



Monitoring the performance of wastewater treatment plants for organic matter removal using excitation-emission matrix fluorescence

Francisco J. Rodríguez-Vidal^{a,*}, María García-Valverde^b, Beatriz Ortega-Azabache^b,
Ángela González-Martínez^b, Ana Bellido-Fernández^b, Victorino Díez-Blanco^c,
M. Olga Ruíz-Pérez^c

^a Department of Chemistry. Higher Polytechnic School. University of Burgos. Av Cantabria s/n, 09006 Burgos, Spain

^b Department of Chemistry. Faculty of Sciences. University of Burgos. Pz Misael Bañuelos s/n, 09001 Burgos, Spain

^c Department of Biotechnology and Food Science. Faculty of Sciences. University of Burgos. Pz Misael Bañuelos s/n, 09001 Burgos, Spain

ARTICLE INFO

Keywords:

Fluorescence
Excitation-emission matrix (EEM)
Industrial wastewater
Wastewater treatment plants
Organic matter removal

ABSTRACT

This study has assessed the usefulness of excitation-emission matrix fluorescence (EEMF) as a fast and simple analytical technique to track changes in dissolved organic matter (DOM) during the sequence of treatment in wastewater treatment plants (WWTPs). Three different industrial wastewaters and treatment plants have been studied in this work: an industrial park wastewater treated in an independent line at the Burgos WWTP (Spain), a food industry wastewater (crisps and snacks manufacturing) that was treated in a MBR (Membrane Biological Reactor) pilot plant (University of Burgos) and a municipal landfill leachate treated in a physicochemical treatment plant within the same landfill.

Removal percentages for the wastewater organic matter at each stage of the treatment plants were successfully obtained by monitoring the main fluorescence peaks: protein-like peaks (tryptophan-like peaks T1, T2 and tyrosine-like peaks B1, B2), humic-like peaks (fulvic-like peak A and humic-like peak C) and microbially-derived peak M. Therefore, EEMF readily allows the assessment of the reactivity of the different types of organic matter towards specific treatments, such as clarification, biodegradation, filtration, etc. Among the wastewaters studied, the food industry wastewater exhibited the greater diversity of fluorescence peaks (B1, B2, T1, T2, A1, A2 and M) whereas the landfill leachate only showed the presence of humic substances (mainly humic-like peak C). This study has demonstrated that EEMF is a useful and user-friendly technique to monitor the performance of wastewater treatment plants for organic matter removal, allowing a rapid response to potential problems in the treatment.

1. Introduction

One of the main objectives in wastewater treatment plants (WWTPs) is to remove organic matter from water, mainly through biological treatments. Wastewater dissolved organic matter (DOM) comprises a diversity of compounds, namely simple sugars, small organic acids such as acetic acid, amino acids, proteins, polysaccharides, fatty acids, humic substances, etc [1,2,3,4,5]. Certain industrial wastewaters, in addition to the aforementioned compounds, can also contain various xenobiotic substances such as organic solvents, chlorinated aromatics, phenols and other chemicals coming from the production process and the cleaning of tanks and reactors [6]. Widely used parameters in WWTPs such as biochemical oxygen demand (BOD) and chemical oxygen demand

(COD) are quite time-consuming techniques that do not allow a rapid response to potential problems in the sequence of treatment. On the contrary, fluorescence is a fast and user-friendly technique that only requires a simple pre-treatment of the sample in most cases [4,7,8].

Excitation-emission matrix fluorescence (EEMF) is nowadays the most used fluorescence technique for studying DOM in water [9,10], the acquisition of an EEMF spectrum only taking around 8 min. Routine analysis in WWTPs requires simple and fast techniques and WWTP staff demands real-time information to act as quickly as possible to solve potential problems in the treatment and fluorescence meets that requirement. EEMF peaks commonly found in wastewaters are the following ($\lambda_{ex}/\lambda_{em}$, in nm) [4,8]:

* Corresponding author.

E-mail address: qpvito@ubu.es (F.J. Rodríguez-Vidal).

<https://doi.org/10.1016/j.microc.2022.107177>

Received 25 March 2021; Received in revised form 2 January 2022; Accepted 3 January 2022

Available online 6 January 2022

0026-265X/© 2022 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

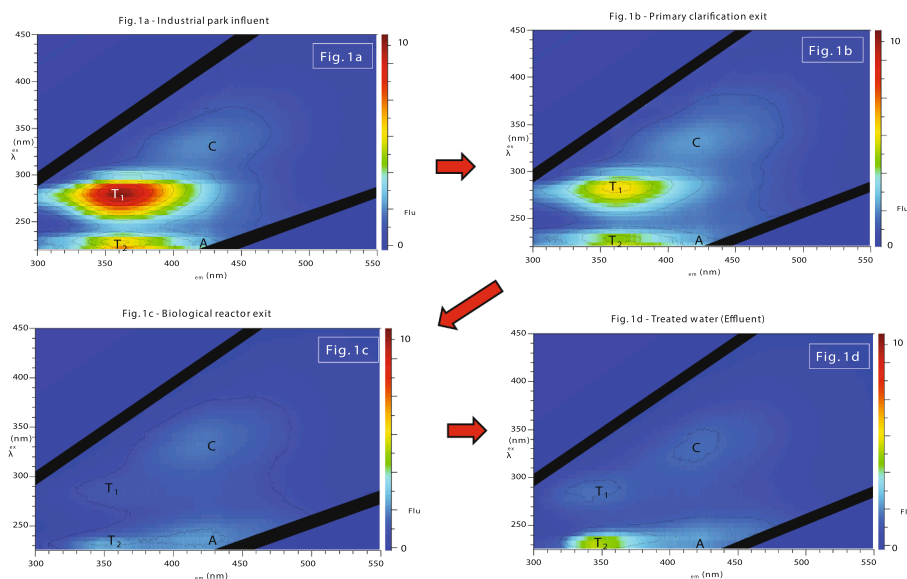


Fig. 1. EEMF spectra for the industrial park wastewater through the main stages of Burgos WWTP (Spain).

