Nitrite and nitrate as electron acceptors for biological sulphide oxidation


* Department of Civil and Environmental Engineering, University of Florence, Via S. Marta 3, 50139 Florence, Italy
(E-mail: alberto.mannucci@dicea.unifi.it)
** Instituto Potosino de Investigacion Cientifica y Tecnologica, Camino a la Presa San José 2055, 78216 San Luis Potosí, Mexico
*** Cerco (Centro Ricerca Reflui Conciari), Consorzio Cuoiodepur, Via Arginale Ovest 56020 San Romano – San Miniato (PISA), Italy

Abstract
Authotrophic denitrification with sulphide using nitrate (R1) and nitrite (R2) as electron acceptor were investigated at bench scale. Different SRT (5 and 20 d) have been tested in R1 while R2 was operated at SRT = 13 d. The results indicated that the process allows to achieve complete sulphide removal in all testes conditions. Tested sulphide loads were estimated form the H₂S produced in a pilot scale anaerobic digester treating vegetable tannery primary sludge; nitrogen loads originated from the nitrification of the supernatant. Average nitrogen removal efficiencies higher than 80% were observed in all the tested conditions once steady state was reached. A maximum specific nitrate removal rate equal to 0.35 g N-NO₃⁻ g SSV⁻¹ d⁻¹ have been reached in R1. Due to sulphide limitation, an incomplete denitrification have been observed and nitrite and thiosulphate tend to accumulate especially in the presence of variable environmental conditions both in R1 and R2. Lower SRT caused higher NO₂⁻ accumulated/NO₃⁻ reduced ratios (0.22 and 0.24, with SRT of 5d and 20 d, respectively) using nitrate as electron acceptor in steady state condition. Temperature decrease caused sudden NO₂⁻ accumulated/NO₃⁻ reduced ratio increasing in R1 and NO₂⁻ removal decreasing in R2.

Keywords
Anoxic processeses, denitrification, denitritation, nitrate, nitrite, sulphide biological oxidation.

INTRODUCTION
Sulphide management represents a major problem in many WWTPs and specific industrial areas as the Tuscan tannery district (Italy), the second largest in Europe, in which more than 5300000 m³ per year of tannery wastewater are produced. In this context, Cuoiodepur WWTP (San Miniato, Pisa-Italy) treats almost exclusively vegetable tannery wastewaters characterized by high COD concentration (12-23 gO₂ L⁻¹), Suspended Solids (6-31 gSS L⁻¹), Ammonium (0.12-0.25 gN-NH₄⁺ L⁻¹), Chlorides (0.3 to 8 g L⁻¹) and Sulphate (1.7-2.7 gSO₄²⁻ L⁻¹) (Mannucci et al., 2010), and produce fertilizers from sludge after thermal drying and mixing with by-product of industrial tannery process. Since European regulation on land application is becoming increasingly stringent, it is important to evaluate alternative options such as anaerobic digestion of primary sludge (Dhayalan et al., 2007; Zupancic and Jemec, 2010; Kameswari et al., 2012). In the last two years, an average of 1200 m³ d⁻¹ of sludge have been wasted from primary settler of the Cuoiodepur WWTP and sent to a thickener to reduce water content and double the solids concentration. A future implementation of the anaerobic digestion phase to treat all the thickened primary sludge will require a 15000 m³ digester operated with an SRT of 25 d.
However, the presence of tannins is critical for methanogenic bacteria and may affect the outcome of the competition between sulphate reducing bacteria (SOB) and methanogens (Mannucci et al., 2014).

The SOB activity causes high sulphide concentration in the produced biogas and make sulphide removal mandatory before methane utilization.

