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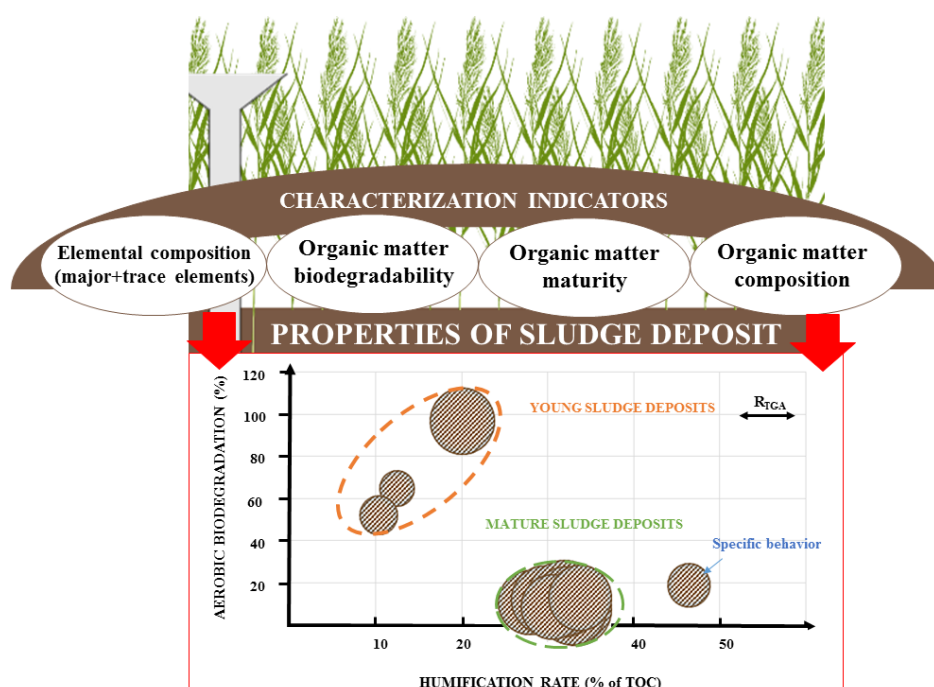
Comparative characterization of surface sludge deposits from fourteen French vertical flow constructed wetlands sewage treatment plants using biological, chemical and thermal indices

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Graphical abstract



Highlights

- Particulate organic matter (POM) in sludge deposits (SD) exhibits specific properties.
- Young deposits (≤ 1 y) and mature de-positions (>3 y) identified as 2 distinct groups.
- Additional pretreatments affected young-age sludge deposits characteristics.
- State of maturation of POM in SD inter-mediate between peat and wet meadows

Abstract

Due to their design and mode of operation, French Vertical Flow Constructed Wetlands (VFCWs) accumulate suspended solids from the inflow wastewater in the form of a sludge layer at the surface of the first filter. In order to maintain the treatment performance over the long term, the characteristics of the sludge deposits and their evolution have to be well described. In this objective, a panel of sludge deposit samples taken from 14 French VFCW sewage treatment plants was investigated. Elemental composition and organic matter content, nature and reactivity were analyzed. Results clearly revealed two categories of sludge deposits, namely the “young-age plants” type (1 year of operation and less) and the “mature plants” type (3 years of operation and more). Sludge deposits from the “mature plants” exhibited same biological, physical and chemical properties. Their organic matter was globally less abundant, more humified and less biodegradable than in the young-age plants type. Their overall contents in trace metals were also higher, although in a limited manner. The effect of additional treatments, particularly FeCl_3 injection for phosphorus precipitation, was observable in the “young-age plants” group. Finally, the sludge deposits sampled from one particular plant with specific operating conditions were found to exhibit very different characteristics from those of either groups identified. This observation underlined the influence of local conditions on the typology of the sludge deposits.

Keywords

Sludge deposits; Constructed wetland; indices; Characterization; Organic matter; Ageing

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1. Introduction

French Vertical Flow Constructed Wetlands (VFCWs) have become in the recent years in France the sewage treatment system most widely used in rural communities of ≤ 2000 Population Equivalent (Bellin, 2016). This rapid development may be explained by the high level of performance of these systems, their good social acceptability and landscape integration, their low energy requirements, easy operation, and low maintenance (Liu et al., 2015a; Semeraro et al., 2015; Wu et al., 2014).

The so-called “French VFCW system” comprises two stages of filters where the first stage is fed with unsettled raw wastewater. This design results in the formation of a sludge layer at the surface of the first filter which has mainly been considered until recently through its hydraulic aspects and its negative consequences with regards to the potential risk of clogging (Hua et al., 2018; Knowles et al., 2011; Nivala et al., 2012; Yang et al., 2018). Yet, the sludge layer may contribute positively to the treatment efficiency. For example, suspended solids removal, water retention and the biodegradation or retention of pollutants may be improved (Chazarenc & Merlin, 2005; Kim et al., 2013; Molle, 2014). On the other hand; the retention of most of feed organic matter within the sludge layer may limit the efficiency of subsequent denitrification (Lee et al., 2009; Van Oostrom & Russell, 1994). The effective roles of the sludge layer in the system may depend on its structural, compositional and reactional properties which are controlled by numerous factors, such as the composition of the influent wastewater, the operating conditions of the plant, or other local environmental factors (Fu et al., 2015). Although extensive work has already been published on similar issues in the fields of natural wetlands (Grasset et al., 2017; Rouwane et al., 2017), anthropic sediment (Badin, 2009; Huot, 2013), activated sludge (Alvarenga et al., 2015; Lachassagne, 2015), or sludge drying reed beds (SDRB) (Collard et al., 2017; Nielsen, 2011; Nielsen & Bruun, 2015; Nielsen & Larsen, 2016; Nielsen & Willoughby, 2005; Vincent et al., 2012), very few studies have focused on VFCWs’

surface sludge deposits (Bois et al., 2015; Chazarenc & Merlin, 2005; Kim et al., 2013; Kim et al., 2015). Molle (2014) and Perron et al. (2007) reported the influence of the VFCW plant design and operating conditions, such as organic and hydraulic loads or feed and rest periodicity. Kim et al. (2013), reported that although mainly organic, sludge deposits contained significant amounts of inorganic constituents originating from the local geological background or generated by possible operations of pretreatment of the influent such as phosphate precipitation by FeCl_3 addition. Kim et al. (2013) and Kania et al. (2018, 2018a) investigated the extent of organic matter evolution within the sludge deposits, such as mineralization. Finally, the authors of this study reported structural properties data of surface sludge deposits in a recent article (Kania et al., 2018b). In the specific context of sludge drying reed bed systems, few studies investigated the mineralization of the sludge and the organic matter stabilization (Masciandaro et al., 2017; Nielsen, 2005; Nielsen et al., 2014; Peruzzi et al., 2017; Peruzzi et al., 2013).

The objective of the present study was to contribute to a better description of the properties of sludge deposits using chemical, biological and thermochemical methods. Samples from fourteen VFCW sewage treatment plants in operation in France were analyzed to assess whether specific typologies would be observed and, if so, identify the dominant factors of influence, especially with regards to the particulate organic matter (POM).

