Reed bed systems for sludge treatment: case studies in Italy

G. Masciandaro, R. Iannelli, M. Chiarugi and E. Peruzzi

ABSTRACT

In recent years, reed bed systems (RBSs) have been widely considered as a valid technology for sludge treatment. In this study are presented results about sludge stabilization occurring within beds in four RBSs, situated in Tuscany (Italy). The results showed that stabilization of the sludge over time occurred in all RBSs, as shown by the low content of water-soluble carbon and dehydrogenase activity, which measures indirectly the overall microbial metabolism, and by the re-synthesis of humic-like matter highlighted by the pyrolytic indices of mineralization and humification. Results about heavy metal fractionation, an appropriate technique to estimate the heavy metal bioavailability and sludge biotoxicity, showed that the process of sludge stabilization occurring in RBSs retains metals in fractions related to the stabilized organic matter, making metals less bioavailable. Moreover, the concentrations of various toxic organic compounds were below the limit of concentration suggested by the European Union’s Working Document on Sludge, for land application. The effectiveness of the stabilization processes in RBs was hence clearly proven by the results that measured mineralization and humification processes, and by the low levels of bioavailable heavy metals and toxic organic compounds in stabilized sludges.

Key words | enzyme activity, heavy metal bioavailability, pyrolysis gas chromatography, sludge stabilization, toxic organic compound

INTRODUCTION

Safe and cost-effective management of sewage sludge is still a worldwide environmental challenge. A common goal in sludge treatment is to reduce toxicity, decrease volume, and convert sludge into useful resources. In recent years, reed bed systems (RBSs) have been widely considered as a valid technology for sludge treatment. However, even though this technology is not widely used in Mediterranean countries, significant experience in the Tuscany region has been gained since 2004.

The RBS is a combination of a traditional sludge drying bed and constructed wetland: Phragmites australis is directly planted in the drying beds, where sludge is frequently applied. This technology involves low construction costs, minimal daily maintenance, water content reduction, and good stabilization of biosolids.

The RBS technology in stabilizing sewage sludges has been proven not only in its effectiveness in dewatering (Stefanakis & Tsihrintzis 2011; Iannelli et al. 2013) and reducing the pollutant content (Peruzzi et al. 2011b; Matamoros et al. 2012) and pathogen content (Nielsen 2007), but also in improving the quality of organic matter in sludges (Uggetti et al. 2010; Peruzzi et al. 2011a; Kolecka & Obarska-Pempkowiak 2013; Nielsen et al. 2014). The stabilization process, resulting from the synergic action of plants, organic matter and microorganisms, is a combination of mineralization and the humification process of organic matter. The organic matter stabilization process occurring in the basins can be successfully studied following parameters usually applied to soil and other environmental matrices.

In this paper, results about sludge stabilization from four different RBSs in Italy after several years of functioning are reported.

MATERIALS AND METHODS

Reed bed systems

Stabilization of sludges was investigated in four urban wastewater treatment plants: Acque S.p.A., RBS 1 La Fontina, RBS 2 Oratoio, RBS 3 Colle di Compito and RBS 4 Pittini, all situated in the Tuscany region (Italy).
In this paper, results from the setup of the plants (2005 for RBS 1 and RBS 2; 2006 for RBS 3 and RBS 4) are reported. For RBS 1, all results for the entire period of operation of the plant have been presented (2005–2012, 72 months), while RBS 2 is still operating (2005–2013, 96 months). Both RBS 3 and RBS 4 have been operating since 2006 (2006–2013, 90 months). All operational details about each RBS are reported in Table 1.

### Methods

Total organic carbon (TOC) was determined by RC-412 multiphase carbon (LECO Corporation, St Joseph, MI, USA) on 50–70 mg of dried sample. The RC-412 employed a state-of-the-art furnace control system, which allowed the temperature of the furnace to be stepped and subjected to ramping. Different sources of carbon (organic and inorganic carbon) were differentiated by the temperature at which they oxidize, decompose or turn into volatiles. Water and carbon dioxide released from the minerals were detected by means of infrared absorption cells.

