

Sewage sludge and waterworks sludge stabilization in sludge treatment reed bed systems

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ABSTRACT

In this study, results about sludge stabilization in sludge treatment reed bed (STRB) systems in two different systems, Hanningfield STRB 1 (England), treating waterworks sludge, and Stenlille STRB 2 (Denmark), treating surplus activated sludge, are presented. The study mainly focused on the effectiveness of the STRBs systems in stabilizing sludge organic matter; in fact, parameters correlated to biochemical and chemico-structural properties of organic sludge matter were determined. Dewatering and sludge stabilization were effective in both STRBs, as highlighted by total and volatile dry solids trend. β -glucosidase, phosphatase, arylsulphatase, leucine amino-peptidase and butyrate esterase activities, enzymes related to C, P, S, N and overall microbial activity, respectively, significantly declined along the profile in both STRBs. The determination of humic carbon highlighted the formation of a stable nucleus of humified organic matter in both STRBs in the deepest layers, thus meaning the successful stabilization of sludge organic matter for both kind of sludges. Similar conclusions can be drawn from pyrolysis gas chromatography analysis (Py-GC), which enables the characterization of soil organic matter quality from a chemical-structural point of view. The pyrolytic indices of mineralization and humification showed that in both STRBs the sludge organic matter is well stabilized.

Key words | enzyme activities, humic carbon, pyrolysis gas chromatography, sludge quality, sludge treatment reed bed system

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INTRODUCTION

Sludge from water treatment works (WTW) represent a typical by-product of water treatment processes, deriving from the processes of chemical coagulation and softening at drinking water treatment plants (Casey 2006). In Europe, several million tons of waterworks sludge are produced every year, with several problems of environmental and economic concern about their disposal (Babatunde & Zhao 2007).

Sludge treatment reed bed (STRB) systems have been widely used in Denmark as a cost-efficient and environmentally friendly technology to dewater and mineralize surplus sludge from conventional wastewater treatment plants (WWTP) (Nielsen *et al.* 2014). In several papers (Nielsen *et al.* 2014; Peruzzi *et al.* 2015), the dewatering and stabilizing sewage sludge effectiveness has been clearly proven. The technology has been applied also in other countries, such as Poland (Kolecka *et al.* 2017), France (Troesch *et al.* 2009), Italy (Masciandaro *et al.* 2015), Greece (Stefanakis

et al. 2011), and Spain (Uggetti *et al.* 2010), both at full and laboratory scales, mainly for surplus sludge stabilization. To the best of our knowledge, few experiences about waterworks sludge stabilization in STRB systems have been carried out (Nielsen & Cooper 2011); some authors have also considered the feasibility of using alum-sludge as a substrate for plant growing in reed bed system for wastewater systems (Zhao *et al.* 2009).

Both sewage sludges and waterworks sludges are characterized by high water content (usually greater than 95% by weight) and by unstable organic matter which readily undergo to active microbial decomposition (Casey 2006). The waterworks sludge in England is generated when potable water is produced from surface water, reservoir water or direct abstraction of river water. For the purification process, coagulants are used to remove impurities. In Hanningfield Water Works, iron based coagulants such as ferric sulphate is used to separate out the silt and

algae contained in the raw reservoir water. This subsequently generates a ferric based liquid sludge (iron sludge).

Treatment principle of STRB systems

STRB systems essentially comprise a series of gravel/sand basins that are planted with *Phragmites australis*. The operation involves cycling through a number of reed bed basins (e.g. 8–12); sludge can be derived from various sources, be it digested or undigested activated sludge, or from other sources such as waterworks sludges. Experience from Orbicon is that STRB are capable of treating many types of sludge with a dry solid content of the inlet sludge up to a concentration between 0.1 and 5%.