2. Materials and methods

2.1. Sites of investigation

Fourteen French VFCW sewage treatment plants located in the south-eastern part of France, were selected for the study. The treatment plants were all equipped with a preliminary gridding stage set between 3 and 30 mm to remove coarse solids and debris. Four of them were also equipped with a sand trap (Table 1). Three typical types of VFCW process designs were represented in the selected plants:

- Process type I, usually referred to as the “classical French system”, where gridded, unsettled raw wastewater was percolated through two successive stages of vertical flow filters.
- Process type II, where an additional stage of aerobic biological trickling filter was used to treat gridded wastewater prior to the two successive stages of vertical flow filters,
- Process type III, comprising the same operations as in type II plus the introduction of a FeCl_3 solution into the outlet of the aerobic trickling filter in order to precipitate phosphorus prior to infiltration through the two successive stages of filters.

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Table 1 Characteristics of the 14 French VFCW sewage treatment plants selected for the study.

VFCW plants	Process type	Process line stages					Number of years of operation at sampling date	Nominal capacity (Population Equivalent)	Average hydraulic load (m³ / y)
		Gridding / sieving	Sand trap	Trickling filter	FeCl ₃ injection	Saturation of 1 st stage filter			
BAG1	Classical French VFCW	Yes	Yes	No	No	Unsaturated	11	1100	56940
BAG2			No				8	500	23725
SABAG			No				7	800	81030
VIL			No				7	350	11315
BAG3			No				4	290	6935
PRE			No				1	700	51830
RAM			No				0.5	350	13505
BAY	AZOE®		No	Yes	Yes	Partially saturated	7	1200	32850
MIN			No				6	1000	25185
NEU	AZOE-N®		Yes				4	1600	75555
VER	AZOE-NP®		No				11	1100	44165
ORB			Yes				5	1500	126290
COR			No				0.5	1000	51830
MAT	AZOE-P®		Yes			Unsaturated	5	1600	68620

2

These processes were further divided into two sub-categories according to the level of saturation of the first-stage filter as shown in Table 1. Full drainage (unsaturated conditions) was implemented to ensure good aeration in all process type I plants (“classical French system”) and in some plants of process types II and III patented as AZOE[®] and AZOE-P[®] respectively. Partially saturated conditions were implemented in the other plants of types II and III (patented as AZOE-N[®] and AZOE-NP[®]) in the objective to allow aeration of the upper unsaturated zone for nitrification, and anoxic conditions in the lower flooded zone for denitrification. More details were given in a previous article (Kania et al., 2018b; Kim et al., 2014).

2.2. Sampling and preparation of the sludge deposits samples

Sludge deposits were sampled in winter time between January and March 2015 from most wetlands, COR was sampled in November 2015 and RAM and PRE in February 2017 due to their more recent dates of implementation of these wetlands. The annual load of dry solids varied from one constructed wetland to another depending on its nominal design and its actual operating conditions. It was estimated between 20 and 35 kg of dry solids per year and m² of first stage filter cells. At the time of the field sampling operations, the sludge layer had not yet been removed from any of the wetlands and it has not been removed since. The lower part of the layer therefore contained deposits from the very beginning of the plant operation, whereas the upper part contained fresh deposits. The entire depth of the sludge layer was sampled on each site in order to obtain average samples representative of the mean composition of the sludge deposits over the whole period of operation of the VFCW plants.

All samples were taken following the same protocol from the stage filters. A few kilograms of deposits were taken by shoveling the entire depth of the sludge layer from 5 to 10 spots (depending on the filter size) at the surface of the cell under resting period for 0-1 week. The sampling spots were randomly located at a distance of at least 5 m one from each other and at least 2 m from the feed pipes. The samples taken from each spot were treated on site to extract

manually reeds rhizomes, and possible traces of gravels. They were then carefully homogenized by quartering and mixing together with shovels. The representative samples from each plant thereby constituted were then taken to the laboratory within a maximum of 4 h. They were transported to the lab by car placed in a non-heated trunk at a maximal temperature of 10°C due to the winter season. There, an aliquot fraction of each sample was used to determine water content and the organic content of the dry solids. The rest was dried in thin layers at 35°C for one day, finely grinded and stored at 4°C.

2.3. Analytical methods

2.3.1. General analytical parameters

Upon reception in the laboratory, a few grams of fresh samples from each plant were dried in triplicates in an oven at 105°C until they reached a constant mass in order to determine their humidity. Dry solids were then burnt in an oven at 550°C for four hours to determine their organic matter contents. The rest of each sample was spread in thin layers of 1-2 cm on a laboratory bench and air-dried for 1 day at 35°C, then grinded down to less than 1 mm to obtain “dry powdered samples” which were stored at 4°C and used for all the other analyses.

Elemental analyses were done by alkaline fusion (LiBO_2) followed by acid digestion (HNO_3) and subsequent analyses of the solutions by inductively coupled plasma atomic emission spectrometry (ICP-AES) (IRIS Advantage ERS, Thermo Scientific). The following elements were analyzed : silicon (Si), aluminum (Al), iron (Fe), manganese (Mn), magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), titanium (Ti) and phosphorus (P). Trace metals chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), cadmium (Cd) and lead (Pb) were analyzed using a Sciex Perkin Elmer ELAN 5000a. ICP-mass spectrometer.

Organic carbon contents were determined by burning at 1400°C 1g of dry powdered samples, previously de-carbonated with acid and under high temperature, and by infrared

absorption with a Carbon - Sulfur EMIA 320V2 (HORIBA). Total nitrogen contents were measured by burning 1g of powdered samples at 1050°C under a flow of helium and oxygen mixture in microanalyzer designed and developed at UMR 5280 Institute of Analytical Sciences which operates according to the Dumas method.

2.3.2. Thermogravimetric and differential thermal analysis (TGA/DSC)

Thermogravimetric analyses (TGA) were carried out using a METTLER TOLEDO TGA analyzer whose temperature precision was $\pm 0.5^\circ\text{C}$ and microbalance sensitivity less than $\pm 0.1\text{mg}$. A dry mass of $\pm 20\text{ mg}$ of each sample was heated from 35°C to 900°C at a rate of $10^\circ\text{C}/\text{min}$, under a flow of air of 50 mL per minute. The analyses were done in duplicates and blank experiments were carried under the same conditions as the assays but without any sample. The mass loss due to thermal degradation of the sample was recorded as a function of the temperature. Heat fluxes were also recorded during the process by differential scanning calorimetry (DSC).

R_{TGA} index was calculated from experimental data as the ratio of the mass loss recorded between 200 and 400°C (Δm_1) to that recorded between 400 and 600°C (Δm_2), as proposed by several authors (Stevenson, 1982 ; Fernandez et al., 2012). In each given range of temperature, the unit mass heat release, also called “energy density” E (in J.mg^{-1}), was also calculated from the ratio between the surface area of the corresponding peak in DSC profiles over the mass loss recorded in the same range of temperatures (E_1 and E_2). The R_{DSC} index was finally calculated as the ratio of E_2/E_1 (Achour, 2008).

2.3.3. Determination of humification rate

The contents in humic-like and fulvic-like substances (referred to as HULIS and FULIS, respectively) were analyzed to evaluate the humification degree of the organic matter in the sludge deposits (Albrecht, 2007). The protocol for the HULIS and FULIS extraction was

adapted from Serra-Wittling et al. (1996). HULIS and FULIS were extracted by suspending for 2 h under constant agitation 1 g of powdered samples in 50 mL of a 0.1 M sodium hydroxide aqueous solution. The suspensions were then centrifuged at 10 000 g for 15 min and vacuum filtered at 0.45 µm using Sartorius cellulose acetate filters. The solutions, containing HULIS and FULIS fractions were analyzed for total organic carbon (TOC) using a Shimadzu TOC-L analyzer, then acidified to pH 1.5 with 1 M sulfuric acid aqueous solution and left at rest overnight at +4°C to allow precipitation of HULIS fraction. FULIS fraction was collected in solution by centrifugation at 6000 g for 15 min followed by filtration at 0.45µm. The solution was analyzed for TOC using the same protocol as for the HULIS + FULIS extracts.

