounds. Organic dyes with aromatic ring structures, lignin and its derivatives, and chlorinated organic compounds, are typical refractory organic compounds, resistant to biodegradation [4,5,13]. However, quantification and characterization of all of the individual organic compounds are practically impossible with any present analytical techniques. Most previous studies only measured a specific group of organic compounds and monitored the ratio of BOD/COD as a relative index of biodegradability [34,35]. Thus, the COD fractionation test was applied in this study to quantify NBDSCOD, as an actual amount of nonbiodegradable organic compounds. The FEEM-PARAFAC technique was applied to classify nonbiodegradable organic compounds, based on the fluorescent characteristics of the compounds. The COD fractionation test and the FEEM-PARAFAC analysis could generate reliable results, supporting the system responses of a biological WWTP (see Figure 6), so they have been proven straightforward and reliable for quantifying and characterizing wastewater organic matter.

4. Conclusions

This study elucidated how industrial discharge with a substantial amount of NBDSCOD affected the characteristics and biodegradability of wastewater organic matter and their fate in a biological WWTP, applying COD fractionation testing, fluorescence spectroscopy, and statistical analysis of the WWTPs' monitoring data. The findings are summarized below.

(1) The COD fractionation tests could quantify an integrated index of the non-biodegradable soluble organic matter (i.e., NBDSCOD), which is abundant in industrial discharges.

(2) FEEM-PARAFAC revealed that domestic wastewater contained biodegradable tryptophan-like components, whereas the industrial discharge contained larger amounts of the non-biodegradable protein-, tyrosine-, and fulvic-like components.

(3) NBDSCOD contained in industrial discharge cannot be treated effectively in a conventional biological treatment process, and hence, escaped treatment.

In short, industrial discharge with a substantial amount of NBDSCOD was found to decrease biodegradability, affecting the fate of wastewater organic matter in a biological WWTP. Amendments to water quality standards, especially with regard to refractory NBDSCOD, might be required to enforce appropriate treatment methods for industrial discharge containing substantial amounts of NBDSCOD. Further application of COD fractionation and fluorescence spectroscopy to various wastewaters will help scientists and engineers to better design and operate a biological WWTP, by understanding the fate of the wastewater organic matter.

Author Contributions: Byung Joon Lee, Jin Hur and Tae-U Lee conceived and designed the experiments; Jae-In Kim, Seung-Ryong Baek and Cheol-Joon Park performed the experiments; Jeong-Woo Choi, Yun-Young Choi and Byung Joon Lee analyzed the results and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yu, S.J.; Park, S.M.; Kwon, O.S.; Park, S.J.; Yeom, I.T. Improvement on Sewerage Effluent Standard of Public Sewerage Treatment Plants. *J. Korean Soc. Water Environ.* **2013**, *29*, 276–287. (In Korean)

2. NIER. *Study on Appropriate Treatment & Management of the Public Sewage Treatment Works Entering the Industrial Wastewater*; Ministry of Environment: Seoul, Korea, 2011. (In Korean)

3. NARS. *Improvement Plans for the Current Industrial Effluent Guidlines*; National Assembly Research Service: Seoul, Korea, 2015. (In Korean)

4. Carmen, Z.; Daniela, S. Textile organic dyes—Characteristics, polluting effects and separation/elimination procedures from industrial effluents—A critical overview. In *Organic Pollutants Ten Years after the Stockholm Convention—Environmental and Analytical Update*; InTech Euro: Rijeka, Croatia, 2012; pp. 55–85.

5. Hubbe, M.A.; Metts, J.R.; Hermosilla, D.; Blanco, A.; Yerushalmi, L.; Haghighat, F.; Lindholm-Lehto, P.; Khodaparast, Z.; Kamali, M.; Elliott, A. Pulp and paper effluent. *Bioresouces* **2016**, *11*, 1–139.

6. Wentzel, M.C.; Mbewe, A.; Lakay, M.T.; Ekama, G.A. Batch test for characterisation of the carbonaceous materials in municipal wastewaters. *Water SA* **1999**, *25*, 327–336.