Up today, chemical scrubbing is the most established technology for H$_2$S removal in WWTPs (Gabriel et al., 2004) due to several advantages: short contact time (1.3 to 2 s), low capital cost, easy operation and the possibility of treating highly variable loads. However, chemical scrubbing requires large amounts of chemicals, primarily NaOH, that increase both the operating costs and carbon footprint of the treatment. Biological processes application, based on the use of aerobic Biotrickling Filters (BTFs) do not require the use of chemicals, can remove H$_2$S under varying operating conditions and require only a few days for biological process start-up (Wu et al., 2001; Namini et al., 2008).

The use of alternative electrons acceptors, instead oxygen, would make the use of BTF technology more favorable for the treatment of the biogas produced from anaerobic digestion of tannery industrial sludge. The supernatant originated from the anaerobic digestion is characterized by high ammonia concentration (up to 900 mg N-NH$_4^+$ L$^{-1}$); its nitrification in a side stream separated from the main treatment train will allow the production of liquid streams with high nitrate and/or nitrite concentration that could be used as electron acceptor in autotrophic denitrification process.

Despite autotrophic denitrification with sulphide as electron donor has been investigated by several researchers with the main applicative purpose of applying it in the treatment of liquid streams (Lu et al., 2009), sulphide removal through denitrification was recently tested on biogas and other gaseous streams (Kleerebezem and Mendez, 2002).

In order to achieve the primary objective of hydrogen sulphide removal, the influent ratio between sulphur and nitrogen has to be considered; nonetheless, it is important to highlight that the stoichiometry of the process is flexible, since sulphide can be oxidized either to elemental sulphur, thiosulphate or sulphate and nitrogen can be used as either nitrite or nitrate, with the result of significantly widening the range of potentially suitable S/N ratio values. Moreover, the successful application of a biotrickling filter for biogas treatment, depend on the possibility of controlling biomass growth (Mannucci et al., 2012). Both of the above mentioned issues definitely depend on the bioprocesses stoichiometry and kinetics, which is, to the best of our knowledge, not clearly quantified in all the reactions of interest. For instance, most of previous work on denitrification with sulphide focused on nitrate as electron acceptor; on the contrary, the use of nitrite as electron acceptor, despite some exception (Moraes et al., 2012), still need to be deepened. Moreover, most of previous work on the kinetics and stoichiometry of sulphur compounds oxidation, were carried out with thiosulphate as electron donor (Artiga et al., 2005), while very few tests (Munz et al., 2009) have been carried out with sulphide due to the difficulty of using a strongly volatile and, at the same time inhibiting, substrate. This work is aimed at investigating denitrification and denitritation with sulphide, in the presence of typical S/N ratio that can derive from the anaerobic digestion of primary sludge of tannery wastewater.

**MATERIALS AND METHODS**

The present experimentation have been conducted CER$^2$CO Lab located at the Cuioidepur WWTP. Two identical bench scale SBR reactors (R1 and R2) with working volume of 3.2 L and HRT of 24 h, were used (Figure 1). Influent nitrogen and sulphide loads were chosen on the basis of the results of an experimentation (data not shown) conducted using a pilot scale anaerobic digester (volume = 150 L, SRT = 25 d) fed with sludge from Cuioidepur WWTP primary settler. The S-SO$_4^{2-}$ concentration in the primary sludge have been monitored daily for more than 250 days (from January 28$^{th}$ to November 17$^{th}$ 2013). The average sulphate concentration and the S-SO$_4^{2-}$/S$_{tot}$ were 740 ± 200 mg L$^{-1}$ S-SO$_4^{2-}$ and 0.96, respectively. Anaerobic digester effluent S-SO$_4^{2-}$ and effluent S-S$^{2-}$ were 15 mg S L$^{-1}$ and 13 mg S L$^{-1}$, respectively. Experimental data confirmed that 96 % of the
influent sulphur (as sulphate) was reduced to H₂S that exits the digester as the biogas. Ammonia concentration in the supernatant resulted 722 ± 60 mg N L⁻¹. Average S/N ratio for the autotrophic denitrification of 1.02 gS gN⁻¹ have been calculated. An S/N ratio of 1.4 constitutes the worst estimated conditions (maximum sulphide and minimum nitrate/nitrite) to the main objective of complete sulphide removal. S/N ratios similar to those obtained during anaerobic digestion experimentation where tested as influent conditions in both SBR reactors.