The proportion of HULIS + FULIS in the organic matter was taken as an indicator of the degree of humification of the sludge organic matter and calculated as follows:

$$\text{Humification rate} = 100 \times \frac{[\text{HULIS}] + [\text{FULIS}]}{\text{TOC}} \quad (1)$$

2.3.4. Biodegradation under aerobic conditions

Respirometric assays were conducted to evaluate the biodegradability of the sludge deposits considered here as an indicator of stability of their organic matter. A given mass of each powdered sample, calculated to contain 75.5 mg of organic matter, was introduced into 500 mL BOD flasks containing 10 mL of a mineral medium made of an aqueous solution of 28.25 g.L⁻¹ KH₂PO₄ ; 146.08 g.L⁻¹ K₂HPO₄; ; 3.66 g.L⁻¹ CaCl₂ ; 28.64 g.L⁻¹ NH₄Cl ; 3.06 g.L⁻¹ MgSO₄, 0.7 g.L⁻¹ FeSO₄ and 0.4 g.L⁻¹ ZnSO₄ (Liu et al., 2015b). The flasks were then inoculated with 10 mL of a seed suspension prepared from biological sludge obtained from a municipal wastewater activated sludge treatment plant located in Villeurbanne (France) and cultivated in the laboratory prior to the beginning of the assays. They were finally spiked with ca. 0.2 mL of a 5 g.L⁻¹ allylthio-urea aqueous solution used as a nitrification inhibitor, hermetically closed by manometric caps to monitor pressure variations, and incubated at 30±1°C in the dark. Carbon

dioxide formed from biodegradation was trapped by sodium hydroxide pellets placed in a specific compartment in the headspace of the test flasks. The recorded pressure therefore decreased proportionally to the oxygen consumed and was subsequently converted into BOD values expressed in mg O₂ per g of sludge TOC.

Biodegradability was finally calculated according to eqn. (2) from the ratio between the cumulated consumption of O₂ (g) over 14 days of incubation (BOD₁₄) and the theoretical oxygen demand (ThBOD) of 2667 mg O₂ per g of TOC (corresponding to a consumption of 32 g O₂ for a complete bio-oxidation of 12 g C based on a stoichiometry of 1 mole of O₂ for 1 mole of C assuming that carbon in the sludge organic constituents was at an average degree of oxidation of zero).

$$\text{Biodegradability (\%)} = \frac{\text{BOD}_{14} \text{ (gO}_2 \text{ per g TOC)}}{2.667} \times 100 \quad (2)$$

3. Results and discussion

3.1. Organic content and elemental composition

3.1.1. Organic content

Table 2 shows the average contents in organic matter and organic carbon analyzed in the surface deposits samples. Organic matter (OM) contents ranged between 50.3 and 86.2% of total dry weight (% w/ w). Regardless of their wetland of origin, the surface deposits were therefore in all cases mostly organic material.

Table 2 General composition of sludge deposits samples.

VFCW labels ^a	OM ^b	TOC ^c	Si ^d	Ca ^d	Al ^e	Fe ^d	Mg ^e	Na ^f	K ^d	Mn ^e	Ti ^e	P ^d	TN ^g	TOC/TN	Cr ^e	Cu ^d	Ni ^d	Zn ^e	Cd ^f	Pb ^d	Σmetals _h
	% w/w of DM		% w/w of DM													mg.kg DM ⁻¹					
BAG1	59.0	24.7	7.7	2.6	2.1	1.0	0.3	0.2	0.5	0.02	0.2	1.2	4.0	6.2	49.8	338.0	23.2	615.6	1.0	43.7	1071
BAG2	57.4	24.2	13.3	2.9	2.0	0.9	0.3	0.2	0.7	0.01	0.2	0.8	3.3	7.2	41.4	363.8	21.7	810.2	1.2	37.2	1276
SABAG	60.3	21.0	11.6	2.6	1.8	1.4	0.3	0.2	0.6	0.04	0.2	1.1	3.6	5.8	46.3	1162.0	23.1	557.4	1.0	33.4	1823
VIL	60.9	22.7	10.5	2.4	2.4	1.2	0.3	0.1	0.5	0.01	0.3	1.2	3.4	6.7	46.5	425.2	18.0	430.0	0.6	31.9	952
BAG3	50.3	18.4	13.2	3.5	2.4	1.4	0.3	0.3	0.7	0.02	0.2	1.0	3.1	5.9	59.3	282.3	25.1	481.4	1.0	34.7	884
PRE	86.2	36.4	2.1	2.5	0.4	0.2	0.1	0.1	0.2	<D.L.	0.1	0.5	1.5	24.7	19.3	216.9	7.7	463.1	0.4	13.6	721
RAM	84.3	32.3	3.4	3.2	0.6	0.3	0.2	0.1	0.2	<D.L.	0.1	0.7	2.1	15.6	18.4	304.7	10.0	481.0	0.6	18.1	833
BAY	70.5	28.5	3.3	4.7	1.5	0.5	0.3	0.1	0.3	0.01	0.2	1.9	4.5	6.4	34.5	232.9	20.3	968.9	1.5	121.5	1380
MIN	73.5	28.2	5.0	3.5	1.3	0.6	0.4	0.2	0.4	0.02	0.3	1.4	4.8	5.9	51.9	195.9	30.3	731.6	0.9	32.2	1043
NEU	58.6	21.7	9.6	3.7	2.3	1.2	0.4	0.3	0.7	0.04	0.2	1.1	3.6	6.0	51.8	223.9	26.9	573.9	1.2	45.4	923
VER	62.0	23.0	5.7	3.8	2.1	3.5	0.3	0.1	0.4	0.02	0.2	2.0	3.8	6.1	57.4	622.5	30.5	1218.5	1.6	64.2	1995
ORB	58.2	22.7	7.3	3.8	2.0	3.5	0.3	0.1	0.5	0.03	0.2	1.6	3.6	6.2	74.0	379.6	36.7	952.9	1.3	48.9	1493
COR	72.0	24.7	2.8	4.6	0.8	5.2	0.3	0.2	0.5	0.02	0.2	2.5	4.1	5.9	51.4	691.4	21.0	908.8	1.0	37.2	1711
MAT	50.6	14.0	11.2	1.6	3.2	5.9	0.4	0.4	1.2	0.5	0.3	2.0	2.5	5.5	57.6	201.7	23.4	744.7	1.1	68.7	1097

OM: Organic Matter; DM: Dry Matter; DL = Detection Limit

^a See Table 1

^b Analytical results from the replicates of each sample did not exceed $\pm 2.5\%$

^c Relative uncertainty of 1-2%

^d Relative uncertainty of 5%

119
120
121
122
123

- 124 ^e Relative uncertainty of 10%
125 ^f Relative uncertainty of 15%
126 ^g Relative uncertainty of 0.3%
127 ^h $\sum \text{metals} = [\text{Cr}] + [\text{Cu}] + [\text{Ni}] + [\text{Zn}] + [\text{Cd}] + [\text{Pb}]$.