- **Protein-like peaks:** tyrosine-like peaks B₁ (275–310/305–320 nm) and B₂ (220–237/305–320 nm), tryptophan-like peaks T₁ (275–285/320–350 nm) and T₂ (tryptophan-like: 215–237/340–381 nm).
- **Humic-like peaks:** humic-like peak C (320–360/420–460 nm) and fulvic-like peak A (230–260/400–480 nm).

Protein-like peaks, which are related to organic matter of anthropogenic origin and organic compounds released by the microorganisms involved in biological treatments at WWTPs, are usually dominant in wastewaters [11,12]. On the contrary, humic-like peaks usually predominate in natural waters [13,14].

Fluorescence has been profusely used to characterize DOM in natural waters (rivers, seawater, etc) [15,16]. As to wastewater, EEMF has been mainly applied to the study of urban WWTPs effluents [6,17,18,19,20,21,22,23] but little attention has been paid to the characterization of industrial wastewaters and even less to the study of the intermediate stages of WWTPs, namely primary clarification and biological reactor stages.

Therefore, this study has three main objectives:

- Demonstrate the usefulness of EEMF as a simple and viable tool to quickly detect and sort out any deficiency in wastewater treatment.
- Study the efficiency of WWTPs to remove DOM from industrial wastewaters using EEMF as an analytical technique.
- Characterize the behaviour and fate of DOM in WWTPs intermediate stages, not only in the effluent.

2. Material and methods

2.1. Wastewaters and wastewater treatment plants.

Three different industrial wastewaters and wastewater treatment plants were studied in this work:

- An industrial wastewater coming from the industrial park of Villalonguejar (Burgos, Spain). Most factories in this industrial site belong to food, pharmaceutical and paper industry. This wastewater is treated at the municipal wastewater treatment plant of Burgos city (see the main features and pictures of the plant at the Supplementary online material). This plant has two different treatment lines: one of them is for treating the domestic wastewater of the city of Burgos and the other one is for the treatment of the wastewater from the

industrial site. Several samples were obtained from March to May 2018 (six samples in total, once a fortnight). Four sampling points within the plant were chosen for this study: Influent (raw wastewater), Primary Clarification exit, Biological Reactor exit (activated sludge supernatant) and Effluent (final treated water after secondary clarification).

- A food industry wastewater coming from crisps and snacks manufacturing in an industry from the industrial park of Burgos (Spain). This wastewater was treated in a pilot plant at the laboratory of the Chemical Engineering Department at the University of Burgos (UBU). The pilot plant consists of a typical MBR (Membrane Biological Reactor) and the sampling points were the following: Influent (raw wastewater), Biological Reactor exit (prior to filtration) and Effluent (treated water after the filtration stage). A total of four different samples were collected from April to May 2018.
- A leachate coming from the urban landfill of the city of Burgos (Spain), which is treated at a wastewater treatment plant within the same landfill. This plant consists of a physicochemical treatment (precipitation of heavy metals using lime followed by sedimentation) and ammonia removal by air stripping at basic pH). In this case, it was not possible to sample any more points other than the influent and the effluent of the treatment plant. Three different samples were collected from March to May 2018.

All wastewater samples were filtered and adjusted to pH 7.

2.2. Fluorescence measurements.

A spectrofluorometer (Varian Cary Eclipse: 1-cm cuvette, room temperature conditions: 19–21 °C) was used to collect fluorescence spectra. The settings of the instrument were: range of emission wavelengths: 350–500 nm (with a wavelength step of 1 nm), range of excitation wavelengths: 220–450 nm (with a wavelength step of 5 nm) and a scan speed of 600 nm/min. The spectra collected were then rearranged and displayed in the form of a two-dimensional contour map of fluorescence intensity as a function of excitation and emission wavelengths (EEM matrix) using QtiPlot software, the variation in fluorescence intensity (triplicate experiments) was less than 9%, the spectrofluorometer being auto-zeroed before each analysis. Fluorescence intensity data provided by the instrument were normalized to Raman units (R.U.) using the Raman peak of Milli-Q water at $\lambda_{ex} = 348$ nm [6] for the purpose of literature comparison. The Raman emission at $\lambda_{em} = 395$ nm

Table 1
Main peaks identified in the wastewaters.