The length of the loading and resting periods depends on the sludge characteristics, climate, and the age of the system/basin, the dry solid content, the thickness of the sludge residue and the intensity of partial loadings during the period of loading. Sludge is loaded to the basins, which are vegetated with common reeds (*P. australis*), and dewatered through passive drainage and evapotranspiration. The sludge residue stays on the surface of the basins and mineralizes through the natural biophysical interaction of plants, microorganisms and air, whilst water is removed from the sludge by both evapotranspiration and drainage through the gravel/sand substrate.

Vegetation and ventilation with air in STRB systems create favourable conditions for the transformation of degradable organic matter to a more stable humic form (Nielsen et al. 2012; Peruzzi et al. 2013). After 8 to 12 years of operation, the mineralized sludge is excavated and used

as fertilizer or as soil conditioners. A schematic of a typical reed-bed treatment system is provided in Figure 1.

In this study, results about sludge stabilization in STRB in two different systems, Hanningfield pilot STRB 1 (England), treating waterworks sludge, and Stenlille STRB 2 (Denmark), treating sewage sludge (surplus activated sludge, SAS), are presented.

MATERIALS AND METHODS

STRB systems

The presented results of the influent and residue sludge in this study are from two different STRBs situated in Hanningfield Water Works (England, 51° 39' 55" N 0° 30' 41" E) and Stenlille WWTP (Denmark, 55° 32' 21" N 11° 34' 29" E). The two systems (Figure 2(a) and 2(b)), built by Orbicon, have the same design comparable to a full-scale plant with reeds, ventilation, sludge input, reject water systems as well as filters and drains. The systems were dimensioned for a treatment capacity of 30–60 kg ds m⁻² y⁻¹. The STRB 2 was constructed with eight basins in 2005 had received sludge from WWTP designed with mechanical, biological and chemical treatment (Table 1). The Hanningfield pilot system (STRB1) was built at Hanningfield WTW in 2008, with six basins each of 20 m². The test period was during 2008–2013. The full scale system was designed and constructed with 16 basins and put into operation in 2012 (Table 1). The height of accumulated sludge in both STRBs were different (0.2 m and 0.4 m in STRB 1 and STRB 2,

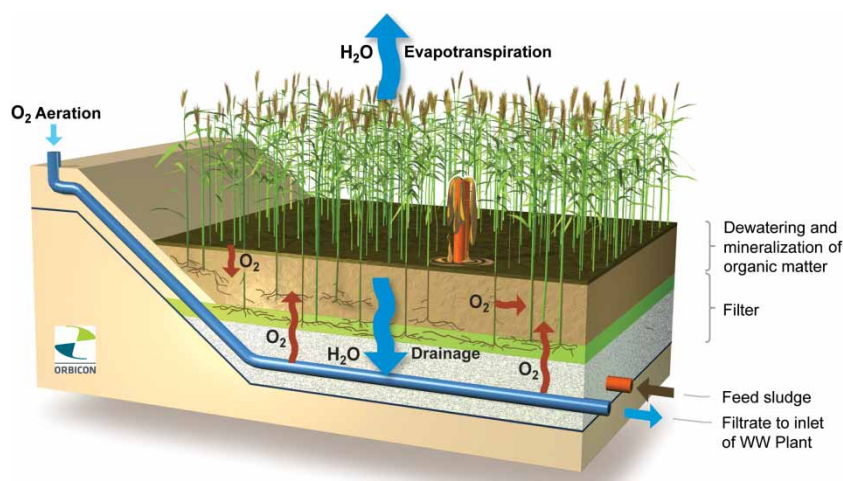


Figure 1 | Layout of STRB system (Orbicon A/S).