Fig. 1 illustrates the plot of organic matter contents of the surface deposits vs. the number of years of operation of the VFCW sewage treatment plants of origin. The graph showed that deposits from young systems with newly established thin sludge layers of about 1-3 cm contained larger concentrations of organic matter than those from older systems of ≥ 3 years with a sludge layer of about 10-25 cm thick. This observation suggested that hydrolysis and /or mineralization of POM progressively reduced the organic matter content of the deposits within the sludge layer. However, although COR, RAM and PRE samples were all taken from young plants (1 year and less), COR sample was less organic than those from RAM and PRE. Time was therefore not the only factor of influence. Other analytical results discussed below have to be considered to explain this observation.

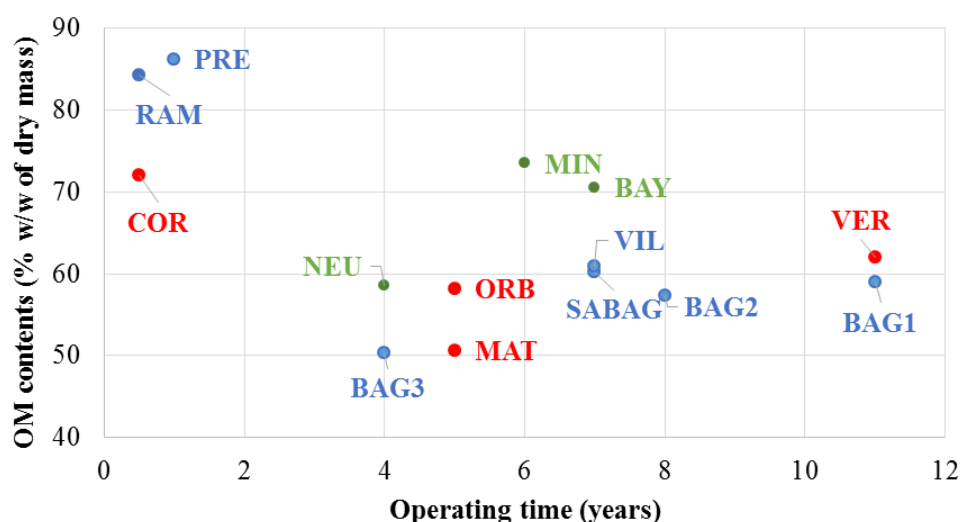


Fig. 1. Organic matter contents of the surface deposits versus operating time of their VFCW plants of origin. Blue dots: Classical French system with no additional treatment. Green dots: additional treatment on trickling filter only. Red dots: trickling filter and FeCl₃ addition.

3.1.2. Elemental compositions

The contents in major elements were quite similar in all the samples, except for MAT which exhibited high Mg, Na, K and Mn concentrations (Table 2), probably due to atypical local conditions as discussed in the sections below. Phosphorus (P) contents in the sludge deposits from plants with FeCl₃ injection (2.0; 1.6; 2.5 and 2.0% w/w in VER, ORB, COR and

MAT, respectively), were two-fold higher than in the plants without any additional treatment where P concentrations ranged between 0.5 and 1.2% w/w of dry matter. This result confirmed the good efficiency of FeCl₃ injection to precipitate phosphorus. The samples from plants with a trickling filter also revealed slightly higher P contents (1.2%; 1.9% and 1.5% in NEU, BAY and MIN samples respectively) than those with no additional treatment at all. This observation could be attributed to the assimilation of phosphorus within the biofilm in the trickling filter, which after detachment would be subsequently retained within the surface sludge layer and induce a relative increase in P contents (Andersson, 2009). Total Nitrogen (TN) contents ranged between 1.5 and 4.8% w/w of dry mass in all the samples. These results were close to those reported in sludges from lagoons sewage treatment plants, but lower than those found in suspended solids from activated sludge systems (Vincent, 2011). COR sample exhibited a TN concentration of 4.1% w/w of dry mass, which was about double of the concentrations measured in the samples of similar age RAM and PRE (1.5 and 2.1% w/w of dry mass, respectively). This observation may be explained by the interactions (complexation, coagulation, precipitation) of soluble N species with FeCl₃ used in COR plant. Other authors (Boucher et al., 1999; Hamdani et al., 2004; Poon & Chu, 1999) reported the effect of FeCl₃ on the removal of TN from dairy effluents. This effect was observable here in young-age plants but not in older plants (Table 2), showing that other ageing effects became predominant over the years of operation.

Table 2 showed that the contents in trace metals were in the same order of magnitude in all the samples. The accumulation of trace metals and other micropollutants is a potential environmental concern when considering land application or nutrients recovery from the dredged sludge (Fang et al., 2016; Gautier et al., 2017; Kim et al., 2016; Peccia & Westerhoff, 2015; Peysson et al., 2013). Total contents in Cd, Cr, Ni, Pb, Cu and Zn in the sludge deposits analyzed here ranged in most cases between ca. 0.9 and 1.6, 35 and 74, 18 and 37, 32 and 69, 196 and 425, 430 and 953 mg.kg DM⁻¹ respectively. These concentrations were in the ranges

classically reported in activated sludge, respectively 1-10 for Cd, 10-100 for Cr, Ni and Pb and 100-1000 mg.kg DM⁻¹ for Zn and Cu (Lachassagne, 2015). A few samples revealed however relatively high contents in some elements. Sludge deposits from SABAG plant exhibited a high copper concentration (1162 mg.kg DM⁻¹), samples from VER a high zinc concentration (1218.5 mg.kg DM⁻¹) and samples from BAY a high lead contents (121.5 mg.kg DM⁻¹). These observations may be related to the specific nature of anthropic discharges (domestic, industrial or both) to the sewage water in the water catchments of the respective constructed wetlands (Chipasa, 2003; Ustun, 2009; Vriens et al., 2017). Table 2 showed the concentrations of trace metals analyzed in the 14 sludge deposit samples. As an informative reference, these concentrations may be compared to the upper limit concentrations of a series of trace metals considered in French regulation for land application of sludge (decree N° 97-1133 of 8/01/1998), expressed in mg of element per kg of DM: Cd and Hg 10 DM; Cr and Cu 1000, Ni 200; Pb 800; Zn 3000; Cr+Cu+Ni+Zn 4000. It was observed that the concentrations increased in time from around 780 mg.kg⁻¹ of DM in young plants RAM and PRE to ≥ 1200 in the older “classical” VFCW plants, suggesting a progressive accumulation of trace metals in the sludge deposits over the years of operation, which may be explained in part by their sorption onto POM and/or mineral fractions of the sludge deposits. The implementation of additional treatment did not induce observable differences of total metal concentration in the samples from mature plants. The average concentrations were 1201 \pm 386 mg.kg DM⁻¹ in samples from classical VFCW (no additional treatments), 1115 \pm 237 mg.kg DM⁻¹ in samples from AZOE[®] treatment plants, and 1529 \pm 450 mg.kg DM⁻¹ in AZOE-NP[®] treatment plants. Regarding sludge deposits from the young plants, the effect of FeCl₃ addition (co-precipitation with and/or sorption onto flocculated mineral and organic phases) may explain here again the observed difference between the samples from COR (ca 1711 mg.kg DM⁻¹) and RAM and PRE (721 and 833 mg.kg DM⁻¹ respectively) as already discussed by El Samrani et al. (2008) and Fu & Wang (2011). The

situation in the sludge layer in the VFCW seems in many aspects comparable with the sludge layer building up in SDRB systems. In sludges from reed bed systems, very similar contents were indeed reported for TOC and TN (Masciandaro et al., 2017; Nielsen et al., 2014 ; Peruzzi et al., 2013), and trace metals (Caicedo et al., 2015; Matamoros et al., 2012; Nielsen & Bruun, 2015).

