



How the performance of a biological pre-oxidation step can affect a downstream photo-Fenton process on the remediation of mature landfill leachates: Assessment of kinetic parameters and characterization of the bacterial communities



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ABSTRACT

The main purpose of this work was to assess the (i) short-term effect of the main nitrification and denitrification variables on the nitrogen's biological removal via nitrite from highstrength leachates, and (ii) the effect of the presence/absence of nitrites/nitrates in a downstream photo-oxidation process. The biological reaction rates were evaluated as a function of several parameters: (i) temperature, dissolved oxygen (DO) concentration and pH, on the nitrification; and (ii) pH, temperature and the addition of phosphate ions, on the denitrification. At the beginning of most nitrification assays, it was verified that the ammonia stripping occurred simultaneously to the nitrification, reaching up to 31% removal of total dissolved nitrogen. The maximum nitrification rate obtained was 37 ± 2 mg $\text{NH}_4^+\text{-N}/(\text{h}\cdot\text{g VSS})$ (25°C , $1.02.0$ mg O_2/L , pH not controlled), consuming 5.3 ± 0.4 mg $\text{CaCO}_3/\text{mg NH}_4^+\text{-N}$. The highest denitrification rate achieved was 27 ± 1 mg $\text{NO}_2^-\text{-N}/(\text{h}\cdot\text{g VSS})$ (pH between 7.5 and 8.0, 30°C , adding 30 mg $\text{PO}_4^{3-}/\text{L}$), with a C/N consumption ratio of 1.6 ± 0.1 mg $\text{CH}_3\text{OH}/\text{mg NO}_2^-\text{-N}$ and an overall alkalinity production of 3.2 ± 0.1 mg $\text{CaCO}_3/\text{mg NO}_2^-\text{-N}$. The denitrification process showed to be sensitive to all studied parameters, while the nitrification reaction did not suffered significant change when DO content was changed. The two most abundant bacterial groups in the nitrification and denitrification processes, as indicated by the 454-pyrosequencing analysis of the 16S rRNA gene, were affiliated to *Saprospiraceae/Nitrosomonadaceae* and *Hyphomicrobiaceae/Saprospiraceae*, respectively. The abundance of *Nitrosomonadaceae* and *Hyphomicrobiaceae* (in particular, *Hyphomicrobium*) in the nitrification and denitrification process, respectively, is in agreement with the nitrifying and denitrifying activity of these bacterial members. The photo-Fenton reaction rate was assessed considering the presence of nitrites and nitrates and the absence of both in a leachate after biological oxidation and coagulation/sedimentation steps. The results showed that for a pre-treated leachate without nitrogen, the DOC degradation rate decreased 28%, while for a bio-treated leachate containing nitrites, the H_2O_2 consumption was 2.4 times higher.

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1. Introduction

Sanitary landfill leachates are characterized as a complex mixture of diverse organic and inorganic contaminants [1,2], which

are usually removed by combining different treatment processes [3,4]. Due to its simplicity, reliability, high cost-effectiveness and to the high nitrogen content (mostly in the $\text{NH}_4^+\text{-N}$ form) inherent in this type of effluent, the activated sludge biological process is almost always applied in leachate treatment plants (LTPs) [5,6]. Up to date, the nitrification reaction followed by a denitrification

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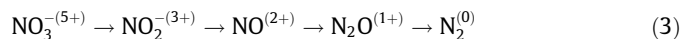
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step is the biological process most used for nitrogen removal from wastewaters [7].

The nitrification reaction is a lithoautotrophic microbiological mechanism, occurring under aerobic conditions, where the carbon dioxide is the carbon source and the molecular oxygen is the final electron acceptor. Usually, nitrification reaction takes place in two steps: (i) first, ammonium nitrogen is oxidised into nitrite (nitritation) by the ammonia-oxidising bacteria (AOB), frequently *Nitrosomonas*, according to Eq. (1); and (ii) then nitrite is converted into nitrate (nitratation) by the nitrite-oxidising bacteria (NOB), such as *Nitrobacter* or *Nitrospira*, in agreement with Eq. (2), under more restrictive operating conditions [8–10]. In wastewater treatment processes with high organic load, the AOB and NOB coexist with organo-heterotrophic bacteria, since these are the most responsible for the conversion of organic nitrogen compounds, such as proteins and amino acids, into simplest products, including the ammonium ions [11–13].