![Figure 1 – Schematic of the used bench scale SBR](image)

R1 was fed filled with 1 L (SST = 8000 mg SST L⁻¹) of activated sludge collected from Cuoiodepur WWTP biological section and diluted with tap water. The reactor was fed with two distinct peristaltic pumps with sulphide (Solution A) and with a solution of micronutrients and either nitrate or nitrite (Solution B and C, respectively). To obtain the sludge capable of autotrophic denitrification, R1 activated sludge was acclimatized by feeding synthetic wastewater (Solution A and Solution B) under anoxic condition for more than 40 days. R1 was operated for 160 days, while R1 biomass was used as inoculum for R2 after 100 days. R1 experimentation was divided into three phases with different operational conditions as reported in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Start up</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>d</td>
<td>0-24</td>
<td>25-45</td>
<td>46-91</td>
<td>92-160</td>
<td>100-160</td>
</tr>
<tr>
<td>S_{IN}</td>
<td>mg S-HS⁻ d⁻¹</td>
<td>34-140</td>
<td>340</td>
<td>550</td>
<td>550</td>
<td>342</td>
</tr>
<tr>
<td>N_{IN}</td>
<td>mg N-NO₃⁻ d⁻¹</td>
<td>65-125</td>
<td>250</td>
<td>350</td>
<td>350</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>mg N-NO₃⁻ d⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>320</td>
</tr>
</tbody>
</table>
Solution A was stored in the absence of headspace, that is, in a storage tank with a variable volume, and at pH =10 to minimize the desorption of hydrogen sulphide. For Solution A $\text{NaHCO}_3$ (1.24 g L$^{-1}$) and $\text{Na}_2\text{S}_3\text{H}_2\text{O}$ (0.41 g L$^{-1}$) diluted in demineralised water were used; for Solution B and C $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (0.66 g L$^{-1}$); $\text{KH}_2\text{PO}_4$ (0.52 g L$^{-1}$); $\text{NH}_4\text{Cl}$ (0.05 g L$^{-1}$); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.063 g L$^{-1}$); $\text{KNO}_3$ (1.63 g L$^{-1}$; B only) and $\text{NaNO}_2$ (0.96 g L$^{-1}$; C only) were diluted in tap water.

The cycle phases for both reactors were as follow: feeding (30 min); mixing (60 min); settling (240 min); decant (30 min). The excess biomass was removed during the last 2 min of the mixing phase. The reactors were maintained at pH between 7 and 8 through the dosage of an HCl solution and at room temperature (between 18 and 28 °C).

Samples were collected three times a week and the following parameters were monitored: COD (soluble and total), VSS (Volatile Suspended Solids), TSS (Total Suspended Solids), N-$\text{NO}_2^-$, N-$\text{NO}_3^-$, S-$\text{SO}_4^{2-}$, Sulphide, Total Sulphur, T, pH. Thiosulphate and elemental sulphur, were estimated indirectly. Soluble and total COD, TSS and VSS have been analyzed according to IRSA-CNR methods (Metodi analitici per le acque, 2003). S-$\text{SO}_4^{2-}$, Sulphide, Nitrite and Nitrate have been measured through ionic chromatography (ICS1000, Dionex, Sunnyvale - U.S.A.) while total sulphur have been measured using plasma spectrophotometry (ICP-OES, Agilent Technology, Santa Clara – U.S.A.). A portable Hach-Lange (Berlin, Germany) probe was used to measure the pH twice a day in the effluent. Elemental sulphur and thiosulphate have been estimated on the results of total and soluble COD in the wasted mixed liquor. Thiosulphate remains in soluble form and contributes to the soluble COD while elemental sulphur remains in particulate and colloidal form and its contribution to the total COD have been estimated on the basis of the difference between total and soluble COD. Thiosulphate and elemental sulphur concentration in the reactors have been estimated according to Eq. 1 and Eq. 2:

