UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento ICEA

Master in Environmental Engineering

Master Thesis

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EVALUATION OF NITROGEN TURNOVER IN AERATED LANDFILLS BY LABORATORY SCALE REACTORS

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A.A. 2012-2013

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PART I

Preliminary Consideration to the Laboratory Test Execution

1. Introduction

The thesis work here presented, is the result of the experimental activity done at the LISA laboratory of the ICEA department of the Padua University.

This thesis is subdivided into three parts. The second part is presented in the form of scientific article and exposes the materials, the methods, the results and their discussion of the experiment in a short and operative ways. The first part is more descriptive, it present a series of preliminary consideration to the test executions that could not be enclosed in the scientific article, because of the need of briefness for publication. Here are also presented the problems encountered in the experimentation starting and criteria that lead to play some choices about the analytical techniques. The third part is composed by annexes which report the experimental data and the calculation preformed during the experiment.

2. Critical review – Framework of the Existing Theories

The ammonia-nitrogen in the landfill leachate is derived from the nitrogen content of the waste. The municipal solid waste, in fact, have an estimated 4% content of proteins, deriving from sources like organic waste, garden waste and biosolids. Hydrolysis and fermentation of the nitrogenous fraction of the biodegradable substrates determines the production of ammonia-nitrogen and such process is called ammonification (Barzal et al., 2002). Removal of ammonia-nitrogen from leachate is necessary because of its aquatic toxicity and oxygen demand in receiving waters. Because ammonia-nitrogen has been implicated as one of the most significant long term pollution problem in landfills, it is likely that its presence will determine, like that of humic and xenobiotic substances, when the landfill is biologically stable and when post-closure monitoring may be reduced or end. Thus, removal of ammonia-nitrogen from leachate is critical to successful and sustainable landfill operations (Kjeldsen et al, 2003).

Ex-situ treatment is one of the possible scenario of ammonia-nitrogen management. Ammonia-nitrogen removal methods often include complex sequens of physical, chemical and/or biological processes, including chemical precipitation, nanofiltration and air stripping (Marttinen et al., 2002; Welander et al., 1997; Cheung et al., 1997). Another possible ex-situ treatment alternative is biological nitrification/denitrification via various reactor configurations. The possibility of realizing an ex-situ nitrification followed by the use of the landfill like an anaerobic reactor for denitrification is widely demonsyrated (Price at al., 2003). The ex-situ treatment implies the presence of a separate treatment system, which inevitably leads to additional costs and further operational difficulties. Therefore the development of in-situ nitrogen removal techniques appears to be an attractive alternative, which could potentially bring social and economical benefits.

Recent laboratory studies have shown the efficacy of in situ nitrogen removal processes in solid waste environments (Jokela et al., 2002; Onay and Pohland, 1998; Youcai et al., 2002). Jokela et al. (2002) conducted a laboratory study demonstrating in-situ denitrification is possible and can result in total oxidized nitrogen concentrations. The ammonia-nitrogen was found to be present in the effluent from the solid waste column, which was attributed to either the release by the waste or the high COD/NO_x ratio, which can promote the reduction of nitrates to ammonia-nitrogen. Youcai et al. (2002) conduced a study in which leachate passes through a biofilter consisting of old waste (8-10 years old) with both anaerobic and aerobic sections. A removal of 99.5% of the ammonia in leachate was observed, coupled with elevated concentrations of nitrate and nitrite, indicating the ammonia was converted biologically. Onay and Pohland (1998) also completed an in situ nitrification/denitrification laboratory study in which high removals of nitrogen in the leachate where observed and attributed to the nitrification and denitrification processes. Additionally, Hanashima (1999) observed accidentally removal of nitrogen in aerobic or semi-aerobic landfills.

3. Bioreactor Landfill Operation

Bioreactor landfills are controlled system in which moisture addition (often leachate recirculation) and/or air injection are used to create a solid waste environment capable of actively degrading the readily biodegradable organic fraction of the waste. Several researchers have documented the benefits associated with bioreactor technology (Murphy et al., 1995; Pohland et al., 1995; Reinhart et al., 1996). One advantage is that increased waste degradation rates characteristic of bioreactor landfills permit the life of a bioreactor landfill to be expanded beyond that of conventional landfills through recovery of valuable airspace. As leachate is recirculated, it is treated in situ, decreasing its organic strength and thus potential impact to the environment. In situ treatment potentially reduces the length of the postclosure care period and associated costs (Reinhart et al., 1998). Additionally, bioreactor landfills stimulate gas production; the majority of the methane is produced earlier in the life of the landfill, allowing for more efficient capture and subsequent use (Berge et al., 2005).

Although the organic strength of the leachate is significantly reduced in bioreactor landfills, ammonia-nitrogen remains an issue. The ammonia-nitrogen concentrations found in leachate from bioreactor landfill are greater than those found in leachate from conventional landfills (Onay et al., 2001). Ammonia-nitrogen tends to accumulate in both system because there is no degradation pathway for ammonia-nitrogen in anaerobic system. However, in bioreactor landfills, moisture addition and recirculating leachate increases the rate of ammonification, resulting in accumulation of higher levels of ammonia-nitrogen, even after the organic fraction of the waste is degraded (Barlaz et al., 2002).

The increased ammonia-nitrogen concentrations intensifies the toxicity of the leachate to aquatic species, potentially inhibiting the degradation processes and necessitating leachate treatment before ultimate disposal to protect receiving waters (Burton et al., 1998). It is been suggested that ammonia-nitrogen is one of the most significant long-term pollution problem in landfills, and it likely that the presence of ammonia-nitrogen will determine when the landfill is biologically stable and when post closure monitoring may end (Price et al., 2003).