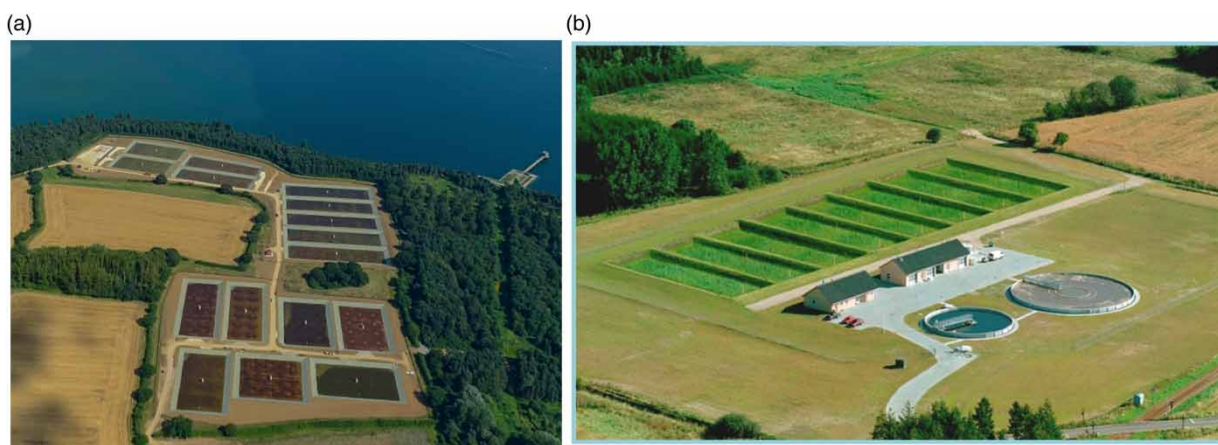


Figure 2 | (a) STRB 1 Hanningfield (England); (b) STRB 2 Stenlille (Denmark).

respectively), were different mainly because of the loading period, area load and the feed sludge quality (Table 1).

The WTW at Hanningfield in Essex treats up to 240 mega litres of drinking water per day, supplying a major part of Essex with potable water.

Methods

Sampling sites were randomly selected within the selected basins in each STRBs at the end of the resting periods of

60 and 55 days, respectively, after the last load in the loading phases. The sampling sites were uniformly distributed along the bed area. Sludges were sampled (one sample day for each system) along the profile within the selected basin with a core sampler. The entire profile of the selected basin was then divided in layers of 10 cm each as follows:

- STRB 1: 0–10 cm and 10–20 cm (1 sample site)
- STRB 2: 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm (10 sub-sample sites from 1 basin)

Table 1 | Treatment plants and loading program

	STRB 1 Hanningfield (England)	STRB 2 Stenlille (Denmark)
Water (production – $\text{m}^3 \text{d}^{-1}$)	240,000	–
Population equivalent (PE)	–	6,800
Basin area (m^2)	42,500 (16 basins)	2,920 (8 basins)
Pilot plant	120 (6 basins)	–
Selected basin in STRB	N° 1 (pilot plant)	N° 1
Capacity (t ds y^{-1}) (full scale)	1,275	175
Theoretical loading rate ($\text{kg ds m}^{-2} \text{y}^{-1}$)	30	60
Real loading rate ($\text{kg ds m}^{-2} \text{y}^{-1}$)	20–30	30–40
Sludge type (feed sludge)	Iron sludge	SAS
• Sludge age (aerobic days)	–	20–25
• Loss on ignition (%)	20–30 ^a	55–65 ^b
• Fat and oil (mg kg ds^{-1})	5–62 ^a	2,050–8,000 ^b
Loading/Resting (days)	2–7/30–45 (Pilot plant)	5–7/45–55
Loading period	2008–2013 (Pilot plant)	2005 –
Sampling (sludge residue)	December 2012	May 2014
Accumulated sludge height at sampling time (m)	0.2	0.4

^aSampling period 2008–2012 (20–25 samples).

^bSampling period 2005–2014 (40–50 samples).

Sampling of the sludge residue in STRB 1 (pilot plant) was carried out in December 2012 and in STRB 2 in May 2014.