\[
S_{\text{thiosulphate}} = \left( \frac{\text{COD}_s - N_{\text{nitrite}}}{gN_{\text{nitrite}}} \right) \frac{g\text{COD}}{gS_{\text{thiosulphate}}} \frac{1}{\text{MW}_\text{thiosulphate}} \text{MW}_\text{sulphur} \quad \text{Eq. 1}
\]

\[
S_{\text{elemental sulphur}} = \left( \frac{\text{COD}_p - VSS}{TSS} \right) \frac{g\text{COD}}{gS_{\text{elemental sulphur}}} \quad \text{Eq. 2}
\]

Where MW = molecular weight.

**RESULTS AND DISCUSSION**

**R1 performance evaluation**

During the whole experimentation sulphide removal efficiency (RE) in R1 was always higher than 99% (data not shown). This results are the same of those reported by Fajardo et al. (2012) in SBR reactors treating sulphide and nitrate in sulphide limiting conditions.

However, a sulphur mass balance based on a complete sulphide oxidation into sulphate does not explain observed RE. The loss of sulphur estimated from the difference between theoretical sulphate production from a complete sulphide oxidation and experimental sulphate production was
17% and 18% during phase II and phase III, respectively. The analysis of R1 effluent in steady state conditions confirms the presence of intermediate sulphur compounds as thiosulphate and elemental sulphur (Table 2).

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Parameter</th>
<th>IN (mg d(^{-1}))</th>
<th>(S_{\text{tot,IN}}) (mg d(^{-1}))</th>
<th>(N_{\text{tot,IN}}) (mg d(^{-1}))</th>
<th>OUT (mg d(^{-1}))</th>
<th>(S_{\text{tot,OUT}}) (mg d(^{-1}))</th>
<th>S-Diff. (%)</th>
<th>N-RE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Phase II</td>
<td>S-SO(_4^{2-}) 109 ± 20</td>
<td>662</td>
<td>--</td>
<td>552 ± 49</td>
<td>645</td>
<td>2.6</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(^{2+}) 552 ± 32</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_2)O(_3)(^{-}) 0</td>
<td></td>
<td>92.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_0) 0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-NO(_2)(^{-}) 0</td>
<td>--</td>
<td>350</td>
<td>53.5</td>
<td>96.5</td>
<td>--</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-NO(_3) 350 ± 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Phase III</td>
<td>S-SO(_4^{2-}) 105 ± 18</td>
<td>695</td>
<td>--</td>
<td>576 ± 80</td>
<td>647</td>
<td>6.9</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(^{2+}) 589 ± 54</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_2)O(_3)(^{-}) 0</td>
<td></td>
<td>64.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_0) 0</td>
<td></td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-NO(_2)(^{-}) 0</td>
<td>--</td>
<td>350</td>
<td>39.8</td>
<td>70.3</td>
<td>--</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-NO(_3) 350 ± 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>S-SO(_4^{2-}) 101 ± 10</td>
<td>434</td>
<td>--</td>
<td>405 ± 24</td>
<td>477</td>
<td>9.1</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(^{2+}) 332 ± 21</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_2)O(_3)(^{-}) 0</td>
<td></td>
<td>72± 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-S(_0) 0</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-NO(_3) 320 ± 15</td>
<td>--</td>
<td>320</td>
<td>75.2</td>
<td>75.2</td>
<td>--</td>
<td>76.5</td>
</tr>
</tbody>
</table>

Table 2 – Sulphur and nitrogen mass balance in R1 and R2 in steady state conditions and characterization of influent and effluent compounds. Difference between influent and effluent total sulphur is reported as S-Diff (%) while nitrogen removal efficiency is reported as N-RE.