Because bioreactor landfill environments are different from conventional landfills, there is a potential for a greater number of nitrogen transformation and removal processes to occur and for them to occur to a greater extend than in conventional landfills. System design of bioreactor landfills provides the flexibility in the location and duration of liquid and air injection, allowing for adjustment of pH, oxidation-reduction potential, and moisture content to create an environment conducive to microbial degradation and biological nitrogen removal (Berge et al., 2005).

Liquid addition to landfill has many advantages associated with it. Leachate recirculation involves the collection and redistribution of leachate trough a landfill. Moisture addition and movement are important factors affecting waste biodegradation, resulting in an increase in the moisture content of the waste and distribution of nutrients throughout the landfill, respectively. Optimal levels of moisture content have been found to be between 40 and 70%, on a weight basis (Barlaz et al., 1990).

Air addition has also been used as an enhancement and has been shown to enhance degradation processes in landfills at both the field and laboratory scale (Leikam et al., 1999). Adding air uniformly throughout the waste is also a challenge. Not only waste heterogeneities and compaction affect the air distribution, the presence of moisture does as well. Air will take the path of least resistance; thus, there will likely be areas of an aerobic landfill in which air does not reach, resulting in anoxic or anaerobic pockets within the waste mass (Berge et al., 2005).

Generally, bioreactor landfills undergo the same degradation processes as conventional landfills, just at faster rate and to a greater extend because of the optimization of in situ conditions. However, degradation pathways may vary depending on the operation of bioreactor landfill. Compared with conventional landfills, bioreactor landfills have shown a more rapid and complete waste conversion and stabilization process (Harper et al., 1988).

3.1 Anaerobic Bioreactor Landfill

Anaerobic bioreactor landfills are those in which moisture additions is practiced. Sources of liquid may include groundwater, stormwater, infiltrating rainfall, or leachate. Moisture content adjustment results in enhanced methane production, which has been repeatedly demonstraded in several laboratory, pilot, and field scale studies. Because waste degradation is enhanced in anaerobic bioreactors and organic material is returned to the landfill via leachate recirculation, methane is produced at a much faster rate. The total volume of gas produced also increases, as organics in the leachate are recycled and then biodegraded within the landfill. The majority of gas production may be confined to a few years, earlier in the life of the landfill, than traditionally occurs in conventional landfills, allowing for a more efficient capture and subsequent use. (Reinhart et al., 1996).

Anaerobic bioreactor landfills are more effective at degrading the solid waste than conventional landfill. However, when compared to other types of bioreactor landfills, anaerobic system tend to have lower temperatures and slower degradation rates (Merz et al., 1970). A disadvantage to operating the landfill as an anaerobic reactor is the accumulation of ammonia-nitrogen. In anaerobic bioreactor landfills, the ammonia-nitrogen present in the leachate is continually returned to the landfill, where there is no degradation pathway for ammonia in anaerobic environments. An advantage of operating the reactors anaerobically when compared with other bioreactor landfill types is that air is not added; therefore the operational cost are less than what would be incurred aerobically and methane can be captured and reused.

3.2 Aerobic Bioreactor Landfills

Adding air to landfill has been shown to enhance degradation processes in landfills, as aerobic processes tend to degrade organic compounds typically found in municipal solid waste (MSW) in shorter periods than anaerobic degradation processes (Leikam et al., 1999). Reported advantages of operating the landfill aerobically include increased settlement, decreased metal mobility, reduced ex situ leachate treatment required, lower leachate management and methane control costs, and reduced environmental liability (Read et al., 2001).

Many of the nitrogen transformation and removal process are favored by aerobic processes, including nitrification and ammonia air stripping or volatilization. Air stripping and volatilization may be favored in aerobic bioreactor landfill because of higher pH levels and temperatures that are inherent in an aerobic environment. The additional gas flow associated with air injection may also induce greater masses of ammonia-nitrogen removal (Berge et al., 2005).

During aerobic degradation of MSW, biodegradable materials are converted mostly to carbon dioxine and water. Little, if any, methane is produced, which may be viewed as either an advantage or disadvantage, depending on whether methane collection and use as an energy source is desired or required. Methane is a potent greenhouse gas; thus, if it can not efficiently controlled and collected in anaerobic landfills, its production can be a local environment concern. Further, the solid waste environment during aerobic degradation has a fairly neutral pH, which decreases metal mobility (Hanashima et al., 1999). Volatile organic acid production is decreased in aerobic bioreactors because the anaerobic fermentation processes are limited. However, volatile acid and methane production may still occur in anaerobic pockets within the landfill (Berge et al., 2005).

The aerobic processes generate a considerable amount of heat, leading to elevated in situ temperatures as high as 66 °C (Stessel et al., 1992). The elevated temperatures increase the evaporation, which results in a significant loss of leachate. As a consequence, there is less leachate to manage. The high temperatures may limit certain biological nitrogen transformation processes from occurring, although no data regarding temperature effects are available. Additionally, the combination of the high temperatures and presence of any air may create a fire potential. However, minimizing methane production and ensuring proper moisture contents, fire potential is lessened (Berge et al., 2005).

Odor often associated with anaerobic systems, such as from hydrogen sulfide and volatile acids, are reduced in aerobic bioreactor landfills. Aerobic processes do have some odor associated with them; however, it is an earthy smell. Some odorous compounds emitted by aerobic composting include methanethiol, which has a pungent sulfide odor (Miller et al., 1992).

4. Nitrogen transformation and removal processes

Currently, ammonia-nitrogen is treated in leachate ex situ to the landfill. Ammonia-nitrogen removal methods often include complex sequence of physical, chemical, and biological processes, including chemical precipitation, nanofiltration, air stripping, and biological nitrification/denitrification processes via various reactor configurations. However, operating the landfill as a bioreactor provides opportunity for in situ nitrogen transformation and removal processes.

When adding air to landfills, biological processes such as nitrification traditionally found and expected only in landfill cover soils as a results of air diffusion may now occur within the waste mass. Additionally, recirculating nitrified leachate allows for denitrification process to occur in anoxic areas found in both anaerobic and aerobic bioreactor landfills.