Total solids (TS) were measured by water loss at 105 °C, while volatile solids (VS) were measured at 550 °C as loss on ignition. Total organic carbon (TOC) and total nitrogen (TN) were analyzed by RC-412 multiphase carbon and FP-528 protein/nitrogen (Leco). Water soluble carbon (WSC) was determined on water extract (1:10 w/v, 1 h, 60 °C), while total extractable carbon (TEC) and the fractionation of humic substances (humic acids, HA; fulvic acids, FA and not humic carbon, NHC) were determined on pyrophosphate extracts (1:10 w/v, 24 h, 60 °C) following Grigatti *et al.* (2004) method. Carbon contents were then measured by RC-412 multiphase carbon. Ammonia (N-NH₃) was determined on aqueous extract (1:10, w/v, room temperature for 1 h) with the ammonia selective electrode (Mettler Toledo Seven Multi™). Nitric nitrogen (N-NO₃) were determined on KCl extract 0.1 M (1:10, w/v, room temperature for 1 h) through a selective electrode (Mettler Toledo Seven Multi™). pH was determined on water extract (1:10 w/v), while electric conductivity (EC) was determined on water extract (1:5 w/v).

Enzyme activity was measured according to Marx *et al.* (2001) and Vepsäläinen *et al.* (2001) based on the use of fluorogenic methylumbelliferyl (MUF) and 7-amino-4-methylcoumarin (AMC) substrates. Samples were analyzed for butyrate esterase (EC 3.1.1.1), β -glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), arylsulphatase (EC 3.1.6.1) and leucine aminopeptidase (EC 3.4.11.1) using 4-MUF-butyrates, 4-MUF- β -D-glucoside, 4-MUF-phosphate, 4-MUF-sulphate and L-leucine-7-amino-4-methylcoumarin as substrates, respectively. A moist sample (equivalent weight to 1 g oven-dry material) was weighed into a sterile jar and treated with 50 mL Na-acetate buffer pH 5.5. A homogenous suspension was obtained by sonicating for 60 s at an output energy of 50 J s⁻¹. Aliquots of 100 μ L were withdrawn and dispensed into a 96-well microplate (three analytical replicates sample – 1 substrate – 1). Finally, 100 μ L of 1 mM substrate solution were added giving a final substrate concentration of 500 μ M. Fluorescence (excitation 360 nm; emission 450 nm) was measured, after 0, 30, 60, 120, 180 min of incubation at 30 °C, with an automated fluorimetric plate-reader (Infinite F200 pro TECAN).

The dried sludge was put into pyrolysis microtubes in a CDS Pyroprobe 190 and pyrolysis was carried out at 800 °C for 10 s, with a heat gradient of 10 °C ms⁻¹. The probe was directly coupled to a Carlo Erba 6000 gas chromatograph

with a flame ionization detector. The pyrograms obtained were quantified by normalizing the areas of the characteristic seven peaks. Identification of pyrogram fragments (acetic acid, acetonitrile, benzene, toluene, furfural, pyrrole and phenol) in the samples was carried out on the basis of the relative retention times compared with standard spectra (Ceccanti *et al.* 2007). Acetonitrile (E1) is derived from the pyrolysis of amino acids, proteins and microbial cells, while acetic acid (K) is principally derived from lipids, fats, waxes, cellulose and carbohydrates.

Benzene (B) is basically derived from condensed aromatic structures of stable-humified organic matter. Pyrrole (O) comes from nitrogenated compounds such as nucleic acids, proteins and microbial cells. Toluene (E3) is derived basically from aromatic compounds with short aliphatic chains. Furfural (N) derives from cellulose and aliphatic organic compounds. Phenol (Y) is derived from fresh and condensed lignocellulosic structures (Ceccanti *et al.* 2007; Macci *et al.* 2012).

RESULTS AND DISCUSSION

In order to evaluate the effectiveness in stabilizing waterworks sludge in STRBs, parameters normally used to estimate the evolution of organic matter in soil were followed (Peruzzi *et al.* 2011). Moreover, the system was compared to an STRB treating SAS.