Nitrate Specific Denitrification Rate (SDR) and Nitrite Accumulation Rate indicates an incomplete denitrification (Figure 2).

![Figure 2 - Nitrate Specific Denitrification Rate, Nitrite Accumulation Rate and Temperature trend in R1 experimentation.](image-url)
An incomplete denitrification have been observed during the whole experimentation. After nitrogen load increase at day 25 (from 80 to 160 mg N-NO$_3^-$ L$^{-1}$) nitrate SDR increase from 0.025 to 0.1 g (N-NO$_3^-$) g (SSV)$^{-1}$ d$^{-1}$. At same time nitrite started to accumulate in R1. During phase III whit an SRT of 5 d, nitrate SDR doubled and reached maximum value of 0.35 g (N-NO$_3^-$) g (SSV)$^{-1}$ d$^{-1}$. This values are similar to those obtained by Fajardo et al. (2012) in similar conditions. Nitrate removal efficiency varied from 67% to 93% during phase II and III and no clear effects of temperature variations were registered.

Starting from day 24, a minimum nitrite concentration of 4 mg N-NO$_2^-$ L$^{-1}$ were registered during the experimentation. Nitrite accumulation rate increased from 0.05 g (N-NO$_2^-$) d$^{-1}$ to 0.17 g (N-NO$_2^-$) d$^{-1}$ at day 130.

At day 130 Nitrite and Thiosulphate increase from 13 mg N-NO$_2^-$ L$^{-1}$ to 73 mg N-NO$_2^-$ L$^{-1}$ and from 5 mg S-S$_2$O$_3^-$ L$^{-1}$ to 25 mg S-S$_2$O$_3^-$ L$^{-1}$, respectively. Nitrite and thiosulphate remain higher than 22 mg N-NO$_2^-$ L$^{-1}$ and 8 mg S-S$_2$O$_3^-$ L$^{-1}$ until day 142. At day 144 thiosulphate was 6.5 mg S-S$_2$O$_3^-$ L$^{-1}$ and remain stable until the end of the experimentation. In the same period nitrite concentration was stable at 5 mg N-NO$_2^-$ L$^{-1}$.

An average NO$_2^-$/NO$_3^-$ ratio of 0.23 was observed. This value is close to the ratio obtained by Fajardo et al. (2012) under sulphide limiting conditions.

Nitrite accumulation might be explained by both sulphide limitations (Fajardo et al., 2012; Manconi et al., 2007) and higher values of the specific utilization rates of nitrite compared to that of nitrite (Mora et al., 2015; Campos et al., 2008).

At steady state conditions, sulphate constitutes more than 90% of the effluent sulphur compounds while more than 74% and 80% of the influent nitrogen was converted into nitrogen gas in phase II and phase III, respectively. In this context, Eq.3 was used to evaluate the effective impact of autotrophic denitrification process on sulfide removal.

$$\text{HS}^- + 0.823\text{NO}_3^- + 0.177\text{HCO}_3^- + 0.667\text{CO}_2 \rightarrow \text{SO}_4^{2-} + 0.169\text{C}_5\text{H}_7\text{NO}_2 + 0.003\text{H}_2\text{O} + 0.326\text{N}_2$$

Eq. 3

Where N/S ratio is 0.36.

The effect of autotrophic denitrification on sulphide removal have been estimated as the difference between the experimental produced $S_{\text{measured}}$ (Eq. 4) and the theoretical one (Eq. 5).

$$S_{\text{sulphate,measured}} = \frac{S - \text{SO}_4^{2-}}{S - \text{SO}_4^{2-} + S_0 + S - \text{S}_2\text{O}_3^{2-}}$$

Eq. 4

$$S_{\text{sulphate, theoretical}} = \frac{\Delta N}{(N/S)} \frac{S - \text{SO}_4^{2-}}{S_{\text{tot}}}$$

Eq. 5

A ratio between $\Delta S_{\text{measured}}/\Delta S_{\text{theoretical}}$ equal to 1.06 and 0.94 resulted in steady state conditions in phase II and III, respectively.