7. Henze, M.; van Loosdrecht, M.C.; Ekama, G.A.; Brdjandnovic, D. *Biological Wastewater Treatment—Principles, Modeling and Design*; IWA Publishing: London, UK, 2008.

8. Takahashi, N.; Kumagai, T. Removal of dissolved organic carbon and color from dyeing wastewater by pre-ozonation and subsequent biological treatment. *Ozone Sci. Eng.* **2006**, *28*, 199–205. [CrossRef]

9. Bae, W.; Won, H.; Hwang, B.; Toledo, R.A.; Chung, J.W.; Kwon, K.; Shim, H. Characterization of refractory matters in dyeing wastewater during a full-scale Fenton process following pure-oxygen activated sludge treatment. *J. Hazard. Mater.* **2015**, *287*, 421–428. [CrossRef] [PubMed]

10. Lindholm-Lehto, P.C.; Knuutinen, J.S.; Ahkola, H.S.J.; Herve, S.H. Refractory organic pollutants and toxicity in pulp and paper mill wastewaters. *Environ. Sci. Pollut. Res.* **2015**, *22*, 6473–6499. [CrossRef] [PubMed]

11. Carstea, F.M.; Bridgeman, J.; Baker, A.; Reynolds, D.M. Fluorescence spectroscopy for wastewater monitoring: A review. *Water Res.* **2016**, *95*, 205–219. [CrossRef] [PubMed]

12. Plosz, B.G.; Vogelsang, C.; Macrae, K.; Heiaas, H.; Lopez, A.; Liltved, H.; Langford, K. The BIOZO process—A biofilm system combined with ozonation: Occurrence of xenobiotic organic micro-pollutants in and removal of polycyclic aromatic hydrocarbons and nitrogen from landfill leachate. *Water Sci. Technol.* **2010**, *61*, 3188–3197. [CrossRef] [PubMed]

13. Ince, B.K.; Cetecioglu, Z.; Ince, O. Pollution prevention in the pulp and paper industries. In *Environmental Management in Practice*; InTech: Rijeka, Croatia, 2011.

14. Wentzel, M.C.; Mbewe, A.; Ekama, G.A. Batch test for measurement of readily biodegradable COD and active organism concentrations in municipal waste waters. *Water SA* **1995**, *21*, 118–124.

15. Yang, L.; Han, D.H.; Lee, B.M.; Hur, J. Characterizing treated wastewaters of different industries using clustered fluorescence EEM–PARAFAC and FT-IR spectroscopy: Implications for downstream impact and source identification. *Chemosphere* **2015**, *127*, 222–228. [CrossRef] [PubMed]

16. Ekama, G.A.; Dold, P.A.; Marais, G.v.R. Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge. *Water Sci. Technol.* **1986**, *18*, 91–114.

17. Grady, C.P.L.; Daigger, G.T.; Love, N.G.; Filipe, C.D.M. *Biological Wastewater Treatment*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2011.

18. Orhon, D.; Cokgor, U.E. COD Fractionation in Wastewater Characterization—The State of the Art. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 283–293. [CrossRef]

19. Hu, Z.; Chanadran, K.; Smets, B.F.; Grasso, D. Evaluation of a rapid physical-chemical method for the determination of extant soluble COD. *Water Res.* **2002**, *36*, 617–624. [CrossRef]

20. Mamais, D.; Jenkins, D.; Pitt, P. A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Res.* **1993**, *27*, 195–197. [CrossRef]

21. Hur, J.; Cho, J. Prediction of BOD, COD, and total nitrogen concentrations in a typical urban river using a fluorescence excitation–emission matrix with PARAFAC and UV absorption indices. *Sensors* **2012**, *12*, 972–986. [CrossRef] [PubMed]

22. Stedmon, C.A.; Bro, R. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. *Limnol. Oceanogr. Methods* **2008**, *6*, 572–579. [CrossRef]