| Wastewater/WWTP | WWTP Stage | T ₁ | T ₂ | A | C | B ₁ | B ₂ | M |
|--|---------------------------|------------------------------|-----------------------------|--|------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Industrial wastewater (industrial park) – Municipal wastewater treatment plant | Influent (raw wastewater) | 280/359 nm (10.42 ± 0.94) | 225/369 nm (7.05 ± 0.63) | 225/422 nm(3.74 ± 0.34) | 335/418 nm (2.16 ± 0.19) | — | — | — |
| | Primary Settlement exit | 280/360 nm (5.92 ± 0.53) | 225/362 nm (5.02 ± 0.45) | 225/422 nm(3.15 ± 0.28) | 330/419 nm (1.90 ± 0.17) | — | — | — |
| | Biological Reactor exit | 285/361 nm (0.99 ± 0.01) | 230/359 nm (2.20 ± 0.20) | 225/422 nm(1.88 ± 0.16) | 330/420 nm (1.64 ± 0.14) | — | — | — |
| | Effluent (treated water) | 290/366 nm (1.06 ± 0.01) | 230/353 nm (2.08 ± 0.17) | 225/423 nm(1.70 ± 0.13) | 330/421 nm (1.45 ± 0.11) | — | — | — |
| Food industry wastewater – MBR pilot plant (University of Burgos) | Influent (raw wastewater) | 275/353 nm (4.78 ± 0.43) | 225/357 nm (2.76 ± 0.25) | A1: 230/423 nm(4.69 ± 0.42)A2: 260/430 nm(6.68 ± 0.60) | — | 275/308 nm (7.71 ± 0.69) | 225/306 nm (2.66 ± 0.24) | 295/425 nm (6.96 ± 0.63) |
| | Biological Reactor exit | 285/360 nm (2.04 ± 0.18) | 230/354 nm (2.16 ± 0.19) | A1: 230/422 nm(4.69 ± 0.42)A2: 255/429 nm(4.83 ± 0.43) | — | 275/306 nm (2.59 ± 0.23) | 225/307 nm (1.72 ± 0.15) | 290/423 nm (3.76 ± 0.34) |
| | Effluent (treated water) | 285/361 nm (1.11 ± 0.10) | 230/352 nm (1.63 ± 0.14) | A1: 230/423 nm(3.81 ± 0.34)A2: 250/429 nm(2.86 ± 0.25) | — | 280/308 nm (0.36 ± 0.03) | 225/307 nm (0.49 ± 0.04) | 290/425 nm (2.17 ± 0.19) |
| Urban landfill leachate – Landfill wastewater treatment plant | Influent (raw wastewater) | — | — | 250/454 nm(5.13 ± 0.46) | 345/426 nm (17.55 ± 1.58) | — | — | — |
| | Effluent (treated water) | — | — | 255/459 nm(2.93 ± 0.26) | 350/432 nm (8.53 ± 0.77) | — | — | — |

$\lambda_{ex} / \lambda_{em}$ (Fluorescence Intensity). λ in nm, Fluorescence Intensity in Raman units (R.U).

was routinely measured to check the stability of the spectrofluorometer, averaging 29.3 ± 1.8 fluorescence intensity units ($n = 28$). Inner-filter effects correction was necessary due to the high organic load of wastewater samples; this correction was made by means of absorbance spectroscopy [24,25]. The following limits of detection (in Raman Units: RU) have been estimated for the EEM fluorescence peaks found in this study: peak A: 0.071 RU, peak C: 0.015 RU, peak T1: 0.026 RU, peak T2: 0.034 RU, peak B1: 0.062 RU, peak B2: 0.092 RU, peak M: 0.053 RU.

3. Results

3.1. Industrial park wastewater/municipal wastewater treatment plant

Fig. 1 shows the EEMF spectra for the industrial park wastewater through the different treatment stages at Burgos WWTP, arranged as follows: Fig. 1.a: industrial raw wastewater (Influent), Fig. 1.b: primary clarification exit, Fig. 1.c: biological reactor exit (activated sludge supernatant) and Fig. 1.d: final treated water after secondary clarification (Effluent). Table 1 shows the fluorescence intensities (Raman units) of the different peaks and their location coordinates ($\lambda_{ex}/\lambda_{em}$) and Fig. 4 shows the percentages of fluorescence removal between two consecutive stages and the total removal in the effluent.

As shown in Fig. 1.a and Table 1, protein-like peaks T1 and T2 clearly predominate in the industrial wastewater influent (peak T1 showing by far the greatest fluorescence), which is in accordance with the majority presence of food sector industries at the industrial park (slaughterhouses, biscuit, dairy and brewery industries). Humic-like peaks A and C, coming from the public water supply of the city, are also present in the influent spectrum but with a remarkably lower fluorescence; this potable water originates from the Úzquiza Reservoir (Burgos) and is mostly composed of fulvic acids [8].

Total removals in the effluent (Fig. 4) are in the following order: peak T1 (90%) > peak T2 (70%) > peak A (54%) > peak C (33%). Despite being the majority peak in the influent, protein-like peak T1 shows the lowest fluorescence in the effluent, due to its noticeably high removal rate through the treatment: this peak shows both the greatest partial removal by biodegradation (biological reactor stage: 83%) and by

sedimentation (primary clarification stage: 43%) of all the peaks (see Fig. 4). Peak T2 also shows good partial and total removal percentages, especially by biodegradation [6,8,22,26], results that were expected since protein-like substances are biodegradable in nature [12,22,27]. The different behavior shown by peaks T1 and T2 through WWTP stages is indicative of their different composition. In fact, some authors make that difference by referring to T1 as “soluble microbial by-products”, whereas T2 is denoted as “aromatic protein-like” [28,29,30].

On the contrary, humic substances are refractory organic matter usually poorly removed by biodegradation processes [6,18,31,32]. Fulvic-like peak A shows a higher removal at the biological reactor stage than humic-like peak C (see Fig. 4), which is in accordance with the lower average molecular weight attributed to fulvic acids [33].