The heterogeneous nature of solid waste complicates the nitrogen cycle in bioreactor landfills. Because the waste is heterogeneous, portions of the landfill may contain different amount of nutrients, be at different temperatures and have different moisture levels. Environmental conditions greatly affect the transformation and removal of nitrogen. Thus, within a landfill cell, there may be many nitrogen transformation processes occurring simultaneously or sequentially. Processes commonly found in wastewater treatment processes and in soils, such as ammonification, sorption, volatilization, nitrification, denitrification, and nitrate reduction, may all occur in bioreactor landfills (Berge et al., 2005).

4.1 Ammonification

Proteins present in the waste are the major source of ammonia-nitrogen. This conversion of organic nitrogen to ammonia-nitrogen by heterotrophic bacteria is termed ammonification. Ammonification is a two-step process consisting of the enzymatic hydrolysis of proteins by aerobic and anaerobic microorganisms relasing amino acids and the subsequent deamination or fermentation of the acids to carbon dioxide, ammonia nitrogen, and volatile fatty acids. During deamination, amine groups are liberated to form ammonia or ammonium, depending on the pH, and alkalinity is slightly elevated (Burton et al., 1998). Once ammonification occurs, the ammonia-nitrogen is dissolved in the leachate and is ready to be transformed and removed via volatilization, sorption, or biological processes when in aerobic environment. The pH also increases during ammonification. Any free ammonia that is present is highly reactive and has been found to combine with organic matter, making them more biodegradable (Barzal et al., 1996). Thus, in landfill, any ammonia that is produced may redissolve and react with organic matter before exiting the landfill.

4.2 Ammonium Flushing

The mass of ammonia-nitrogen that can be leached from the waste is controlled by the volume of water passed through the landfill, the nitrogen content of the waste, and the ammonia-nitrogen concentration in the bulk liquid. Reducing ammonia-nitrogen concentrations by washout and dilution to acceptable levels within the landfill requires the addition of large volumes of water.

Flushing results in the remove of ammonia-nitrogen from landfills by adding large volumes of water, which must be treated externally. When operating the landfill as a bioreactor, leachate is very recycled, and hence ammonia-nitrogen is continually reintroduced to the landfill while additional ammonia is solubilized into the leachate (Berge et al., 2005).

4.3 Ammonium Sorption

Sorption of ammonia-nitrogen to waste may be significant in bioreactor landfill because of the high ammonium concentrations present. Ammonium is known to sorb onto various inorganic and organic compounds. The amount of ammonium sorbed on some organics has been reported to exceed the mass found in the bulk liquid (Nielson et al., 1996). Sorption of ammonium to the waste will allow for temporary storage of ammonium prior to it being used in other processes, such as nitrification or volatilization, and may also result in the slow dissolution of ammonium over time (Heavey et al., 2003).

Sorption is dependent on pH, temperature, ammonium concentration, and ionic strength of the bulk liquid. For ammonia to sorb to waste particles, it must be in the form of ammonium (NH_4^+). At a pH levels expected in a landfill, the dominant form of the ammonia species is the ammonium ion. As ionic strength of the bulk liquid increases, sorption of ammonium tends to decrease due to ion-exchange effects. The sorbed ammonium is released and exchanged with other ions present in the bulk liquid, especially those with higher selectivity or concentration. A common procedure to extract sorbed ammonium from solid particles involves the addition of a sodium or potassium sulfate solution. The sodium or potassium ions exchange with the ammonium, allowing for the ammonium to desorb from the waste. The conductivity of landfill leachate is generally high and thus may influence ammonium sorption (Berge et al. 2005).

It seems probable that more sorption occurs in older solid waste than in younger waste because older waste has a smaller particle size and thus a larger surface area, yielding more available reactive sites for sorption. Additionally, older waste contains more recalcitrant organic particles to which ammonium may sorb. Further, as waste ages, there may be changes in the surface charges of the waste, resulting in higher levels of sorption. The presence of complex organics has been shown to influence ammonium sorption; the ammonium ions may fix irreversibly to these molecules (Heavey et al., 2003).

4.4 Volatilization

In conventional landfills, ammonia makes up approximately 0.1 to 1.0% of landfill gas exiting the landfill (Tchobanoglous et al., 1993). Ammonia is not a greenhouse gas, so its impact on the environment is not as harmful as methane; however, there are some adverse health effects that may result from exposure to the gas. Ammonia has a pungent odor and is respiratory-tract irritant. Also, ammonia gas can dissolve in the moisture on skin and form ammonium hydroxide, a corrosive chemical that can cause skin irritation (Matheson et al., 2002).

Volatilization only occurs when free ammonia is present. At pH levels above 10.5 to 11.5, the majority of the ammonia-nitrogen present in solution is in the form of free ammonia gas (NH_3). As temperature increases, more of the ammonia is converted to free ammonia gas because of temperature dependence of the acid dissociation constant. At a pH level of 7, under standard conditions, 0.56% of ammonia present is in the form of free ammonia. When the temperature increases to 60° C, a temperature commonly found in aerobic landfills, the percentage of free ammonia present at pH 7 increases to 4.90 % (Berge et al., 2005).

Airflow also plays an important role in ammonia-nitrogen volatilization. As air is introduced, it begins to agitate the leachate, creating a removal pathway for dissolved free ammonia to volatilize and leave the landfill. Airflow also dilutes the concentration of gas-phase ammonia-nitrogen above the leachate, increasing the driving force for dissolved ammonia nitrogen to partition to the gaseous phase (Henry et al., 1999).