3.2. Biodegradability of sludge deposits' organic matter

Fig. 2 shows the results of cumulated O₂ consumption measured over 14 days of incubation at 30°C of the sludge deposits samples. It can be seen that the samples from the mature plants exhibited respiration levels ranging between 200 and 500 mgO₂.TOC⁻¹ in 14 days of incubation, which may be considered as low values according to the scale used for compost (Wood et al., 2009) and with respect to the theoretical maximum of 2667 mg O₂ per g of TOC. These results therefore revealed the poor biodegradability and/or bioavailability of the organic matter in these samples. The samples from the youngest three plants PRE, RAM and COR exhibited drastically higher respiration levels (1729, 1387 and 2577 mg O₂ per g of TOC respectively), indicating that their organic matter was readily biodegradable and bioavailable in the conditions of the assays. Vincent (2011) reported that fresh organic matter from raw wastewater was responsible for the major part of O₂ consumption in “classical French” VFCWs.

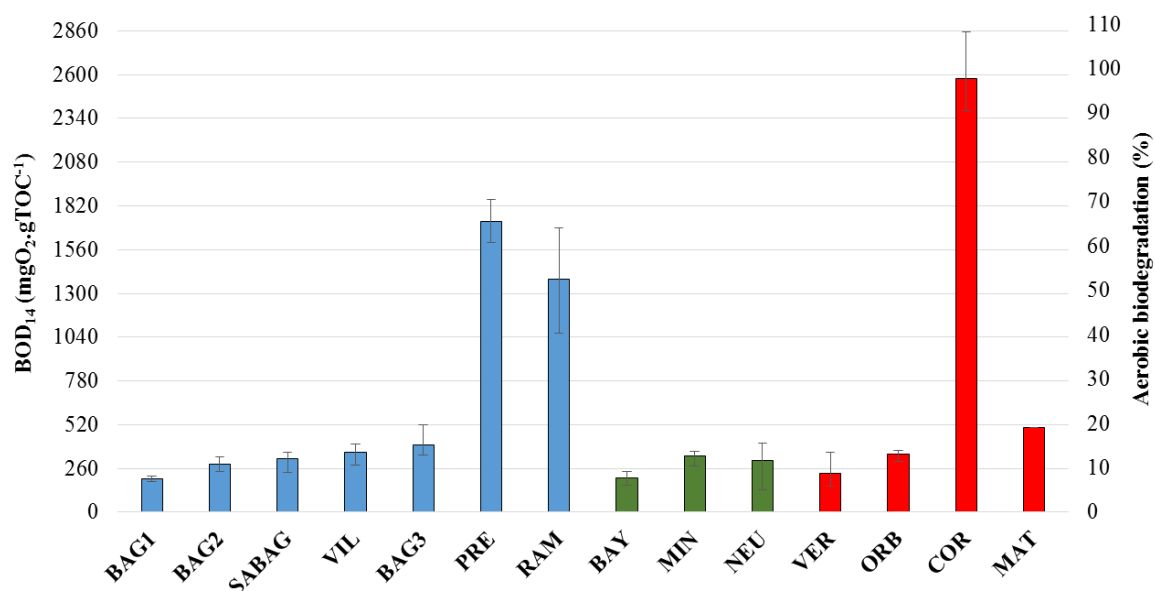


Fig. 2. Aerobic biodegradation measured over 14 days of incubation at 30°C on the surface sludge deposits sampled from French VFCW sewage treatment plants of different types. Classical system with no additional treatment (blue bars); additional treatment on trickling filter only (green); trickling filter and FeCl₃ addition (red).

Results obtained from the analysis of sludge deposits from VFCW sewage treatment plants of increasing ages therefore showed that both the organic contents of the sludge deposits and the biodegradability of their organic matter decreased over time, suggesting the occurrence of both mineralization and humification processes. Similar phenomena were reported in other environmental media (Albrecht, 2007; Francou, 2003).

3.3. Indicators of maturation of particulate organic matter

3.3.1. TOC/TN ratio

TOC/TN ratio is a parameter classically used to monitor the progress of biodegradation in the processes of organic waste composting (Iglesias Jiménez & Perez Garcia, 1989; Zahra El Ouaquoudi et al., 2015). The ratio normally decreases over time since biodegradation releases carbon (in the form of CO₂) whereas nitrogen remains within the waste material during composting operations. Compost is considered mature when the ratio falls below 15 (Zahra El Ouaquoudi et al., 2015). Grimaud (1996) also reported that secondary sludge from conventional

domestic wastewater treatment would exhibit a TOC/TN ratio between 6 and 12, whereas primary sludge would be above 12.

Based on these studies, the ratio was tentatively used here as an indicator of the evolution of organic matter in the sludge deposits. Table 2 showed that low values between 5.5 and 7.2 were measured in sludge deposits taken from VFCW plants of three years and more, whereas samples from the young plants RAM and PRE exhibited much higher values of 24.7 and 15.6 respectively. COR sample however, although also originating from a young plant, revealed a low TOC/TN value of 5.9. The ratio TOC/TN ratio was however considered non-relevant as an indicator of POM evolution in VFCW. As a matter of fact, unlike composting where N is not exported from waste material, the conditions of evolution of surface sludge deposits in VFCW allowed both the release of N in the form of soluble and leachable species and/or the accumulation of N species from wastewater via complexation and/or adsorption. Other authors (Brewer & Sullivan, 2003; Huang et al., 2006) also reported that TOC/TN ratio was not sufficient or not always relevant to discriminate the level of maturity of OM. Other indicators widely used in published studies, such as the humification rate, were therefore considered.

3.3.2. Humification rate

The contents in HULIS and FULIS fractions analyzed in the different samples, expressed as % w/w of organic carbon, and the calculated humification rate are shown in Fig. 3. The humification rate of almost all the samples from mature plants were found to be relatively high and very similar, ranging from 27.9 ± 0.5 to $33.8 \pm 0.9\%$ w/w of sludge TOC. This indicator was therefore consistent with the results discussed above relative to the biostability of their organic matter. Masciandaro et al. (2017) obtained very similar values (humification rate between 23.7 and 30.4%) in sludge treatment reed bed systems and concluded to the effectiveness in stabilizing sludge organic matter. One sample however, also collected from a

mature plant, MAT, exhibited on this indicator a much higher value than all other samples, as high as ca. $46.4 \pm 2.8\%$, suggesting that the organic matter in this sample was particularly humified, although it did not exhibit the smallest biodegradability in Fig. 2. The particular properties of this sample are discussed below.