Total nitrogen (TN) was determined by FP-528 protein/nitrogen (LECO Corporation, St Joseph, MI, USA). An encapsulated sample (60–80 mg) was placed into the loading head of the FP-528, where it was sealed and purged of any atmospheric gases that had entered during sample loading. The sample was then dropped into a hot furnace and flushed with pure oxygen for very rapid combustion. By-products of combustion (CO₂, H₂O, NOₓ, and N₂) passed through the furnace filter and thermoelectric cooler for subsequent collection in a ballast apparatus. These collected gases in the ballast were equilibrated, and a small aliquot was then used for further conversion of the gases. The remaining aliquot was reduced and then was measured by the thermal conductivity cell for nitrogen.

Water soluble carbon (WSC) was determined according to the method of Yeomans & Bremner (1988) by dichromate oxidation on aqueous extract (1:10, w/v, 1 hour at 60 °C) (Garcia et al. 1991). Dehydrogenase activity (DHase) (EC 1.1.1.1) was determined by the reduction of 2-p-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) using 0.2 g of sludge at 60% of field capacity, exposed to 0.2 ml of 0.4% INT in distilled water for 20 hours at 22 °C in darkness. The INTF formed was extracted with 5 ml of a mixture of 1:1.5 tetrachloroethylene/acetone by shaking vigorously for 1 minute. INTF was measured spectrophotometrically at 490 nm. DHase activity was expressed as mg INTF/kg dw h (dw: dry weight) (Masciandaro et al. 2000).

For the pyrolysis gas chromatography (Py-GC) analysis, the dried sludge (about 0.2 g) was put into pyrolysis microtubes in a CDS Pyroprobe 190 (Oxford, PA, USA) and pyrolysis was carried out at 800 °C for 10 seconds, with a heat gradient of 10 °C/ms. The probe was directly coupled to a Carlo Erba (Milan, Italy) 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; Macci et al. 2012).

The heavy metal fractionation was determined using the Mocko & Waclawek method (2004). The concentrations of different metal fractions were transformed from mg/kg to mEq/kg and then the concentration for each fraction was summed to give the total for the metal.

Linear alkylbenzene sulfonates (LAS), nonylphenols (the sum of nonylphenol, NP; nonylphenol ethoxylate with the one ethoxy group, NP1EO; and nonylphenol ethoxylates with two ethoxy groups, NP2EO (NPEs)) and di-2-ethylhexyl-phthalate (DEHP) were simultaneously
extracted from air-dried samples with methanol by microwave-assisted extraction (5 ml of methanol, 10 minutes and 250 W microwave irradiation; Villar et al. 2007). Chromatographic analysis was performed on an Agilent 1100 series high performance liquid chromatograph with an ultraviolet diode array (wavelength excitation 1/4 226 nm) and fluorescence detectors (wavelength excitation 1/4 226 nm; wavelength emission 1/4 301 nm). Organic compounds separation was carried out using a ZORBAX Eclipse XDB-C8 (150 × 3 mm i.d., 5 μm). A mobile phase of acetonitrile and 10 mM ammonium acetate solution was used at different gradients (30:70 for 8 minutes, 65:35 for 7 minutes, 100:0 for 10 minutes and 70:30 for 5 minutes). A column equipped with a thermostat at 28 °C and a flow rate of 1 ml/minute were adopted (Fountoulakis et al. 2005; Santos et al. 2007; Pakou et al. 2009).

**Sampling**

Sludge sampling was carried out every 3 months for the first year of operation, then every 6 months for the rest of the experimental period. For each bed of each RBS, five subsamples were taken, which were mixed in order to obtain a representative sample of each bed. The samples were collected near the gravel layer. The plant material was removed from samples. About 20 days before the sampling, the sludge applications were stopped.

**Statistical analysis**

All results reported in the text are the means of determinations for each bed in each RBS. One-way analysis of variance was carried out to compare the means between times of sampling, in order to follow the progress of sludge stabilization. Where significant F-values were obtained, differences between individual means were tested using Tukey-HSD (honest significant difference) tests at 0.05 significance level, using the Statistica 7.0 software (StatSoft, Inc., USA).