Dewatering process seemed to be more effective in STRB 1 with respect to STRB 2: TS, in fact, significantly increased along the profile in STRB 1, with respect to STRB 2 (Figure 3). However, the obtained results about STRB 2, which treated SASs, were comparable to results presented by other authors (Uggetti *et al.* 2010) for systems treating similar sludges. VS, which normally represent the amount of organic matter, and consequently, TOC decreased both significantly in STRB 2, while they remained unchanged in STRB 1; this trend highlighted the extent of mineralization process of sludge organic matter occurred in STRB 2 (Figure 3, Table 2). Also, TN significantly decreased in STRB 2, while it remained unchanged in the profile of STRB 1, even though at lower concentrations (Table 2). These results are in agreement with the previous findings in similar RBS (Peruzzi *et al.* 2011). However, it is worth recalling that sewage sludge are mainly constituted by fresh organic matter, while waterworks sludge are characterized by relatively low biodegradable organic fractions (Casey 2006).

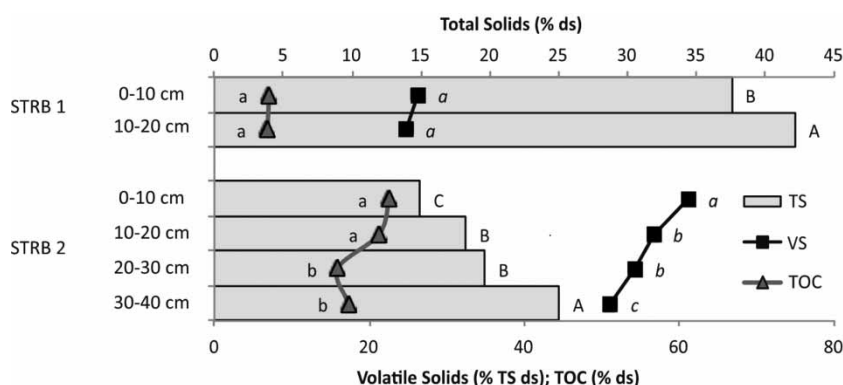


Figure 3 | TS (% ds), VS (% ds) and TOC (% ds) in STRB 1 and STRB 2. Different letters mean values significantly different ($P < 0.05$) within each STRB.

Table 2 | Electrical conductivity (EC, dS m^{-1}), pH, TN (%), phosphatase $\text{mmol MUB kg ds}^{-1} \text{h}^{-1}$, aryl-sulphatase $\text{mmol MUB kg ds}^{-1} \text{h}^{-1}$, in STRB 1 and STRB 2

	EC, dS m^{-1}	pH	TN, %	Phosphatase, $\text{mmol MUB kg ds}^{-1} \text{h}^{-1}$	Arylsulphatase, $\text{mmol MUB kg ds}^{-1} \text{h}^{-1}$
STRB 1					
0–10 cm	0.250 a	7.7 a	0.47 a	1,132 a	283 a
10–20 cm	0.249 a	7.8 a	0.42 a	323 b	28.1 b
STRB 2					
0–10 cm	0.779 a	7.6 a	3.32 a	4,563 a	2,770 a
10–20 cm	0.368 c	7.0 b	2.73 b	4,052 b	2,468 b
20–30 cm	0.344 c	6.6 c	2.01 c	4,524 a	2,308 b
30–40 cm	0.508 b	6.5 c	2.18 c	2,930 c	947 c

Different letters mean values significantly different ($P < 0.05$) within each STRB.

Electrical conductivity (EC) in both systems reached low values, comparable to values obtained during sludge composting (Moretti et al. 2015); the reduction was mainly a consequence of the formation of more stable bonds between the ions and organic matter during the stabilizing process, which reduced the solubility of the salts present in the sludge residues. In STRB 1, pH remained unchanged at sub-alkaline values; conversely, in STRB 2, pH decreased significantly within the profile, reaching sub-acid values in the deeper layers, as a consequence of organic matter stabilization process, as noticed by Stefanakis et al. (2011) (Table 2).