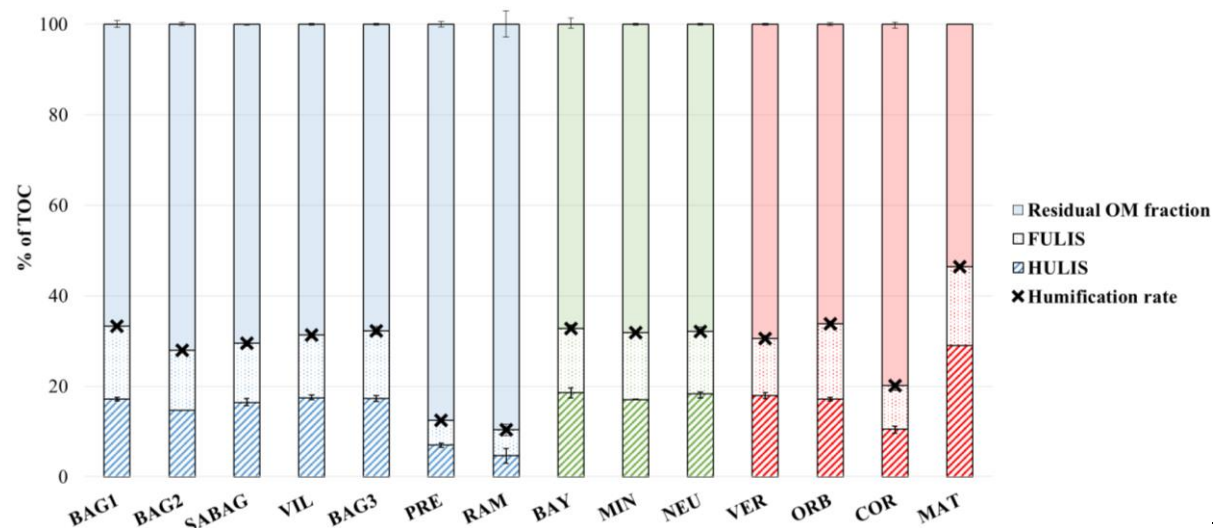


Fig. 3. Distribution of organic carbon in sludge deposits (SD) organic fractions, and humification rate measured in the 14 VFCWs sludge deposits. Classical system with no additional treatment (blue); additional treatment on trickling filter only (green); trickling filter and FeCl_3 addition (red).

Samples from the young plants RAM and PRE showed lower values of humification rates, 12.5 ± 0.6 and $10.3 \pm 2.9\%$ respectively, which were relatively close to level reported for activated sludge (16.5% in Riffaldi (1982) and 10.9% in Li et al. (2011)). COR sample however exhibited a humification rate of ca $20.1 \pm 1.6\%$ w/w of TOC which was comprised between the levels measured in the other two young plants RAM and PRE and in mature plants. This observation was attributed to the transformation of the organic matter through the trickling filter and the detachment of biofilm fragments which may favor the formation of humic-like substances as reported by other authors (Maeng et al., 2015; Zhao et al., 2013).

3.4. Thermal analyses

TGA and DSC profiles obtained from thermal analyses of the sludge deposits samples are shown in Fig. 4 in the range of temperatures between 200° and 600°C known to induce

thermal degradation of organic compounds of natural origins (Torres-Climent et al., 2015). The first part of the graphs, from 200°C to 400°C, was considered to correspond to the degradation of relatively labile organic compounds, such as hemicelluloses, cellulose and some aliphatic compounds (Chen et al., 2015; Gomez et al., 2007; Som et al., 2009; Zahra El Ouaqoudi et al., 2015). The second part between 400 and 600°C was considered associated to more complex and stable organic compounds such as lignin and other high molecular weight poly-aromatic structures such as humic and fulvic substances (Chen et al., 2015; Cuypers et al., 2002; Melis & Castaldi, 2004; Zahra El Ouaqoudi et al., 2015).

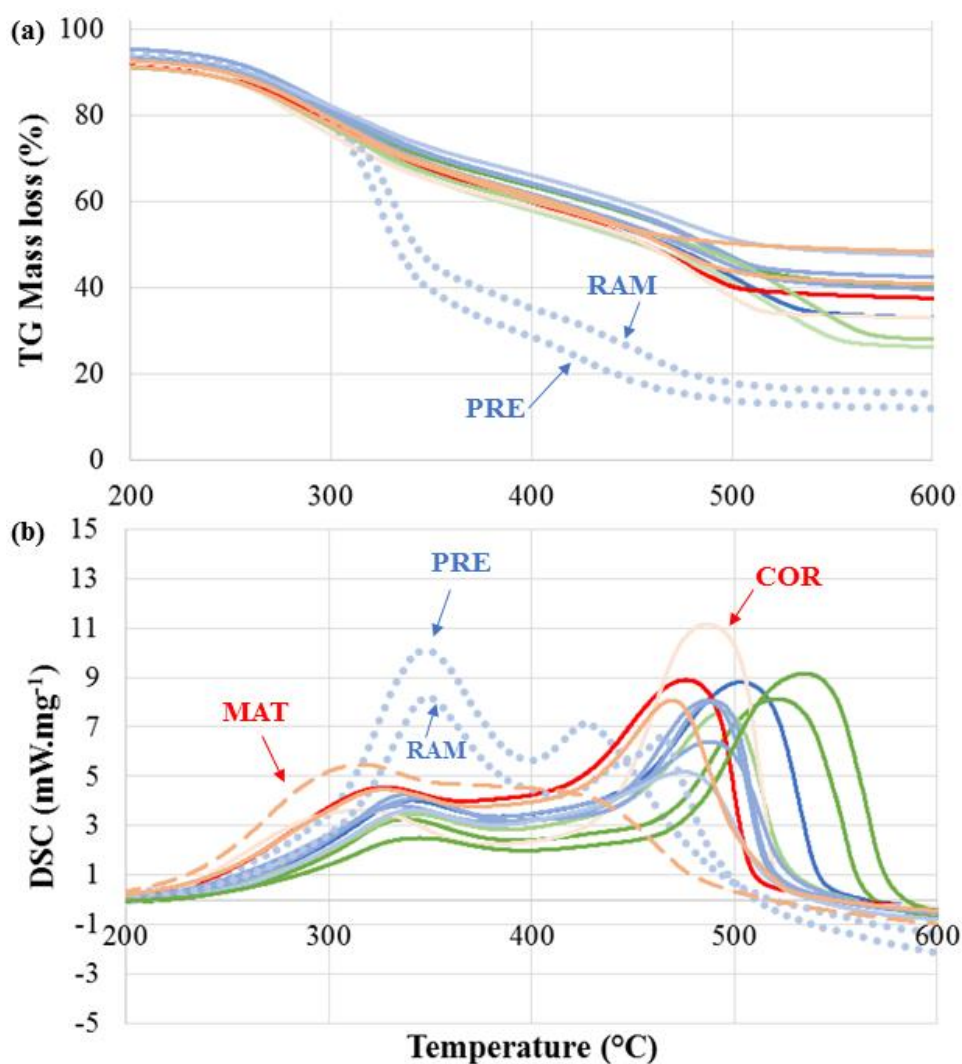


Fig. 4. Mass loss (a) and differential scanning calorimetry (b) profiles recorded from surface sludge deposits from 14 French VFCW sewage treatment plants. Classical system with no additional treatment (blue); additional treatment on trickling filter only (green); trickling filter and FeCl₃ addition (red).

For all samples, except RAM and PRE, TGA profiles showed that around 1/4 to 1/3 of the initial dry mass (26.2 to 35.1% w/w of dry mass) was degraded between 200 and 400°C. The mass losses between 400 and 600°C were in the same order of magnitude as from 200 to 400°C, ranging between 19.8 and 32.4% w/w of dry mass. RAM and PRE samples, taken from the youngest VFCW plants, exhibited different patterns, with about 2/3 of initial dry mass degraded between 200 and 400°C (66.4 and 66.1% w/w of dry mass respectively), and only around 16% w/w of initial dry mass degraded between 400 and 600°C. Similar results were reported for activated sludge by Magdziarz et al. (2013), with around 50% degradation of initial dry mass between 200 and 400°C and 20% w/w of dry mass above 400°C. MAT sample exhibited an atypical profile. Although between 200 and 400°C the mass loss was 32.0% w/w of dry mass as in almost all samples, above 400°C the mass loss was the lowest of all samples (11.1%).