4.5 Nitrification

Nitrification is a two-step aerobic process in which ammonia-nitrogen/ammonium is microbially oxidized to nitrite and nitrate via obligate aerobe, autotrophic, chemolithotrophic microorganisms. Because nitrification is an aerobic process, it is almost non-existent in conventional landfills and in bioreactor landfills in which air is not added. In those systems, nitrification is restricted to upper portions of the landfill or the cover where air may infiltrate (Burton et al., 1998). In landfills in which air is purposely added, nitrification can be a significant nitrogen removal pathway.

During the first step of nitrification, *Nitrosomonas* bacteria oxidize ammonia-nitrogen to nitrite, according to the following reaction (Rittman et al., 2001):

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$$

The second step of the nitrification process is the oxidation of nitrite to nitrate by *Nitrobacter* bacteria according to the following reaction (Rittman et al., 2001):

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^-$$

Nitrifiers must fix and reduce inorganic carbon to use as their carbon source, resulting in low cell yields and thus small maximum specific growth rates. Additionally, nitrification results in the consumption of alkalinity as nitrous acid is formed.

Nitrification may occur in bioreactor landfills in which air is added. Although the metabolic processes associated with nitrification may be essentially the same in landfills and wastewater treatment processes, the operation, control, and potential extent of such processes is not the same. Nitrification in landfill environments is complicated by oxygen and temperature limitations, heterotrophic bacteria competition, and potentially pH inhibition. Oxygen is a required element for nitrification. Adding air to a landfill would be dual-purpose: to nitrify, removing the ammonia-nitrogen, and to enhance the degradation of solid waste. However, maintaining and controlling sufficient oxygen levels within the landfill, especially considering the heterogeneous nature of solid waste and the high temperatures characteristic of aerobic landfills, may be difficult and may result in oxygen limitations and thus reduced nitrification rates. Additionally, oxygen may become limiting to nitrifiers in areas within the landfill containing large amounts of organic carbon due to competition with heterotrophs. Under oxygen-limiting conditions, autotrophic ammonia-oxidizing bacteria may produce nitric and nitrous oxides, which would be a distinct disadvantage of this technique as they are potent greenhouse gases (Burton et al., 1998).

It is suspected that *in situ* nitrification may be optimized when operated in landfill cells containing older waste, because, as in composting, as the age of the waste increases, the temperature of the system decreases due to reduced biological activity (Tchobanoglous et al., 1993). Additionally, since older waste contains fewer biodegradable organics, less competition with heterotrophs for oxygen will occur.

pH may also be a complication during nitrification processes in landfills. The pH of leachate in aerobic landfills is generally near neutral, or slightly above (Read et al., 2001). The alkalinity of leachate is generally in the range of 1,000 to 10,000 mg/L as calcium carbonate (Tchobanoglous et al., 1993). Because nitrification destroys alkalinity, there may not be sufficient alkalinity present to buffer pH changes that would result from nitrification of high ammonia-nitrogen leachates. It is possible that alkalinity may need to be added to the landfill to buffer the leachate.

4.6 Denitrification

In situ denitrification is also complicated in solid waste systems, although it may be easier to implement than nitrification. Denitrifiers are more robust than nitrifiers, however they require a sufficient organic carbon source for high nitrate removal rates. Because of the carbon needs, denitrification may occur most efficiently in young waste, rather than in older, partially oxidized waste.

Typically, in-situ denitrification occurs in anoxic bioreactor landfills. However, because of the potential for anoxic pockets to be present in aerobic systems, denitrification may also occur in portions of aerobic bioreactor landfills that air does not reach (Berge et al., 2005).

4.6.1 *Heterotrophic Denitrification*

Denitrification is an anoxic process that reduces nitrate to nitrite, nitric oxide, nitrous oxide, and finally nitrogen gas, as shown in the following reactions (Rittman et al., 2001):

$$NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O$$
$$NO_2^- + e^- + 2H^+ \longrightarrow NO + H_2O$$
$$2NO + 2e^- + 2H^+ \longrightarrow N_2O + H_2O$$
$$N_2O + 2e^- + 2H^+ \longrightarrow N_2(g) + H_2O$$

Tipically, denitrifying bacteria are heterotrophic, facoltative aerobes, which use nitrate as an electron acceptor when oxygen is absent or limiting. A potential advantage of heterotrophic denitrification is the simultaneous carbon and nitrate destruction without requiring oxygen input (Grady et al., 1999). Denitrification also recovers half of the alkalinity consumed during nitrification. It is important to note that processes in which nitrate is used as a terminal electron acceptor are energetically favoured over acetogenic, sulphate reduction, and methanogenic processes. Thus in landfills in anaerobic/anoxic environments in which nitrate reduction occurs, inhibition of such processes may occur (Berge et al., 2005).

4.6.2 *Autotrophic Denitrification*

Nitrate removal in wastewaters containing high sulphur concentrations or reduced sulphur sources, such as hydrogen sulphide, may occur via autotrophic denitrification. Thiobacillus denitrificans use an inorganic sulphur source (H_2S, S, SO_3^{2-}) rather than an organic carbon source when reducing nitrate to nitrogen gas according to reaction (Onay and Pohland, 2001):

$$2NO_3^- + 1{,}25HS^- + O{,}75H^+ \longrightarrow N_2 + 1{,}25SO_4^{2-} + H_2O$$

This nitrate removal mechanism produce sulfate. At low carbon to nitrogen ratios this removal mechanism is favored over heterotrophic denitrification (Koenig et al., 1996). Autotrophic denitrification may occur in landfill, expecially in order landfills or older portion of landfills where the carbon to nitrogen ratio may be low. The increased sulfate concentration may have an adverse effect on methane production rates by limiting the amount of organic carbon available to the methanogens due to competition with sulfidogens (Berge et al. 2005).

5. Experimental background

The previous experiment, that started at the beginning of 2011, was conducted in two distinct and successive phases. The first phase duration was 104 days; in this phase, air was not insufflated inside the columns, which were maintained in this way under anaerobic conditions. The reactors were maintained in this experimental period at 35°C.