The breakdown of organic matter can be noticed following suitable parameters related to carbon and nitrogen cycles and overall microbial activity (Figures 4 and 5).

WSC, a soluble form of carbon correlated to fresh organic matter, and β -glucosidase enzyme, a hydrolytic enzyme involved in the C cycle, significantly decreased in both systems, especially in the deepest layers of STRB 2, highlighting the progress of sludge stabilization process. Moreover, butyrate esterase activity, a non-specific esterase

involved in the cycling of carbon related to live biomass content (Wittmann et al. 2004), which represented an indirect measure of overall microbial activity, dramatically decreased in both systems, underlining the extent of stabilization process, especially in the deepest layers.

In addition, parameters related to nitrogen cycle presented some analogies to carbon ones: soluble forms of nitrogen (N-NO_3 and N-NH_3) reached significant small values, especially in STRB 1. Moreover, the obtained values demonstrated the presence of aerobic conditions, especially in the deepest layers of STRB 2 and in the whole sludge residue in STRB 1. In addition, leucine-amino peptidase, a proteolytic enzyme that hydrolyses the peptide bond adjacent to a free amino group, significantly decreased with increasing depth, thus highlighting the effectiveness of STRB systems in stabilizing sludge organic matter. The trend of different enzymatic activities in environmental samples (soil, litter, lignocellulose or other matrices) represents, in fact, a useful tool for assessing the functional diversity of microbial communities or organic mass turnover (Kandeler et al. 1999; Baldrian

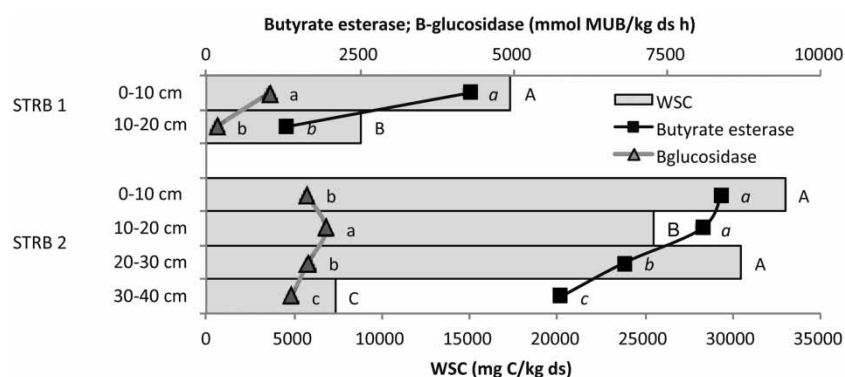


Figure 4 | WSC (mg C kg ds⁻¹), butyrate esterase (mmol MUB kg ds⁻¹ h⁻¹) in STRB 1 and STRB 2. Different letters mean values significantly different ($P < 0.05$) within each STRB.

2009). Besides butyrate esterase, β -glucosidase and leucine amino-peptidase activities, also phosphatase and sulphatase enzymes, activities involved in phosphorus and sulphur cycles, respectively (Tabatabai & Fu 1992), presented a vertical stratification, where the minimum activity was reached in the deepest layers of both systems.

These results are in agreement with Nielsen *et al.* (2014) findings, where three different STRBs treating several kinds of sludges were investigated. As available substrate decreased, in fact, the enzyme activity decreased as well, thus indicating the success of stabilization process for waterworks and sewage sludges (Diaz-Burgos *et al.* 1992; Peruzzi *et al.* 2011).

A further argument in support of the successful sludge stabilization is the trend of parameters related to humic substances. Humic carbon is commonly constituted by two different components: FA, which represent the less stable part of humic matter, and HA which represent the more stable fraction of humic matter.