The thermal degradation processes were logically exothermic since the analyses were made in the presence of air (DSC profiles in Fig. 4). The heat released per unit mass of organic matter oxidized in the assays was related to the energy of inter-atomic bounds and the degree of oxidation of carbon in the organic constituents. It can be observed that the ratio E_1 between 200 and 400°C was much smaller than E_2 between 400 and 600°C (Table 3). The thermal degradation was therefore much less exothermic per unit mass of organic constituents below 400°C than above 400°C. It was therefore concluded that the POM constituents degraded below 400°C were composed of molecules such as carbohydrates with relatively weak inter-atomic bounds such as C–O, C–C and C–N (activation energy of 350–412 kJ.mol⁻¹) (Leinweber & Schulten, 1992), and a relatively high degree of oxidation of carbon indicative of the oxygenation index of the molecules (Barré et al., 2016; Soucémarianadin et al., 2018). The organic constituents degraded above 400°C were considered to contain more C=C bounds of higher energy (activation energy of 520-840 kJ.mol⁻¹) such as in humic-like substances, lignin

and lignocellulosic complexes (Magdziarz & Wilk, 2013), with carbon at a lower degree of oxidation, such as in (poly)-aromatic compounds (Leinweber & Schulten, 1992).

Table 3 R_{TGA} , E_1 , E_2 and R_{DSC} values for 14 VFCW sludge deposits.

	R_{TGA}	E₁	E₂	R_{DSC}
		J.mg ⁻¹		
BAG1	0.95	8.6	20.7	2.4
BAG2	0.75	10.5	16.7	1.6
SABAG	0.83	9.2	15.4	1.7
VIL	0.77	8.9	19.1	.2
BAG3	0.76	10.2	21.2	2.1
PRE	0.24	8.9	24.1	2.9
RAM	0.28	7.9	24.6	3.2
BAY	0.92	6.6	16.3	2.5
MIN	1.05	6.0	17.3	2.4
NEU	0.85	8.0	17.7	2.2
VER	0.76	11.7	22.0	1.9
ORB	0.76	9.5	15.6	1.7
COR	0.79	8.5	18.7	2.2
MAT	0.35	13.6	18.7	1.4

The thermal indices R_{TGA} and R_{DSC} were calculated and the results given in Table 3. For all samples except those from the youngest plants PRE and RAM, the R_{TGA} index was between 0.8 and 1.1, indicating the presence of relatively stable organic constituents. For comparison, compost is considered mature for an R_{TGA} index above 0.8 (Som et al., 2009; Zahra El Ouaquoudi et al., 2015). R_{TGA} indexes of PRE and RAM samples were 0.2 and 0.3 respectively, confirming the previous discussions related to the predominance of labile organic compounds in these samples and the lower humification rate. R_{DSC} values were found between 1.6 and 3.2. Samples from the young plants RAM and PRE showed a higher index than those from the mature plants, confirming their lower stability (Achour, 2008).

COR sample exhibited again a particular profile, with R_{TGA} and R_{DSC} indexes closer to the group of mature plants than young plants. These results therefore confirmed the effects of the additional treatments, notably the trickling filter, on the characteristics of sludge deposits in young-age VFCW plants.

Finally, the TGA/DSC analysis allowed to highlight the particular behavior of MAT sample which did not feature a clear bimodal pattern but showed instead a peak around 320°C followed by a shoulder at 445°C. The particularities of MAT sample are further discussed below.

3.5. Multi-parameter cross-analysis of sludge deposits characteristics

3.5.1. Inter-comparison of the 14 sludge deposits

A correlation analysis was conducted on the different parameters of OM maturity and stability used in this study. Results gathered in Table 4 showed that OM content was relatively correlated with aerobic biodegradation and R_{DSC} , and anti-correlated with the humification rate. A high organic content was typical of young-age VFCW plants, where fresh organic matter was readily biodegradable and poorly humified. R_{DSC} index was anti-correlated with the humification rate and R_{TGA} was not significantly correlated to any other indexes.

Table 4 Correlation coefficients (and P-value) between the parameters of biological, chemical and thermal characterization. Correlation coefficients shown in bold were considered statistically significant, with $P < 0.05$.

	OM	Humification rate	Aerobic biodegradation	R_{TGA}	R_{DSC}
OM	1	-0.834 (2.10⁻⁴)	0.623 (0.02)	-0.363	0.828 (3.10⁻⁴)
Humification rate		1	-0.702 (0.01)	0.427	-0.750 (2.10⁻³)
Aerobic biodegradation			1	-0.467	0.436
R_{TGA}				1	-0.253
R_{DSC}					1

To allow a relevant and discriminant graphical representation of the different SD samples with respect to their characteristics, the most discriminant parameter within each type of analyses was selected (namely biological, thermochemical, and chemical analyses). Based on the results shown in the previous sections, biodegradability and humification were selected as the biological and chemical parameters. Based on the statistical correlation assessment, R_{TGA} index was selected as the thermochemical parameter because it did not show significant

correlations with the first two parameters according to Table 4, and was therefore considered to provide independent additional information. The corresponding 3-D representation shown in Fig. 5 clearly revealed distinct typologies of samples. A first group was identified with the samples from the young-age VFCW plants RAM, PRE and COR. COR sample was positioned slightly apart from RAM and PRE samples, underlining the influence of the additional treatments on the characteristics of the sludge deposits within the young-age of the VFCW plants. A second group was clearly formed with the samples from the mature plants. This group was very homogeneous, revealing the very strong similarities in the characteristics of the sludge deposits of the VFCW plants of three years of operation and more, regardless of the implementation or not of additional treatments and other local conditions. Sludge samples from mature VFCW plants exhibited a humification rate between 20 and 40%, an OM biodegradation rate between 0 and 35%, and a R_{TGA} between 0.75 and 1. Finally, MAT sample was excluded from both previous groups in the graphical representation of Fig. 5. Its overall characteristics were therefore distinct from either groups of young-age and mature VFCW plants. MAT plant was actually an atypical VFCW plant as compared to the others. For example, a predominance of baby's wipes was observed at the surface of the first filter when the sludge deposits were sampled. These wipes are made of about 60% polyester and 40% cellulose for most of them and affect the composition of the sludge deposits. Fig. 5 therefore allowed to clearly identify a third group of samples whose characteristics revealed a particular situation.

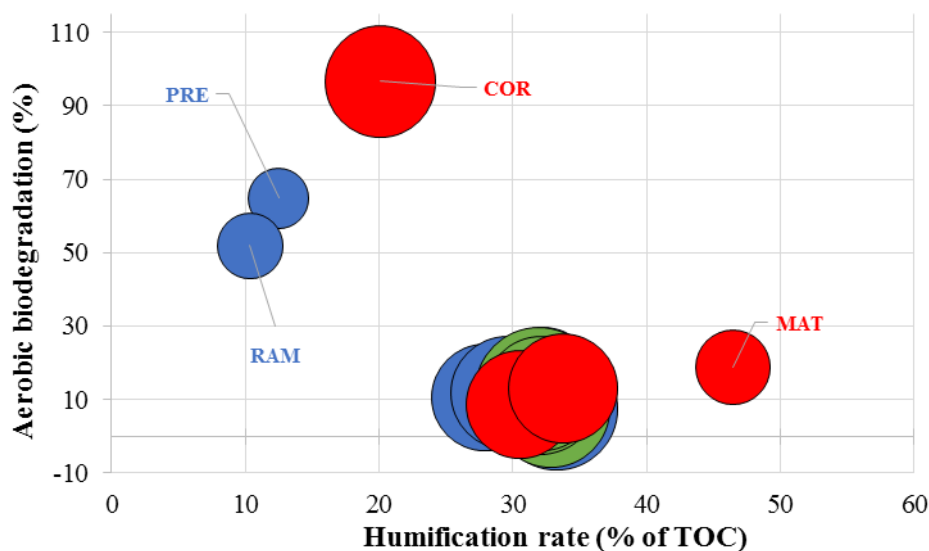


Fig. 5. Differentiation of sludge samples according to their indices related to the stability and maturity of the OM. Each sample is represented with a circle whose center is positioned according to the biodegradation rate and humification rate of the samples, and diameter is proportional to their R_{TGA} indices. Classical system with no additional treatment (blue); additional treatment on trickling filter only (green); trickling filter and $FeCl_3$ addition (red).