**RESULTS AND DISCUSSION**

**Sludge stabilization: mineralization and humification processes**

The sludges were stabilized and transformed within the RBSs by sludge–plant–microorganism interactions (Peruzzi et al. 2009). The two main concomitant processes taking place are mineralization and humification of organic matter.

In general, the content of TOC and TN, the main nutrients present in sludges, slowly decreased over time, following a similar trend in all four RBSs. During the first phase of operation (24–36 months), the TOC and TN concentrations were generally higher than concentrations found during the last period (72–96 months) (Figure 1).

![Figure 1](https://iwaponline.com/wst/article-pdf/72/7/1043/466769/wst072071043.pdf)

**Figure 1** | TOC (%C) and TN (%N) in stabilized sludges. Means followed by the same letter(s) (lowercase for TOC and uppercase for TN) are not significantly different according to Tukey’s test at P < 0.05 over time.
These results were in agreement with the findings of Gagnon et al. (2013). This overall constant level of organic matter mineralization was more clearly highlighted by the trend of two sensitive parameters to biological stabilization status, DHase and WSC: the latter represents a readily biodegradable substrate for microorganisms, while the former, occurring intracellularly in all living microbial cells, represents the overall microbial activity (Masciandaro et al. 2000) (Figure 2). As a consequence of mineralization processes occurring in the basins, WSC and DHase activity dramatically decreased in all RBSs during the first months of operation, and immediately after reached significantly low and stable concentrations, even though fresh organic matter was continuously added to the basins.

The occurrence of stabilization is also evident in the significant changes in the chemical structural properties of organic matter, measured by the Py-GC technique (Ceccanti et al. 2007; Macci et al. 2012) (Figure 3). The index of

![Figure 2](https://iwaponline.com/wst/article-pdf/72/7/1043/466769/wst072071043.pdf)

**Figure 2** | WSC (mg C/kg dw) and DHase (mg INTF/kg dw h) in stabilized sludges. Means followed by the same letter(s) (lowercase for WSC and uppercase for DHase) are not significantly different according to Tukey’s test at $P < 0.05$ over time.

![Figure 3](https://iwaponline.com/wst/article-pdf/72/7/1043/466769/wst072071043.pdf)

**Figure 3** | Pyrolytic indices of mineralization (O/N) and humification (B/E3) in stabilized sludges. Means followed by the same letter(s) (lowercase for B/E3 and uppercase for O/N) are not significantly different according to Tukey’s test at $P < 0.05$ over time.
mineralization \( (O/N) \) expresses the ratio between pyrrole (a heterocyclic aromatic organic compound derived from nitrogenous compounds, humified organic matter, and microbial cells) (Song & Farwell 2008) and furfural (a pyrolytic product coming from degradation of polysaccharides). The higher the ratio, the higher the extent of mineralization of organic matter, meaning that a high concentration of labile organic compounds remains (Ceccanti et al. 2007; Macci et al. 2012). The mineralization index generally decreased over time in all RBSs, even though with some fluctuations. Lower values of \( O/N \) were noted during the final phases, meaning that the rate of the mineralization process slowly decreased over time, as the sludge organic matter becomes more stabilized. As the mineralization process slowed, the humification process tended to be the dominant stabilization process for sludge organic matter. The index of humification \( B/E3 \) (benzene to toluene, the former deriving basically from stable condensed aromatic structures, the latter deriving from pseudo-stable aromatic structures with short aliphatic chains) increases when organic matter is becoming more mature. The humification index significantly increased over time, especially during the final phases in all RBSs, reaching significant values that demonstrated the re-synthesis of humic-like matter (Song & Farwell 2004, 2008; Macci et al. 2012).

### Heavy metal and toxic organic compounds

The total heavy metal content (Table 2) remained below the level established by law for the reuse of sewage sludge in agriculture (Italian regulation D. Lgs. 99/92 for disposal on agricultural lands: Cu 1,000, Ni 300, Cd 20, Pb 750, Zn 2,500 mg/kg dw). Similar concentrations of heavy metals

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were reported by Fytili & Zabaniotou (2008) for sewage sludge content in European countries.