There is a slight increase in peak T1 fluorescence (7%) at the secondary clarifier stage in comparison to the previous stage (see Fig. 4). This apparent contradiction might be explained by the release of soluble microbial products (SMP) from the biomass present in the secondary clarifier, SMP that are closely related to peak T1 fluorescence as was aforementioned [34,35]. Actually, some authors indicate that effluent organic matter in urban WWTP is mostly composed of SMP [1,12,36,37].

It is interesting to note that protein-like peaks shift their location coordinates ($\lambda_{ex}/\lambda_{em}$) when comparing the influent and the effluent (see Table 1). Peak T1 shows a shift towards longer wavelengths (red-shift) in both λ_{ex} and λ_{em} (from 280/359 nm in the influent to 290/366 nm in the effluent), whereas peak T2 shows a mixed behavior: a red-shift for λ_{ex} but a blue-shift for λ_{em} (from 225/369 nm in the influent to 230/353 nm in the effluent). As suggested earlier, these shifts may be indicative of a change in the composition of those fluorophores following treatment, which is in line with the release of SMP to the effluent by the microorganisms present in the secondary clarifier. However, this shift is not observed for the humic-like peaks (see Table 1).

3.2. Food industry wastewater/MBR pilot plant

A food industry (crisps and snacks manufacturing) wastewater was treated in a MBR pilot plant at Burgos University. EEMF spectra for the

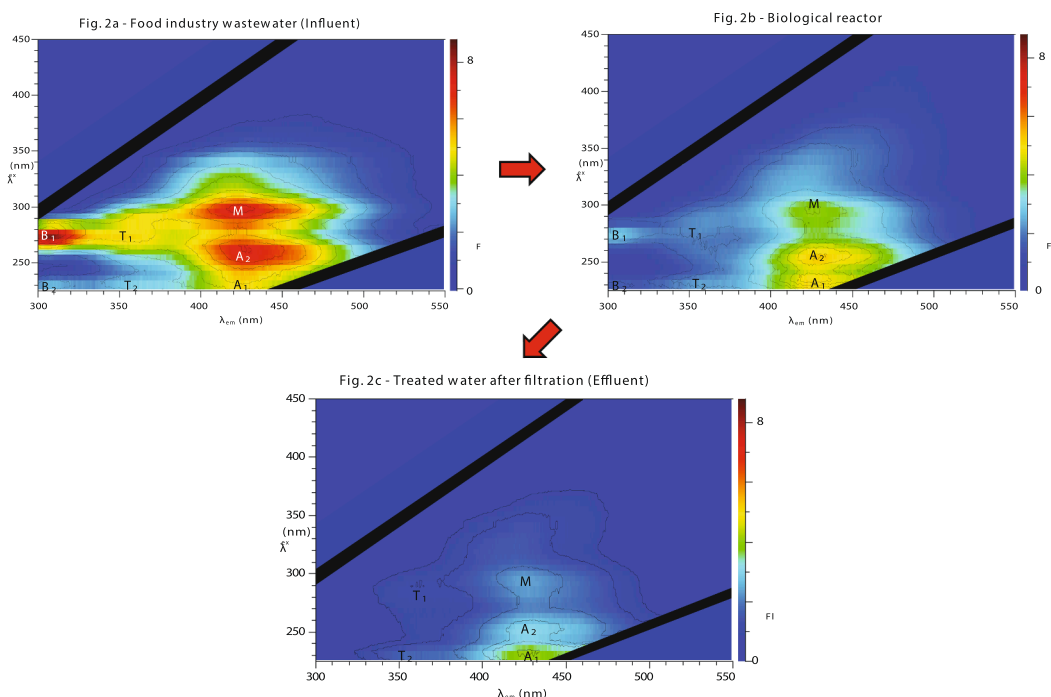


Fig. 2. EEMF spectra for the food industry wastewater through the main stages of the MBR pilot plant.

different stages are shown in Fig. 2, total and partial removals are depicted in Fig. 4 and peak information (location coordinates and fluorescence intensity) is in Table 1.

As shown in Fig. 2.a, this wastewater influent is quite different from the previous one. There is a greater diversity with regard to protein material content: in addition to tryptophan-like peaks (T1 and T2), tyrosine-like peaks (B1 and B2) appear on the left side of the spectrum. As to humic-like peaks, there are also significant differences in comparison with the industrial park influent: fulvic-like peak A is found subdivided into two peaks (A1 and A2) and humic-like peak C is absent but a new peak M is detected instead (see Fig. 2.a). Peak M is indicative of the presence of organic material from recent biological activity (microbially-derived organic compounds) [9,26,32,38]. The main peaks in the influent are in the following order of fluorescence intensity: $B1 > M > A2$.

The peaks showing the greatest total removal are the tyrosine-like peaks B1 and B2 (95% and 82%, respectively) (see Fig. 4). This result is in line with others reported in the literature, which indicate that tyrosine-like fluorescence is preferentially removed by treatments including oxic processes [12,32,39]. Additionally, peaks B1 and B2 also show the highest removal at the filtration stage (ultrafiltration membrane), which suggests that these fluorophores should be composed of high molecular-weight protein-like molecules.

On the contrary, fulvic-like peak A1 is by far the least removed

through the whole treatment (only 19% of total removal), showing virtually no biodegradability and a remarkably poor removal by membrane filtration (the least removal of all the peaks). These results suggest that peak A1 should comprise non-biodegradable, low-molecular-weight fulvic-like material.