During the second phase, lasting 62 days, all six reactors were operated under aerobic conditions by means of air insufflations.

The injections and the temperatures performed in that experiment are represented in table 1.

Table 1 – *Injections and temperatures performer on the previous experiment*

Reactor	Anaerobic phase (104 days)			Aerobic phase (62 days)		
	1^ Injection	2^ injection	Temperature (ºC)	3^ injection	4^ injection	Temperature (ºC)
R1	NH_4Cl	NH_4Cl	35	NH_4Cl	NH_4Cl	35
R2	$NH_4Cl + NaS_2O_3$	NH_4Cl	35	NH_4Cl	NH_4Cl	35
R3	NH_4Cl	NH_4Cl	35	NH_4Cl	NH_4Cl	45
R4	NH_4Cl	NH_4Cl	35	NH_4Cl	NH_4Cl	55
R5	CH_3COONH_4	CH_3COONH_4	35	CH_3COONH_4	CH_3COONH_4	35
R6	-	-	35	NH_4Cl	NH_4Cl	35

6. Methodology

6.1 Preliminary operations to the test execution

Previous to start the experiment some maintenance and set up operation had to be done. The leachate recirculation pipes had shown to be worn, and so they were replaced with new pipes, due to prevent their degradation. These pipes were Tygon Standard pipes, having an inner diameter of 6 mm, they are constituted of nontoxic material and have a good base, acids, inorganic substances and high temperature resistance.

The recirculation flow was maintained to 6 l/d, leachate was recirculated by peristaltic pumps of Heidolph model PD 5001, driven by an analogical timer. Peristaltic pumps are suitable for the dosage of corrosive, abrasive or aggressive solvents because the liquid comes into contact only with the pipes and not with the mechanical parts of the pumps. Pumps were calibrated before to be started. Timer was set up to ensure a pumps operational time of 15 minutes. The pumps were turned on 3 times per day, at 7:00 h, 12:00 h and 18:00 h, in this manner the laboratory activity was not affected by the recirculation operation. Therefore the required flow rate was assumed to be 0,13 l/min in order to pump 2 liters in 15 minutes.

The reactors were then set to a temperature of 35 °C and maintained in aerobic condition.

Figure 1 – *Reactors with thermal insulated armor, provided with bags for the biogas collection*

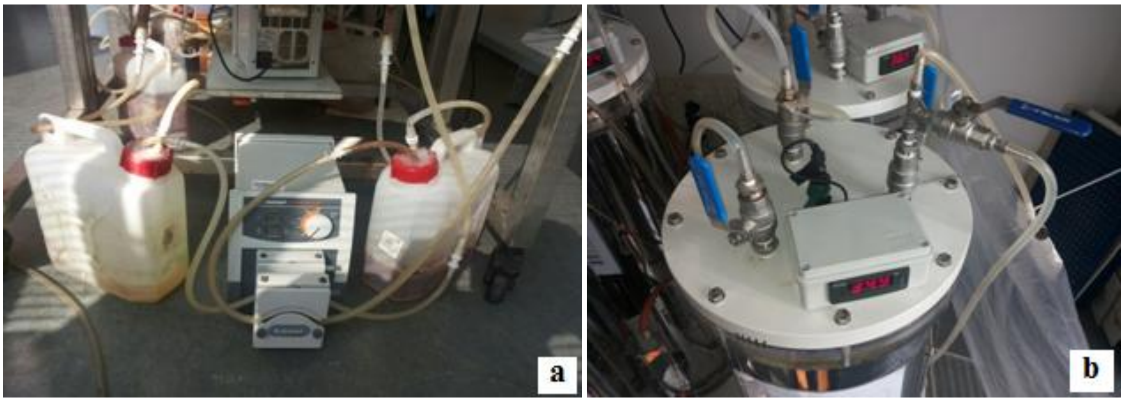

Figure 2 – (a) – *Leachate storage container and recirculation pump detail*
(b) – *Particular of the valves installed on the superior flange of every column*

Then six flowmeters (model Sho-Rate GT1335 of Brooks Instruments) were connected to two pumps (model Air Professional 360 of Prodac).

Then it was set the air distribution system. In practice, the pump supplies air through the flowmeter, which regulates the flow. After that, air flows through a bottle containing water to saturate itself of water vapor, and so to compensate eventual losses due to evaporation. Then air enters in the column and going out passes through the scrubber containing a boric acid solution, which collect the ammonia passed into the gas phase.

The scrubbers working principle is hereinafter briefly exposed, as is hereinafter synthetically reported the boric acid volume selection criteria.

Ammonia is a weak base easily volatile and therefore can be quantitatively separated from an aqueous solution by distillation at a pH around 9.5. Since natural waters have generally different pH values and different buffering capacities, to maintain the necessary pH during the distillation process is added to the test sample a buffer solution of borate. Ammonia collected in the distillate, is determined by titration with a reference solution of a strong mineral acid (sulfuric acid), using an indicator with turning point at around pH 5 (methyl red and methylene blue). Using boric acid like receiver solution the chemical reaction that takes place is the following:

$$NH_3 + H_3BO_3 \rightarrow (NH_4^+ + H_2BO_3^-) + H_3BO_3$$

Boric acid captures the gaseous ammonia forming a complex ammonia-borate ($NH_4^+ + H_2BO_3$). If the solution contains ammonia the receiver solution color changes (from purple to green). Once the turning occurs in the scrubber is done the titration, to determine the amount of ammonia contained in the boric acid solution. The reaction that take place is the following:

$$(NH_4^+ + H_2BO_3^-) + H_3BO_3 + H_2SO_4 \rightarrow NH_4^+ + H_3BO_3 + HSO_4^-$$

Titration ends when turning point is achieved (from green to purple). In this experiment the scrubber was filled with 100 ml of boric acid that can contain 800 mg of ammonia (each liter of boric acid contain 8000 mg of NH_3). This amount of boric acid can be adjusted if the volatilization of NH_3 increases.