The extractable (TEC), humified (HA and FA) and NHC contents are detailed in Figure 6. As expected, content of humic substances were significantly higher in the STRB 2, treating sewage sludges with respect to STRB 1 treating waterworks sludges. However, both systems presented comparable levels of humification rate, the ratio between humic carbon and TOC (26.16–24.68% and 30.38–23.69% for STRB 1 and STRB 2, respectively), thus demonstrating the effectiveness in stabilizing sludge organic matter, in terms of mineralization and humification process (Peruzzi *et al.* 2013). However, the value of HA and, consequently, the value of humification rate could be overestimated for the presence of interfering substances (mainly proteins and lipids), especially in systems treating sewage sludges (Cecanti & Garcia 1994; Grigatti 2004).

A more authentic evaluation of sludge stabilization process in terms of mineralization and humification process can be obtained by the organic matter chemical-structural characterization obtained by Py GC technique (Figure 7 and Table 3).

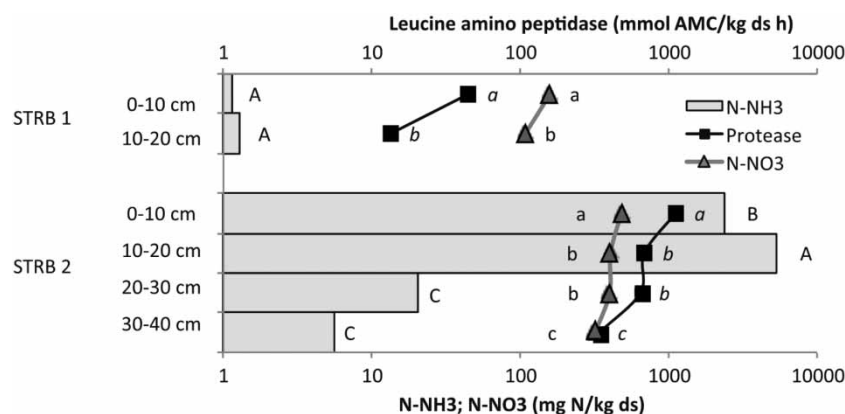


Figure 5 | Nitric nitrogen (N-NO₃, mg N kg ds⁻¹), ammonia nitrogen (N-NH₃, mg N kg ds⁻¹) and leucine amino peptidase (mmol AMC kg ds⁻¹ h⁻¹) in STRB 1 and STRB 2. Different letters mean values significantly different ($P < 0.05$) within each STRB.

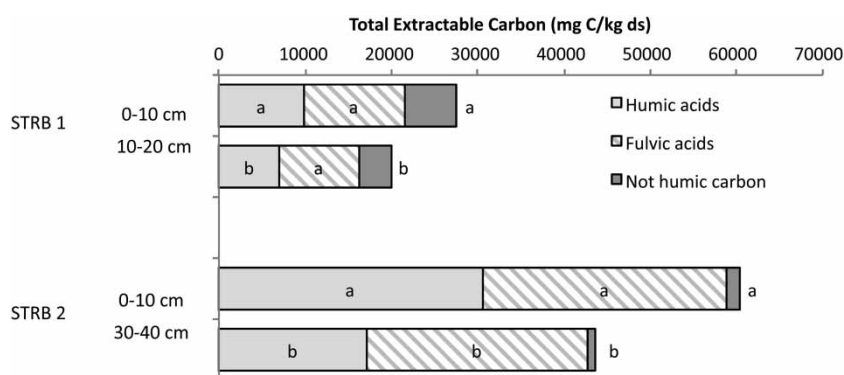


Figure 6 | TEC (mg C kg ds⁻¹), HA (mg C kg ds⁻¹), FA (mg C kg ds⁻¹) and NHC (mg C kg ds⁻¹) in STRB 1 and STRB 2. Different letters mean values significantly different ($P < 0.05$) within each STRB.