The procedure for fractionation differentiated the sludge heavy metals into four fractions with different degree of bioavailability:

1. Exchangeable fraction associated with carbonated phase (Fraction 1). This is the most mobile fraction potentially toxic for plants. Metals are adsorbed on the sludge components and Fe and Mn hydroxides.

2. Reducible fraction (Fraction 2). Heavy metals are strongly bound to Fe and Mn oxides, but they are thermodynamically unstable in anoxic and acidic conditions.

3. Oxidizable fraction bound to organic matter (Fraction 3). Heavy metals complexed by humic substances become soluble when organic matter is degraded in oxidizing conditions. This fraction is not considered to be bioavailable and mobile.

4. Residual fraction (Residual Fraction). Heavy metals are included in crystalline structures in the residual solids. They are considered to be not extractable and in an inert form.

In this paper are reported results for Fraction 1 and Fraction 3 (Figure 4). Fraction 1 content significantly declined over time, as noticed previously in other papers (Kolecka & Obarska-Pempkowiak 2013; Peruzzi et al. 2014), in particular during the first phases, as the stabilization proceeded in RBSs. Other authors found higher content of metals associated with Fraction 1 in sewage sludge stabilized with traditional methods (thickening, heat treatments, anaerobic digestion, etc.) (Walter et al. 2006; Fuentes et al. 2008). Conversely, Weng et al. (2014) reported similar results in sludge dewatered by heat treatment. As the bioavailable fraction dropped, the Fraction 3 bound to organic matter significantly rose, as a consequence of the humification process (Peruzzi et al. 2014). This increase is particularly evident during the last phases of operation.

Results for toxic organic compounds are reported in Figure 5. The concentration found in the RBSs reflects the concentration of toxic organic compounds present in sewage sludges: RBS 2 showed, in fact, higher values of LAS, NPEs and DEHP with respect to other RBSs.

In all RBSs, the concentration of LAS in the sewage sludge was lowered to the limit of concentration suggested by the European Union’s Working Document on Sludge (2000) for land application (2,600 mg/kg dw). Because of their relatively high biodegradability in the aerobic environment of RBSs, LAS were immediately degraded in all RBSs during the stabilization process in the basin.

For NPEs, the concentrations found in sewage sludge, even though similar to results found by previous authors (Roig et al. 2012), remained above the limit of 50 mg/kg dw suggested for land application. The concentrations remained high during the first 2–4 years of operation, confirming the resistance of NPEs to biodegradation (González et al. 2010; Ömeroğlu et al. 2015), then tended to significantly decrease in all RBSs, thus demonstrating the presence of aerobic conditions needed to degrade NPEs (Chang et al. 2007). The behaviour of DEHP was quite similar, even though a small nucleus seemed to remain persistent in
DEHP is a hydrophobic contaminant with lipophilic properties (Aparicio et al. 2009), which needs aerobic conditions to be successfully degraded. The concentrations found in sewage sludge were similar to results found by other authors (Pakou et al. 2009) and were lower than the limit suggested for land application (100 mg/kg dw). Similar observations about the persistence, occurrence and degradation of toxic organic compounds in RBSs for sludge treatment were reported by Nielsen & Willoughby (2005) and Matamoros et al. (2012).

CONCLUSIONS

The stabilization of sludge organic matter was clearly proven by a long-term monitoring of parameters expressing mineralization and humification processes. In particular:

- A long period of monitoring allowed the complex dynamics of organic matter stabilization occurring in RBSs to be followed.
- The content of TOC and TN slowly decreased over time as a consequence of the mineralization process.
- The mineralization process was clearly demonstrated by the dramatic decrease of water-soluble carbon and dehydrogenase activity found in all four RBSs.
- The mineralization process was predominant during the first period of operation, while the humification process became predominant during the last phases, as highlighted by chemical structural characterization of sludge organic matter.
- The degradation of toxic organic compounds was a consequence of the mineralization process.
- The bioavailability of heavy metals in sludges was greatly affected by the humification process occurring in RBSs.
- In all RBSs, similar patterns of organic matter mineralization and humification processes were noticed.

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