Figure 3– *Scrubbers filled with boric acid for the outflowing gas washing in order to quantify the content of ammonia-nitrogen*

6.2 Sample analysis

After the set up phase, a sample for each column must be taken in order to understand the current characteristic of the waste mass. This step has the objective to give the first data and to set the most correct strategy for the experiment. Two leachate samples for each reactor has been taken and analyzed every week for all the duration of the experiment. The measured parameters were:

- pH
- Alkalinity
- $N\text{-}NH_4^+$
- $N\text{-}NO_3^-$
- SO_4^{2-}

The pH was measured with a pH-meter. The pH is essential to be measured because it is a limiting parameter in every biological process which is in study.

Alkalinity is measured by titration. Alkalinity is an important parameter because it allows to understand the leachate buffer capacity and to prevent the pH decrease, which is inconvenient to nitrification and denitrification processes.

Ammonium nitrogen ($N\text{-}NH_4^+$) was analyzed by a spectrophotometric method. NH_4^+ needs to be determined in order to evaluate the ammonium nitrogen in the system, which permits to know the quantity of nitrogen to be nitrified and denitrified.

Nitrate ($N\text{-}NO_3^-$) was analyzed by a spectrophotometric method. The nitrates are an intermediate in the nitrification/denitrification process. Therefore knowing that nitrification and denitrification may occur simultaneously, the determination of the nitrates is useful only to know if denitrification is occurring or not. If an accumulation of nitrate is observed, it is probably that the denitrification process is not occurring. In the reactors in which was performed a nitrate injection, the determination of the nitrate is essential.

Sulphate (SO_4^{2-}) was analyzed by a spectrophometric method. Sulphate needs to be determined in order to evaluate the autotrophic denitrification, knowing that in this process NO_3^- is converted to N_2 with production of sulphate

After the measurement of the ammonia-nitrogen, it was determined the ammonia-nitrogen removal rates, calculated using a central difference method of analyses (Berge et al., 2006):

$$R_R = \frac{C_{t-1} - C_{t+1}}{t_{t+1} - t_{t-1}}$$

where R_R is the rate of ammonia change at time t (mgN/day), C is the total $N\text{-}NH_4^+$ mass (mgN), and *t* is the time (days). This rate was calculated to understand the capacity of the old waste to remove ammonia nitrogen.

Also the removal rate of nitrates was calculated, using the same method adopted to calculate the ammonia-nitrogen removal rate.

7. Addition of Ammonia-Based and Nitrate-Based solutions

The research plan involved the addition of solutions containing ammonia and nitrate. In columns in which the focus was the autotrophic denitrification, it was decided to introduce ammonium chloride (NH_4Cl) in the column 4 (R4), and potassium nitrate (KNO_3) in the column 1 (R1). In the columns in which the focus was the heterotrophic denitrification, it was chosen to introduce ammonium acetate (CH_3COONH_4) in the column 5 (R5), and sodium acetate (CH_3COONa) in the column 2 (R2).

The purpose of the injections of ammonium chloride and ammonium acetate was to raise the concentration of ammonia, so as to have in course of the tests change in the concentration appreciable. The objective preparing the solution has been to have a concentration of ammonia of about 1000 $mgN\text{-}NH_4^+/l$ in the columns. The first injection of ammonium chloride in the reactor 4 was made on June 26th, 2013, after 64 days from the beginning of the test. The first injection of ammonium acetate in the reactor 5 was made on May 29th, 2013, after 36 days from the beginning of the test. During the first phase of addition of the solutions containing ammonia, the liquid volume inside the column was reintegrated, in order to reintroduce the liquid that was taken for the leachate sample analysis. To do this was used distilled water.

As regard the calculation of the quantity of ammonium chloride (NH_4Cl) and ammonium acetate (CH_3COONH_4) to be introduced in the columns to bring the concentration of ammonia to 1000 mgN-NH_4^+/l, we proceed as shown respectively in table 2 and table 3, which shows by way of example, the calculation done to determine the quantities to be introduced during the injection.

Table 2 – *Calculation table of the quantity of ammonium chloride which is introduced in column 4 on June 26, 2013*

COLUMN 4	
Date	26-giu
Wanted N-NH_4^+ concentration (mg/l)	1000
Nitrogen atomic weight (g/mol)	14
NH_4Cl molecular weight (g/mol)	53,5
Liquid inside the column (l)	4,6
Volume of the solution (l)	1
Volume for the buffer solution (l)	0,5
Total volume of water (l)	6,1
Required amount of N-NH_4^+ (mg)	6100
Initial N-NH_4^+ concentration (mg)	33
N-NH_4^+ to be added (mg)	6067
Substance to be added (mg)	23185
Quantity of NH_4Cl to be added (g)	23,2

Table 3 – *Calculation table of the quantity of ammonium acetate which is introduced in column 5 on May 29, 2013*

COLUMN 5	
Date	28-mag
Wanted N-NH_4^+ concentration (mg/l)	1000
Nitrogen atomic weight (g/mol)	14
CH_3COONH_4 molecular weight (g/mol)	77,08
Liquid inside the column (l)	7,3
Volume of the solution (l)	1
Total volume of water (l)	8,3
Required amount of N-NH_4^+ (mg)	8300
Initial N-NH_4^+ concentration (mg)	25
N-NH_4^+ to be added (mg)	8275
Substance to be added (mg)	45559
Quantity of CH_3COONH_4 to be added (g)	46

The calculations were carried out starting from the premise that the desired initial ammonia-nitrogen concentration was 1000 mgN-NH_4^+/l. First of all was calculated the required amount of N-NH_4^+ multiplying the desired initial ammonia-nitrogen concentration times the total volume of water. To this value was then subtracted the initial N-NH_4^+ concentration in order to obtain the amount of N-NH_4^+ to be added to the columns. Finally, this value was then divided by the atomic weight of nitrogen and multiplied by the molecular weight of the substance to be introduced (NH_4Cl or CH_3COONH_4), so as to obtain the concentration to be added to the columns.