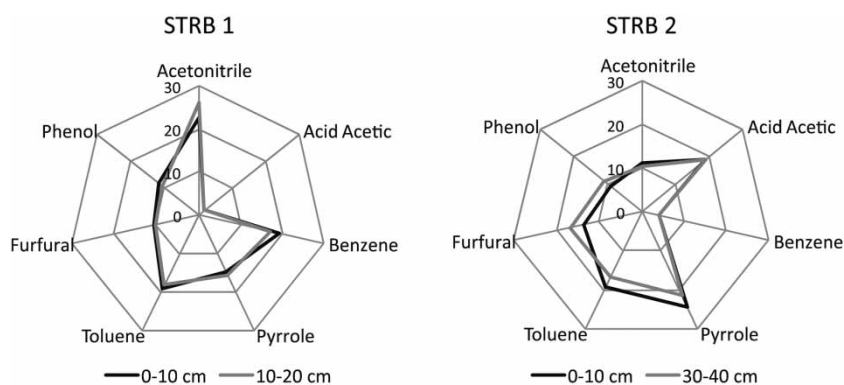


Figure 7 | Pyrolysis-GC results: pyrolytic fragments in STRB 1 and STRB 2. Acetonitrile (%), acid acetic (%), benzene (%), pyrrole (%), toluene (%), furfural (%) and phenol (%).

Acetonitrile (E1), which derived from the pyrolysis of amino acids, proteins, and microbial cells, and benzene (B), which indicated the presence of condensed aromatic structures of stable organic matter, were prevalent in STRB 1 with respect to STRB 2, thus highlighting the presence of mineralization process involving fresh organic matter and the instauration of a significant humification process. On the other hand, in STRB 2 acid acetic (K),

which resulted from the pyrolysis of lipids, fats, and waxes, and furfural (N), which rose from carbohydrates ligno-cellulosic materials, proteins and other aliphatic organic compounds, were consistent with respect to STRB 1, thus highlighting the presence of a mineralization process involving rapidly metabolizable organic substances. However, also pyrrole (O) derived from nitrogenated compounds such as nucleic acids, proteins, microbial cells and condensed humic structures, was prevalent in STRB 2 with respect to STRB 1, thus underlining the beginning of a humification process mediated by microbial activity. In addition, the similar content of toluene (E3), deriving pseudo-stable aromatic substances with long aliphatic chains, and phenol (Y), arising from condensed humic structures, indicated the establishment of a humified stable organic matter nucleus in both STRBs (Ceccanti et al. 2007; Macci et al. 2012). It is noteworthy that the prevalence of humification process in STRB 1, with respect to STRB 2 was underlined by the trend of humification index (B/E3), which increases when organic matter becomes more mature; moreover, in STRB 2, both the

Table 3 | Pyrolysis-GC results: mineralization index of fresh organic matter (O/N), humification index (B/E3), and mineralization index of stable organic matter (O/Y)

	O/N	B/E3	O/Y
STRB 1			
0–10 cm	1.40 a	1.02 a	1.24 a
10–20 cm	1.51 a	0.96 a	1.45 b
STRB 2			
0–10 cm	1.77 b	0.21 a	2.74 b
30–40 cm	1.29 a	0.26 a	1.93 a

Different letters mean values significantly different ($P < 0.05$) within each STRB.

mineralization index (O/N) about fresh organic and mineralization index (O/Y) about stable organic matter presented higher values ($p < 0.05$) with respect to STRB 1, thus confirming the presence of a mineralization process involving fresh and stable organic matter. These indices, in fact, increased when the degradation of organic matter is high, thus confirming in STRB 2 the mineralization process is still occurring, even though in both STRBs the sludge organic matter can be considered stabilized.

CONCLUSIONS

The study demonstrated that STRBs were effective in dewatering waterworks sludge and sewage sludge: stabilization was successful both for inorganic and organic sludges as well.

Chemical and biochemical characterization revealed that both kind of sludges were successfully stabilized in terms of low levels of nutrients and microbial activity.

A stable nucleus of humic organic matter was noticed in both systems; moreover, the chemical structural characterization highlighted that sewage sludges were mainly stabilized by mineralization process involving fresh and stable organic matter, while waterworks sludges were also stabilized by humification process.

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