R2 performance evaluation

Figure 3 shows that, when nitrite alone was used as electron donor (R2) and steady state conditions are achieved, denitrification reached up to 80% of nitrite removal. In this conditions, thiosulphate partially accumulated in R2, as observed in R1. Nitrite reduction to nitrogen gas seems to be more sensitive to temperature variations than nitrate reduction to nitrite.

Due to lower nitrite (Krishnakumar and Manilal, 1999), nitrate (Chung et al., 2014), thiosulphate (Claus and Kutzner, 1985; Campos et al., 2008) and sulphate (Chung et al., 2014; Claus and
than threshold values, the effect of gradual and sudden temperature variation seems to be cause of nitrite and thiosulphate accumulation both in R1 and R2.

Figure 3 – Nitrite RE (A) and effluent thiosulphate (B) in R2

Autotrophic denitrification is highly influenced by temperature decrease: N-NO$_2^-$ RE decreases and the increase of CODsol indicates an increase in thiosulphate concentration. Thiosulphate production, probably due to chemical processes (Can-Dogan et al., 2010), cause a reduction of sulphate in the effluent and an increase of the difference between the sulphate theoretically produced from the oxidation of the total influent sulphide and the measured one. On these basis, it is possible to hypothesize that sulphide oxidation to elemental sulphur and/or thisulphate take place with kinetics significantly higher that oxidation of thiosulphate to sulphate; similarly, the reduction of nitrate to nitrite proceeds at higher transformation rates than denitritation.

Sulphur compounds both in the influent and in the effluent of R2 referred to steady state (from day 45) are reported in Table 2.

The effect of autotrophic denitrification on sulphide removal have been estimated as reported for R1 using Eq. 6 (N/S = 0.55).

\[
HS^- + 1.255NO_2^- + 0.15HCO_3^- + 0.45NH_4^+ \rightarrow SO_4^{2-} + 0.03C_8H_7NO_2 + 1.28H_2O + 0.815N_2
\]

Eq. 6

A ratio between $\Delta S_{\text{sulphate, measured}}/\Delta S_{\text{sulphate, theoretical}}$ equal to 0.95 resulted in steady state conditions.

CONCLUSIONS

The simultaneous sulphur and nitrogen removal efficiency through autotrophic denitrification have been tested in operational conditions similar to those that will be obtained from the anaerobic digestion of tannery primary sludge and nitrification of the supernatant.
The results from this study indicate that both denitrification and denitrification with sulphide as electron donor are effective for the removal of sulphide: sulphide removal efficiency was higher than 99% in all the tested conditions what concern SRT, Temperature and influent S/N ratio. Sulphide removal kinetics are extremely high, while nitrite tends to be partially accumulated as a function of SRT and T probably due to sulphide limiting conditions and slower kinetics of nitrite reduction respect to nitrate reduction to nitrite. The load of nitrogen present in tannery wastewater is compatible with the application of biogas biological treatment through autotrophic denitrification. In steady state conditions nitrogen removal efficiencies higher than 80% were obtained using nitrate or nitrite loads similar to those obtained by nitrification of the supernatant of an anaerobic digester treating vegetable tannery primary sludge.

ACKNOWLEDGMENTS
The authors acknowledge the EU Life+ program (Life Env/IT/075 Biosur), the Toscana Region (Meta POR 2007-2013) activities and the Marie Curie program (Irses 295176 Carbala).

REFERENCES


Metodi analitici per le acque 2003, Agenzia per la protezione dell’ambiente e per i servizi tecnici/ Istituto di Ricerca sulle Acque Consiglio Nazionale delle Ricerche, Rome, Italy


Moraes B.S., Souza T.S.O., Foresti E. 2012 Effect of sulfide concentration on autotrophic denitrification from nitrate and nitrite in vertical fixed-bed reactors. Process Biochemistry, 47 1395–1401