The calculation steps for the ammonium-chloride injections, are the following:

$$\text{Required } [\text{N-}NH_4^+] = \text{Wanted } [\text{N-}NH_4^+] \times \text{Total volume of water}$$

$$[\text{N-}NH_4^+] \text{ concentration to add} = \text{Required } [\text{N-}NH_4^+] - \text{Initial } [\text{N-}NH_4^+]$$

$$[NH_4Cl] \text{ concentration to add} = \frac{[\text{N-}NH_4^+] \text{ concentration to add}}{\text{N atomic weight}} \times [NH_4Cl] \text{ MW}$$

The purpose of the injection of potassium nitrate (KNO_3) in reactor 1 was to create a syntethic leachate to denitrify under autotrophic conditions and to raise the concentration of nitrate, so as to have in course of the tests change in the concentration appreciable. The calculation steps to know the quantity of KNO_3 to be add are the same of the ones followed to know the quantity of ammonia-base solutions to be add, starting from the premise that the desired initial nitrate concentration inside the system was always 1000 mgN-NO_3^-/l.

The following table shows, by the way of example, the calculation done to determine the quantities of CH_3COONa to be introduced during the injection.

Table 4 – *Calculation table of the quantity of potassium nitrate which are introduced in column15 on May 28, 2013*

COLUMN 1	
Date	28-mag
Wanted N-NO_3^- concentration (mg/l)	1000
Nitrogen atomic weight (g/mol)	14
KNO_3 molecular weight (g/mol)	101
Liquid inside the column (l)	6,8
Volume of the solution (l)	1
Volume for the buffer solution (l)	1
Total volume of water (l)	8,8
Required amount of N-NO_3^- (mg)	8800
Initial N-NO_3^- concentration (mg)	2176
N-NO_3^- to be added (mg)	6624
Substance to be added (mg)	47787
Quantity of KNO_3 to be add (g)	47,8

8. Issues and Criteria to consider

When a complex experimentation, characterized by some innovative elements, is set off, is normal to confront itself with some phenomena, that initially appear unclear or some problematic aspects.

During the first half of the experiment, the sulphates analysis were subject to doubts due to an oscillatory trend regarding almost all the columns. The sulphates trends in reactors R2, R3, R4, that were the reactors more subjected to the oscillations, are reported in figure 4, 5 and 6 below.

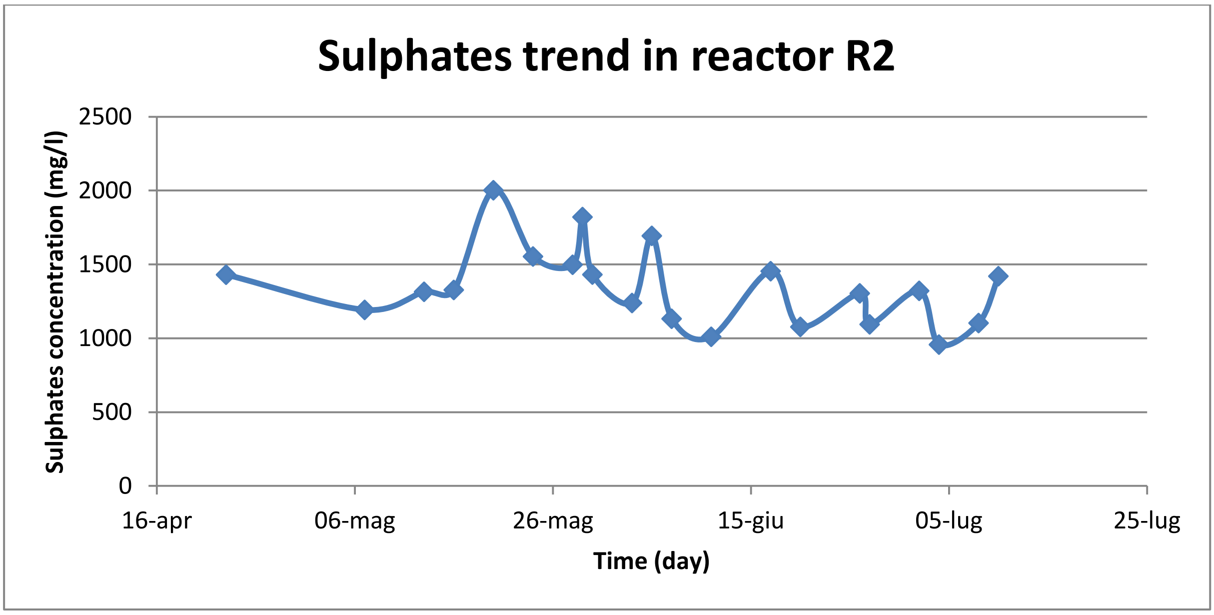

Figure 4 – *Sulphates trend in column R2. The values reported refers to the analysis realized from April 23, 2013 to July 10, 2013*