Total removals for the whole set of peaks are as follows (see Fig. 4): $B1 > B2 > T1 > M > A2 > T2 > A1$. Concerning the biodegradable nature of the fluorophores, the peaks showing the highest removal at the biological reactor stage are: $B1 > T1 > M > B2$. As in Section 3.1, a shift in the location coordinates of peaks T1 and T2 is observed when moving from the influent to the effluent but this behavior is not detected for the rest of the peaks.

3.3. Urban landfill leachate/ physicochemical treatment plant

Urban landfill leachates comprise many pollutants, such as heavy metals, dissolved organic matter (humic substances, carbohydrates, peptides, fatty acids, etc) and several persistent organic pollutants (POPs) from plastics, pharmaceuticals and pesticides [40]. As a landfill works as a giant anaerobic reactor, leachate composition closely depends on landfill age; that is why some researchers proposed a classification of landfills on the basis of their age [41,42]: young (*less than 5* years), medium-aged (5–10 years) and old landfills (>10 years) (these limits are slightly variable). Young landfill leachates usually show

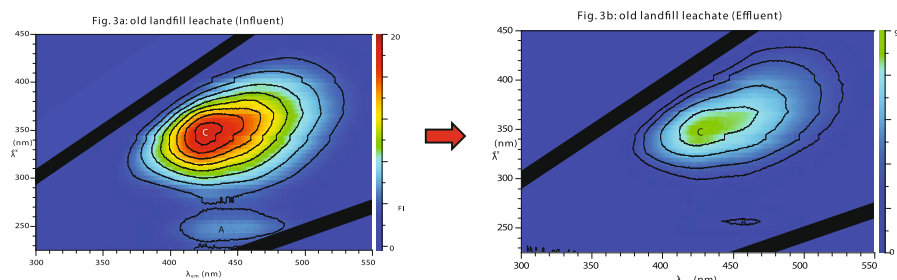


Fig. 3. EEMF spectra for an old landfill leachate through a physicochemical treatment plant.

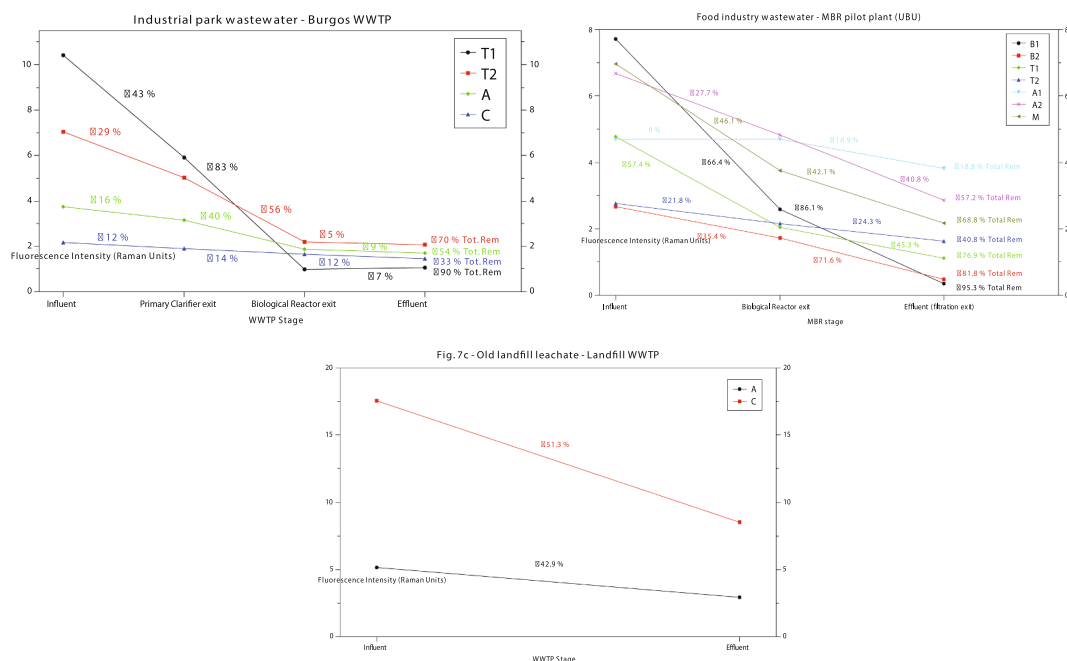


Fig. 4. Partial and total removals for the different wastewaters and treatment plants.

protein-like peaks (T and B) as the main fluorescence (most organic matter is still composed of labile compounds, such as carbohydrates and proteins) whereas humic-like fluorescence (mainly peak C) is predominant in medium-aged and old landfill leachates (organic matter has spent enough time within the landfill to undergo humification processes) [4,43,44,45,46,47,48]. Fig. 3 shows the EEMF spectra for an old landfill leachate, humic-like peaks being the only fluorescence present in the spectra: a greatly intense peak C (notice its high fluorescence intensity in comparison to wastewater fluorescence levels in previous sections) and a minority peak A, which is in line with the literature reported earlier. In this case, only two points (influent and effluent) could be sampled at the physicochemical treatment plant located within the landfill (see Section 2.1). Total removals for both peaks are alike, peak C showing a slightly greater removal (51%) than peak A (43%) (see Fig. 4). This result is in accordance with the treatment used at the landfill plant (sedimentation with lime, only physicochemical treatments with no biological processes), since humic acids (peak C) are macromolecules with a higher molecular weight and more readily removed by sedimentation than fulvic acids (peak A) [6,18,31,32].