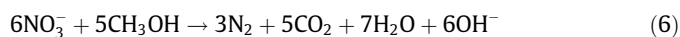
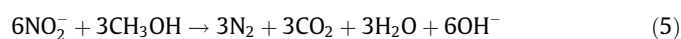
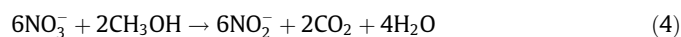


In contrast, the denitrification reaction is widely distributed among prokaryotic microorganisms, being coupled with chemo-organoheterotrophy or chemo-litho-autotrophy. It occurs under anoxic conditions, because an ionic nitrogen oxide compound acts as the final electron acceptor. The denitrification process involves the dissimilatory reduction of nitrate and/or nitrite into atmospheric nitrogen, through a sequential production of gaseous nitrogen oxide intermediates. The linear pathway of the reductive steps is shown in Eq. (3) (where the values between parenthesis represent the oxidation states of each nitrogen species) [8,9,14]:



Each oxidised nitrogen substrate is catalysed by a specific enzyme (NO_3^- , NO_2^- , NO or N_2O reductase) and attends as electron acceptor on the denitrifying bacteria respiration, commonly coupled with the oxidation of a biodegradable organic compound as electron donor for energy generation [8,9]. When compared with the aerobic metabolism, the energy-yielding of this anoxic metabolism is low. Hence, efficient denitrification usually requires the presence of high concentrations of electron donors.

Although, in general, leachates contain a high organic matter content, their biodegradable fraction decreases with aging, due to the releasing of recalcitrant molecules (mainly humic and fulvic acids) from the solid wastes deposited in the landfills [5]. This fact combined with that of denitrification step being preceded by nitrification (where the biodegradable carbon is already oxidised) often leads to the need of adding an external carbon source to accomplish the denitrification reaction. Several carbon sources have been used on this process, e.g., methanol, ethanol, glucose, methane, acetic and benzoic acid [15–19]. Among the various carbon sources commercially available, methanol is widely used, mainly because it is the cheaper, it reduces the sludge production and its residual content can be easily removed by aeration [15,17,20]. Eqs. (4)–(6) represent the energy-yielding reactions involved in denitrification, where the methanol provides energy to bacteria, such as *Hyphomicrobium*, and the gaseous dinitrogen is released to the atmosphere [12]:



The biological nitrogen removal via nitrite, instead of nitrate, could be a good option since nitrite is an intermediate of both nitrification and denitrification reactions. Thus, the nitrification could be stopped at nitrite and then denitrification could start from there, by reducing the nitrite into nitrogen gas, thereby saving about 25% in the oxygen demand for the nitrification, 40% in the needs of external carbon source for the denitrification and 50% in the size of the anoxic reactor [21,22]. This shortcut was effectively implemented by Spagni and Marsili-Libelli [23], on the treatment of sanitary landfill leachate with an average initial concentration of ammonium nitrogen of 1199 mg $\text{NH}_4^+\text{-N/L}$, using a sequencing batch reactor (SBR), at bench-scale. The authors achieved efficiencies of 98 and 95% on the nitrification and denitrification reactions, respectively, saving about 35% on the external carbon source.

The extension of the nitrification reaction can be influenced by diverse abiotic factors, being the most important the temperature, pH, dissolved oxygen (DO) deficiency, the presence of toxic or inhibitory substances and the substrate concentration. The nitrite build-up occurs when, individually or in combination, certain deviations in the abiotic factors repress the action of the NOB in detriment of the AOB. The denitrification process can be affected by the energy source, the temperature, the pH and the presence of oxygen, since the bacteria begin to respire with oxygen and stop to denitrify [8].

In general, there is no technology that, acting alone, is able to treat effluents with such recalcitrant organic fraction and high nitrogen load as in the leachate. The best solution is based on combined systems being able to achieve a cost/effective treatment technology. The combination of biological processes with physical-chemical technologies minimizes the drawbacks of each individual treatment, optimizing the effectiveness of the overall process. Accordingly, our research group in cooperation with EFA-CEC, Engenharia e Sistemas, S.A. company have published an European Patent (EP 2784031) [24] disclosing a methodology for the treatment of landfill leachates, which comprises the following sequential steps: (i) preliminary biological oxidation; (ii) coagulation/sedimentation process; (iii) photo-Fenton reaction (combining solar and artificial radiation); and (iv) final biological oxidation. One of the main drawbacks of this technology is the high consumption of H_2O_2 during the photo-Fenton process. Thus, a better knowledge on how the performance of the biological pre-oxidation step influences the efficiency of the photo-Fenton step can be a good way to optimize the high reactant consumption.

The main goal of the present work was to assess: (i) how the sudden variation of certain operating conditions may affect the reaction rates of nitrification and denitrification via nitrite on the treatment of a mature leachate, since this process is almost always incorporated in LTPs; and (ii) how the absence or presence of nitrogen, under the form of nitrites or nitrates, can affect the reaction rates of a downstream photo-Fenton process. To achieve our goal, the influence of: (i) temperature, DO concentration and pH, on the nitrification of a leachate collected at the outlet of an aerobic lagoon; (ii) pH interval, operating temperature and phosphate addition, on the denitrification of a nitrified leachate; (iii) presence of nitrites or nitrates and the absence of both nitrogen species, on the photo-treatment of a leachate pre-treated by biological oxidation and coagulation/sedimentation processes, was assessed. The biological sludge used in both nitrification and denitrification reactions was previously adapted to aerobic and anoxic conditions, respectively. Furthermore, the composition and structure of the corresponding bacterial communities were assessed, using 454pyrosequencing of the 16S rRNA gene.

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