3.5.2. Comparison to other samples at different levels of POM evolution

Although some debates still remain on the subject, it is usually considered in the literature that ageing of natural particulate organic matter (POM) occurs via (i) the hydrolysis and mineralization of at least part of the most readily biodegradable constituents and (ii) the rearrangement of the remaining constituents into complex supra-structures usually called humus and usually considered as made of humic-like and fulvic-like substances. These phenomena are considered to increase (i) the proportion of HULIS+FULIS within the particulate organic matter and (ii) the proportion of HULIS vs. FULVIC within humus fraction. To assess the extents to which these processes occurred in the sludge deposits, Fig. 6 illustrates the plot of the ratio FULIS concentration/HULIS concentration of the samples as a function of the overall proportion of HULIS+FULIS within the total organic carbon of the samples. Data reported by Grasset et al. (2017) for different types of samples from natural systems were included in Fig. 6, along with other data calculated for activated sludges using the results of other studies (Li et al., 2011; Riffaldi, 1982). Fig. 6 illustrated the maturation pattern of natural organic matter from the early stages of evolution (fresh OM) in the top left part to the mature

state in the bottom right of the graph. The VFCW sludge deposits samples appeared as two major groups in Fig. 6. The samples from young VFCW plants (RAM, PRE and to a lesser extent COR) constituted a first group situated at an earlier stage of maturation from the second group made of the samples from the mature VFCW plants. COR sample was actually positioned between the young and mature groups, revealing the effects of the additional treatments discussed previously.

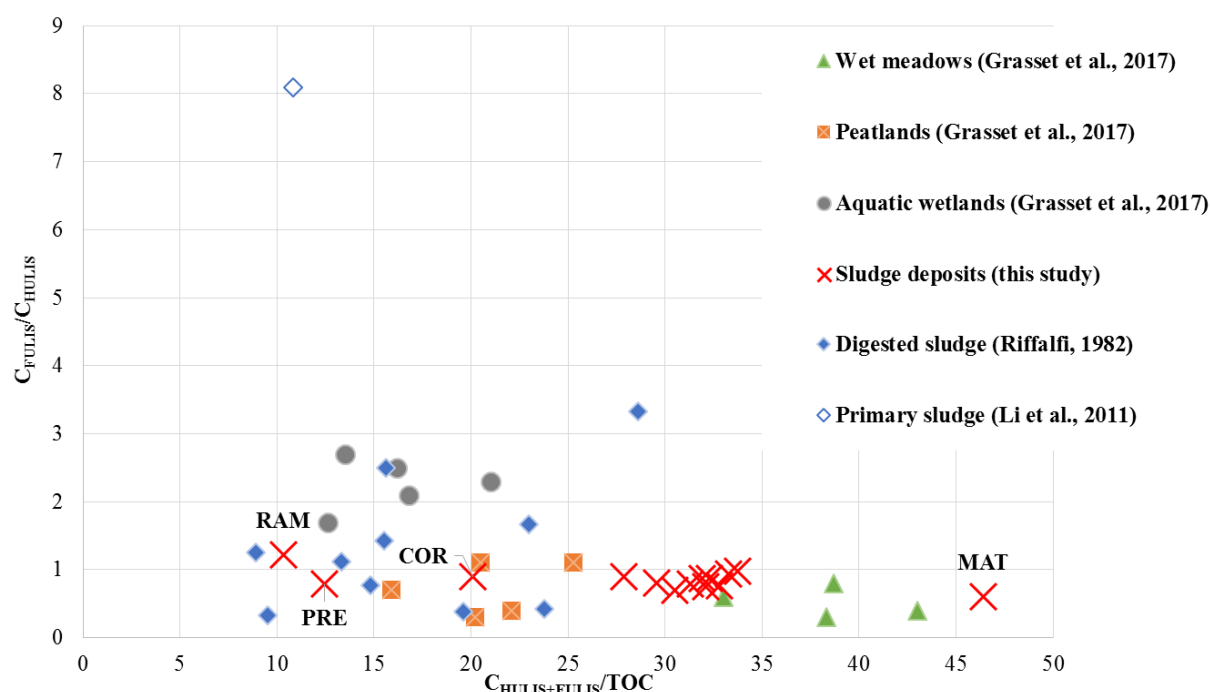


Fig. 6. $C_{\text{FULIS}}/C_{\text{HULIS}}$ against $C_{\text{HULIS}+\text{FULIS}}/\text{TOC}$ for natural wetlands (aquatic wetlands, peatlands and wet meadows), activated sludges and the 14 sludge deposits of this study.

Altogether, the spots showing the VFCW sludge deposits exhibited a specific state of evolution of the organic matter as compared to the other samples. Their position in the graph revealed a specific typology of samples, despite the differences existing amongst them. Most sludge deposits samples were positioned between the spot of digested sludge samples and peatlands and the spot of wet meadows. On the contrary, RAM, PRE and COR samples were positioned close to the area of peatlands and digested sludge samples.

4. Conclusions

Biological, chemical and thermochemical methods were applied to characterize surface sludge deposits sampled from 14 French VFCW sewage treatment plants. Results clearly revealed the existences of two groups of samples. The dominant factor of distinction between the two groups was the age of the VFCW plant of origin. The first group corresponded to sludge deposits taken from VFCW plants which were still in their first year of operation at the time the samples were collected. These samples exhibited a high content of organic matter of high biodegradability, high thermal lability, and low humification. Although the number of samples in this category (3) was low to permit a straightforward generalization, it was observed that the implementation of additional treatments in one of the three VFCW plants of this group modified the characteristics of its sludge deposits in terms of humification rate and thermal behavior. This observation showed that the presence of additional treatments modified the characteristics of the sludge deposits into a signature close to that of samples from mature systems. The second group gathered samples from 10 distinct French VFCW plants in a remarkably homogenous manner. The plants of origin were three years of age or more. Their sludge deposits were characterized by a lower organic content, lower biodegradability and thermal lability, and higher humification than those of the first group. This common set of properties was favorable to a relative accumulation of trace metals, which were analyzed at higher concentrations in this group of samples. However, the concentrations measured were found compatible with a land application practice should the sludge have to be dredged out from the filters for maintenance. One last sample, although originating from a mature VFCW plant, revealed quite different properties as compared to the other ten samples of the group. This observation could not be explained by the implementation of additional treatments. Rather, it revealed that the sample was identified as “atypical”, suggesting a particular situation in the VFCW plant of origin.

This study therefore showed that the organic matter captured within the surface sludge layer in French VFCW sewage treatment plants was submitted to a relatively rapid evolution

process over the very first year of operation of the plants. After one to three years of operation, a certain equilibrium was established, resulting from the balance between the flow of fresh POM continuously brought to the surface and the evolution of the rest of the deposits within the depth of the layer. This equilibrium state was found to be remarkably similar in all the plants of 3 years and more, regardless of the implementation of additional treatments, local conditions, and time. Surface sludge deposits therefore appeared as integrative tracers of the evolution of the system.

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