4. Conclusions

This work studies the usefulness of EEMF as a fast and simple analytical technique to track changes in DOM during the sequence of treatment in wastewater treatment plants. Three different wastewaters and treatment plants have been studied in this work.

The industrial park wastewater is characterized by the majority presence of protein-like peaks (T1 and T2), which show the greater total removals at Burgos WWTP. EEMF also enables us to draw conclusions about the release of soluble microbial products (SMP) from the secondary clarifier biomass and a different fluorophore composition when comparing the influent and the effluent. The food industry wastewater (crisps and snacks manufacturing) treated at a MBR pilot plant exhibited the greater diversity of fluorescence peaks (B1, B2, T1, T2, A1, A2 and M), tyrosine-like peaks B1 and B2 showing the greatest total removals and fulvic-like peak A1 showing virtually no biodegradability. Humic-like peak C clearly dominates in the landfill leachate EEMF spectrum, showing around 50% removal in the physicochemical treatment plant.

This study has demonstrated that EEMF is a useful and user-friendly

technique to monitor the performance of wastewater treatment plants for organic matter removal, allowing a rapid response to potential problems in the treatment. The acquisition of a full EEMF spectrum usually takes around 15 min and the pre-treatment of the sample is very simple: pH adjustment, filtration (especially for wastewater influents) and dilution (for samples with high organic matter load if inner filter effects are not corrected by absorbance spectroscopy). EEMF could complement and partially substitute other traditional and time-consuming techniques such as Chemical Oxygen Demand (COD: time analysis = 2 h) and Biochemical Oxygen Demand (BOD: time analysis = 5 days) in routine analysis at WWTPs. Therefore, the implementation of the EEMF technique in WWTPs laboratories is highly recommended.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2022.107177>.

References

- [1] M. Esparza-Soto, S. Núñez-Hernández, C. Fall, Spectrometric characterization of effluent organic matter of a sequencing batch reactor operated at three sludge retention times, *Water Res.* 45 (19) (2011) 6555–6563, <https://doi.org/10.1016/j.watres.2011.09.057>.
- [2] F. Huang, F. Meng, M. Fan, Y. Zhao, X. Wu, L. Shen, Fluorescence enhancement effect of Eu(III)-thenoyltrifluoroacetone-cetyltrimethyl ammonium bromide in water-dissolved organic matter extracted from wheat straw, *Spectrochim. Acta Part A.* 151 (2015) 302–307, <https://doi.org/10.1016/j.saa.2015.06.090>.
- [3] K. Komatsu, T. Onodera, A. Kohzu, K. Syutsubo, A. Imai, Characterization of dissolved organic matter in wastewater during aerobic, anaerobic and anoxic treatment processes by molecular size and fluorescence analyses, *Water Res.* 171 (2020) 115459, <https://doi.org/10.1016/j.watres.2019.115459>.
- [4] F.J. Rodríguez-Vidal, M. García-Valverde, B. Ortega-Azabache, A. González-Martínez, A. Bellido-Fernández, Characterization of urban and industrial wastewaters using excitation-emission matrix (EEM) fluorescence: Searching for specific fingerprints, *J. Environ. Manag.* 263 (2020) 110396, <https://doi.org/10.1016/j.jenvman.2020.110396>.