23. Choi, E.; Rhu, D.; Yun, Z.; Lee, E. Temperature effects on biological nutrient removal system with weak municipal wastewater. *Water Sci. Technol.* **1998**, *37*, 219–226. [CrossRef]

24. Mikola, A.M.K.; Vahala, R.; Rautiainen, J.A. Factors affecting the quality of the plant influent and its suitability for prefermentation and the biological nutrient removal process. *J. Environ. Eng.* **2011**, *137*, 1185–1192. [CrossRef]

25. EnviroSim Associates. *General Activated Dludge-Digestion Model (General ASDM)*; EnviroSim Associates: Hamilton, ON, Canada, 2007.

26. Ni, B.J.; Fang, F.; Xie, W.M.; Sun, M.; Sheng, G.P.; Li, W.H.; Yu, H.Q. Characterization of extracellular polymeric substances produced by mixed microorganisms in activated sludge with gel-permeating chromatography, excitation–emission matrix fluorescence spectroscopy measurement and kinetic modeling. *Water Res.* **2009**, *43*, 1350–1358. [CrossRef] [PubMed]

27. Shen, Y.X.; Xiao, K.; Liang, P.; Sun, J.Y.; Sai, S.J.; Huang, X. Characterization of soluble microbial products in 10 large-scale membrane bioreactors for municipal wastewater treatment in China. *J. Membr. Sci.* **2012**, *415*, 336–345. [CrossRef]

28. Fan, J.; Li, H.; Shuang, C.; Li, W.; Li, A. Dissolved organic matter removal using magnetic anion exchange resin treatment on biological effluent of textile dyeing wastewater. *J. Environ. Sci.* **2014**, *26*, 1567–1574. [CrossRef] [PubMed]

29. Yu, H.B.; Song, Y.H.; Tu, X.; Du, E.D.; Liu, R.X.; Peng, J.F. Assessing removal efficiency of dissolved organic matter in wastewater treatment using fluorescence excitation emission matrices with parallel factor analysis and second derivative synchronous fluorescence. *Bioresour. Technol.* **2013**, *144*, 595–601. [CrossRef] [PubMed]

30. Cohen, E.; Levy, G.J.; Borisover, M. Fluorescent components of organic matter in wastewater: Efficacy and selectivity of the water treatment. *Water Res.* **2014**, *55*, 323–334. [CrossRef] [PubMed]

31. Murphy, K.R.; Hambly, A.; Singh, S.; Henderson, R.K.; Baker, A.; Stueyz, R.; Khan, S.J. Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC model. *Environ. Sci. Technol.* **2011**, *45*, 2909–2916. [CrossRef] [PubMed]

32. Li, W.T.; Chen, S.Y.; Xu, Z.X.; Li, Y.; Shuang, C.D.; Li, A.M. Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scan: A comparative study. *Environ. Sci. Technol.* **2014**, *48*, 2603–2609. [CrossRef] [PubMed]

33. Ou, H.S.; Wei, C.H.; Mo, C.H.; Wu, H.Z.; Ren, Y.; Feng, C.H. Novel insights into anoxic/aerobic1/aerobic2 biological fluidized-bed system for coke wastewater treatment by fluorescence excitation–emission matrix spectra coupled with parallel factor analysis. *Chemosphere* **2014**, *113*, 158–164. [CrossRef] [PubMed]

34. Kreetachat, T.; Damrongsri, M.; Punsuwon, V.; Vaithanomsat, P.; Chiemchaisri, C. Effects of ozonation process on lignin-derived compounds in pulp and paper mill effluents. *J. Hazard. Mater.* **2007**, *142*, 250–257. [CrossRef] [PubMed]

35. Ghaly, M.Y.; Jamil, T.S.; EL-Seesy, I.E.; Souaya, E.R.; Nasr, R.A. Treatment of highly polluted paper mill wastewater by lolar photocatalytic oxidation with synthesized nano TiO_2. *Chem. Eng. J.* **2011**, *168*, 446–454. [CrossRef]