- [5] H. Yu, F. Qu, L. Sun, H. Liang, Z. Han, H. Chang, S. Shao, G. Li, Relationship between soluble microbial products (SMP) and effluent organic matter (EfOM): Characterized by fluorescence excitation emission matrix coupled with parallel factor analysis, *Chemosphere* 121 (2015) 101–109, <https://doi.org/10.1016/j.chemosphere.2014.11.037>.
- [6] L. Yang, J. Hur, W. Zhuang, Occurrence and behaviors of fluorescence EEM-PARAFAC components in drinking water and wastewater treatment systems and their applications: a review, *Environ. Sci. Pollut. Res.* 22 (9) (2015) 6500–6510.
- [7] C. Qian, W. Chen, W. Li, H.Q. Yu, A chemometric analysis on the fluorescent dissolved organic matter in a full-scale sequencing batch reactor for municipal wastewater treatment, *Frontiers Environ. Sci. Eng.* 11 (2017) 12.
- [8] F.J. Rodríguez-Vidal, M. García-Valverde, B. Ortega-Azabache, Á. González-Martínez, A. Bellido-Fernández, Using excitation-emission matrix fluorescence to evaluate the performance of water treatment plants for dissolved organic matter removal, *Spectrochimica Acta A* 249 (2021) 119298, <https://doi.org/10.1016/j.saa.2020.119298>.
- [9] K.R. Murphy, A. Hambly, R.K. Henderson, A. Baker, S.J. Khan, Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC mode, *Environ. Sci. Technol.* 45 (2011) 2909–2916.
- [10] H. Yin, Y. Wang, Y. Yang, J. Huang, Z. Xu, Tryptophan-like fluorescence as a fingerprint of dry-weather misconnections into storm drainage system, *Environ. Sci. Eur.* 32 (2020) 61, <https://doi.org/10.1186/s12302-020-00336-3>.
- [11] W.-T. Li, S.-Y. Chen, Z.-X. Xu, Y. Li, C.-D. Shuang, A.-M. Li, Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scan: A comparative study, *Environ. Sci. Technol.* 48 (5) (2014) 2603–2609, <https://doi.org/10.1021/es404624q>.
- [12] H. Yu, Y. Song, X. Tu, E. Du, R. Liu, J. Peng, 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.* 144 (2013) 595–601, <https://doi.org/10.1016/j.biortech.2013.07.025>.
- [13] M. Borisover, Y. Laor, I. Saadi, M. Lado, N. Bukhanovsky, Tracing organic footprints from industrial effluent discharge in recalcitrant riverine chromophoric dissolved organic matter, *Water Air Soil Pollut.* 222 (1-4) (2011) 255–269.
- [14] H. Yu, Y. Song, E. Du, N. Yang, J. Peng, R. Liu, Comparison of PARAFAC components of fluorescent dissolved and particular organic matter from two urbanized rivers, *Environ. Sci. Pollut. Res.* 23 (2016) 10644–10655.
- [15] D. Markechová, M. Tomková, J. Sádecká, Fluorescence excitation-emission matrix spectroscopy and parallel factor analysis in drinking water treatment: a review, *Pol. J. Environ. Stud.* 22 (2013) 1289–1295.
- [16] Y. Shutova, A. Baker, J. Bridgeman, R.K. Henderson, Spectroscopic characterization of dissolved organic matter in drinking water treatment: from PARAFAC analysis to online monitoring wavelengths, *Water Res.* 54 (2014) 159–169, <https://doi.org/10.1016/j.watres.2014.01.053>.
- [17] J. Bridgeman, A. Baker, C. Carliell-Marquet, E. Carstea, Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy, *Environ. Technol.* 34 (23) (2013) 3069–3077.
- [18] E. Cohen, G.J. Levy, M. Borisover, Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment, *Water Res.* 55 (2014) 323–334, <https://doi.org/10.1016/j.watres.2014.02.040>.
- [19] X. Guo, H. Yu, Z. Yan, H. Gao, Y. Zhang, Tracking variations of fluorescent dissolved organic matter during wastewater treatment by accumulative fluorescence emission spectroscopy combined with principal component, second derivative and canonical correlation analyses, *Chemosphere* 194 (2018) 463–470, <https://doi.org/10.1016/j.chemosphere.2017.12.023>.
- [20] J. Li, L. Wang, J. Geng, S. Li, Q. Yu, K.e. Xu, H. Ren, Distribution and removal of fluorescent dissolved organic matter in 15 municipal wastewater treatment plants in China, *Chemosphere* 251 (2020) 126375, <https://doi.org/10.1016/j.chemosphere.2020.126375>.
- [21] K. Xiao, J. Yu, S. Wang, J. Du, J. Tan, K. Xue, Y. Wang, X. Huang, Relationship between fluorescence excitation-emission matrix properties and the relative degree of DOM hydrophobicity in wastewater treatment effluents, *Chemosphere* 254 (2020) 126830, <https://doi.org/10.1016/j.chemosphere.2020.126830>.
- [22] L. Yang, H.S. Shin, J. Hur, Estimating the concentration and biodegradability of organic matter in 22 wastewater treatment plants using fluorescence excitation emission matrices and parallel factor analysis, *Sensors* 14 (2014) 1771–1786, <https://doi.org/10.3390/s140101771>.
- [23] J. Yu, K. Xiao, W. Xue, Y.-X. Shen, J. Tan, S. Liang, Y. Wang, X. Huang, Excitation-emission matrix (EEM) fluorescence spectroscopy for characterization of organic matter in membrane bioreactors: Principles, methods and applications, *Frontiers Environ. Sci. Eng.* 14 (2) (2020), <https://doi.org/10.1007/s11783-019-1210-8>.
- [24] D.M. McKnight, E.W. Boyer, P.K. Westerhoff, P.T. Doran, T. Kulbe, D.T. Andersen, Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity, *Limnol. Oceanogr.* 46 (1) (2001) 38–48.
- [25] T. Ohno, Fluorescence inner-filtering correction for determining the Humification Index of dissolved organic matter, *Environ. Sci. Technol.* 36 (2002) (2002) 742–746.
- [26] M. Riopel, F. Caron, S. Siemann, Fluorescence characterization of natural organic matter at a Northern Ontario Wastewater Treatment Plant, *Water Air Soil Pollut.* 225 (2014) 2126–2143.
- [27] L. Gao, F. Han, X. Zhang, B. Liu, D. Fan, X.u. Sun, Y. Zhang, L. Yan, D. Wei, Simultaneous nitrate and dissolved organic matter removal from wastewater treatment plant effluent in a solid-phase denitrification biofilm reactor, *Bioresour. Technol.* 314 (2020) 123714, <https://doi.org/10.1016/j.biortech.2020.123714>.
- [28] F. Meng, Z. Zhou, B.-J. Ni, X. Zheng, G. Huang, X. Jia, S. Li, Y.a. Xiong, M. Kraume, Characterization of the size-fractionated biomacromolecules: tracking their role and fate in a membrane bioreactor, *Water Res.* 45 (15) (2011) 4661–4671, <https://doi.org/10.1016/j.watres.2011.06.026>.
- [29] N. Peng, K. Wang, N. Tu, Y. Liu, Z. Li, Fluorescence regional integration combined with parallel factor analysis to quantify fluorescent spectra for dissolved organic matter released from manure biochars, *RSC Adv.* 10 (2020) 31502.
- [30] J. Zhou, J.-J. Wang, A. Baudon, A.T. Chow, Improved fluorescence excitation-emission matrix regional integration to quantify spectra for fluorescent dissolved organic matter, *J. Environ. Qual.* 42 (3) (2013) 925–930.
- [31] K. Xiao, Y. Shen, J. Sun, S. Liang, H. Fan, J. Tan, X. Wang, X. Huang, T.D. Waite, Correlating fluorescence spectral properties with DOM molecular weight and size distribution in wastewater treatment systems, *Environ. Sci. Wat. Res. Tech.* 4 (12) (2018) 1933–1943.
- [32] H. Yu, Y. Song, R. Liu, H. Pan, L. Xiang, F. Qian, Identifying changes in dissolved organic matter content and characteristics by fluorescence spectroscopy coupled with self-organizing map and classification and regression tree analysis during wastewater treatment, *Chemosphere* 113 (2014) 79–86, <https://doi.org/10.1016/j.chemosphere.2014.04.020>.
- [33] F.J. Rodríguez, L. Marcos, L. Núñez, Effects of Ozonation on Natural Organic Matter Reactivity in Adsorption and Biodegradation Processes – A Case Study: The Úzquiza Reservoir Water, *Ozone Sci. Eng.* 33 (2011) 185–193.
- [34] B.J. Ni, R.J. Zeng, F. Fang, W.M. Xie, G.P. Sheng, H.Q. Yu, Fractionating soluble microbial products in the activated sludge process, *Water Res.* 44 (2010) (2010) 2292–2302, <https://doi.org/10.1016/j.watres.2009.12.025>.
- [35] M.L. Quaranta, M.D. Mendes, A.A. MacKay, Similarities in effluent organic matter characteristics from Connecticut wastewater treatment plants, *Water Res.* 46 (2) (2012) 284–294, <https://doi.org/10.1016/j.watres.2011.10.010>.
- [36] C. Jacquin, G. Lesage, J. Traber, W. Pronk, M. Heran, Three-dimensional excitation and emission matrix fluorescence (3DEEM) for quick and pseudo-quantitative determination of protein- and humic-like substances in full-scale membrane bioreactor (MBR), *Water Res.* 118 (2017) 82–92, <https://doi.org/10.1016/j.watres.2017.04.009>.
- [37] Y. Li, A.-M. Li, J. Xu, W.-W. Li, H.-Q. Yu, Formation of soluble microbial products (SMP) by activated sludge at various salinities, *Biodegradation* 24 (1) (2013) 69–78.
- [38] S.K.L. Ishii, T.H. Boyer, Behavior of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review, *Environ. Sci. Technol.* 46 (4) (2012) 2006–2017.
- [39] C. Musikavong, S. Wattanachira, F. Nakajima, H. Furumai, Three dimensional fluorescent spectroscopy analysis for the evaluation of organic matter removal from industrial estate wastewater by stabilization ponds, *Water Sci. Technol.* 55 (11) (2007) 201–210.
- [40] Q.-Q. Zhang, B.-H. Tian, X. Zhang, A. Ghulam, C.-R. Fang, R. He, Investigation on characteristics of leachate and concentrated leachate in three landfill leachate treatment plants, *Waste Management* 33 (11) (2013) 2277–2286, <https://doi.org/10.1016/j.wasman.2013.07.021>.
- [41] A. Gupta, R. Zhao, J.T. Novak, C.D. Goldsmith, Variation in organic matter characteristics of landfill leachates in different stabilisation stages, *Waste Manag. Res.* 32 (12) (2014) 1192–1199.
- [42] W. Li, Q. Zhou, T. Hua, Removal of Organic Matter from Landfill Leachate by Advanced Oxidation Processes: A Review, *Int. J. Chem. Eng.* 2010 (2010) 1–10.
- [43] W. Chen, A. Zhang, G. Jiang, Q. Li, Transformation and degradation mechanism of landfill leachates in a combined process of SAARB and ozonation, *Waste Manag.* 85 (2019) 283–294, <https://doi.org/10.1016/j.wasman.2018.12.038>.
- [44] X.-S. He, B.-D. Xi, X. Li, H.-W. Pan, D.a. An, S.-G. Bai, D. Li, D.-Y. Cui, Fluorescence excitation-emission matrix spectra coupled with parallel factor and regional integration analysis to characterize organic matter humification, *Chemosphere* 93 (9) (2013) 2208–2215, <https://doi.org/10.1016/j.chemosphere.2013.04.039>.
- [45] Z. Li, Q. Yang, Y. Zhong, X. Li, L. Zhou, X. Li, G. Zeng, Granular activated carbon supported iron as a heterogeneous persulfate catalyst for the pretreatment of mature landfill leachate, *RSC Adv.* 6 (2016) 987.
- [46] W. Sun, D. Yue, J. Song, Y. Nie, Adsorption removal of refractory organic matter in bio-treated municipal solid waste landfill leachate by anion exchange resins, *Waste Manag.* 81 (2028) 61–70.
- [47] L. Wang, Q. Yang, D. Wang, X. Li, G. Zeng, Z. Li, Y. Deng, J. Liu, K. Yi, Advanced landfill leachate treatment using iron-carbon microelectrolysis-Fenton process: Process optimization and column experiments, *J. Hazard. Mat.* 318 (2016) 460–467, <https://doi.org/10.1016/j.jhazmat.2016.07.033>.
- [48] H.W. Wang, X.Y. Li, Z.P. Hao, Y.J. Sun, Y.N. Wang, W.H. Li, Y.F. Tsang, Transformation of dissolved organic matter in concentrated leachate from nanofiltration during ozone-based oxidation processes (O₃, O₃/H₂O₂ and O₃/UV), *J. Environ. Manag.* 191 (2017) 244–251, <https://doi.org/10.1016/j.jenvman.2017.01.021>.