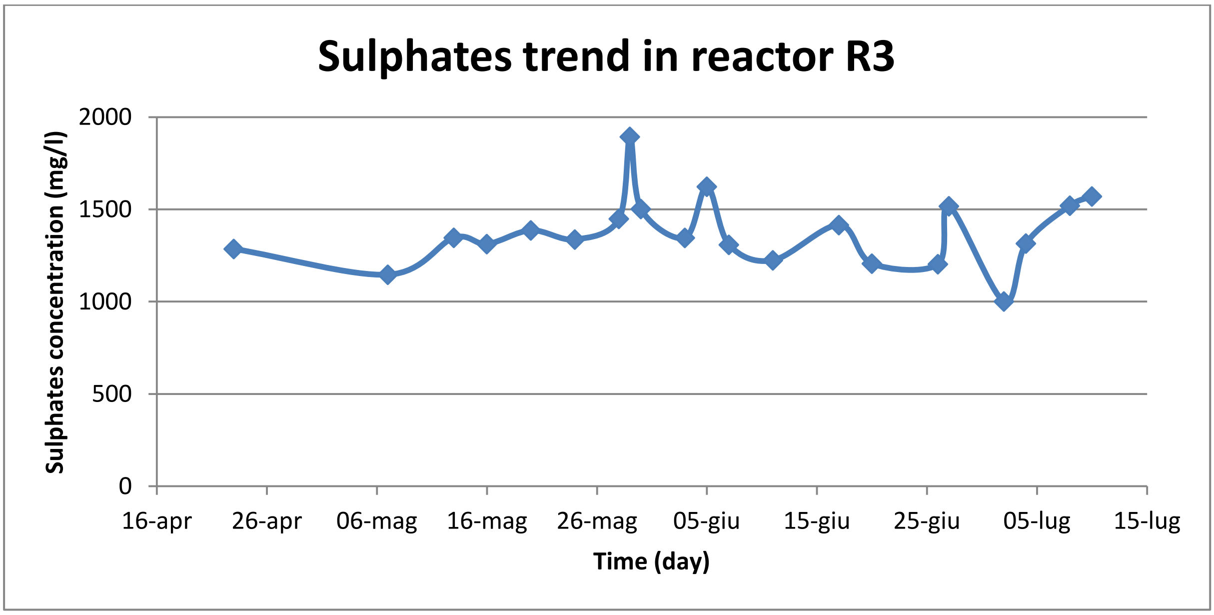

Figure 5 – *Sulphates trend in column R3. The values reported refers to the analysis realized from April 23, 2013 to July 10, 2013*

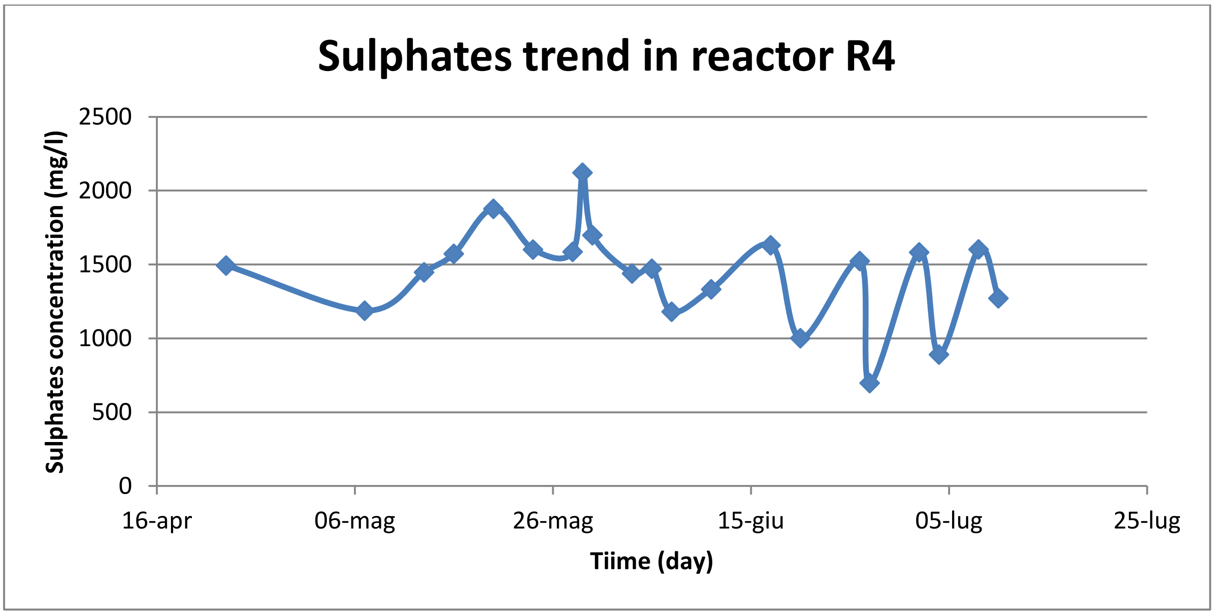

Figure 6 – *Sulphates trend in column R4. The values reported refers to the analysis realized from April 23, 2013 to July 10, 2013*

It was hypothesized the presence of an analytical error, therefore to evaluate this supposition, an external laboratory (EUROFINS laboratory) was commissioned to carried out the sulphates analysis on sample I analyzed too. It was also decided to commission to the EUROFINS laboratory to analyzes also the nitrate, in order to have a comparison with the analysis performed in the LISA laboratory. Therefore we delivered to the EUROFINS lab a leachate sample of 250 ml for each columns on dates 23/07/2013, 29/07/2013 and 05/08/2013.

The EUROFINS laboratory perform the leachate analysis only on filtered sample, so we had to filter the leachate samples before delivering them to the external laboratory. To evaluate if the analysis performed on filtered and non filtered samples give different results, we decided, before the delivering to the external lab, to take a sample of leachate for each columns and to filtered a part of it in order to compare the analysis of sulphates and nitrates of the filtered and non filtered samples. The results that we obtain were approximately equal, and this is why in the leachate of the columns there is a negligible concentration of solids. Therefore we conclude that the analysis on non filtered samples was not a possible cause of the sulphates oscillation.

The reason why we choose to commission the EUROFINS lab was that the analysis are realized by ion chromatography, while in the LISA lab are performed by spectrophotometric method.

Spectrophotometric analysis, is based on the comparison between the color enveloped by an unknow amount of a know substance with the color produced by a standard sample containing a know amount of this substance. When a monochromatic light passes through the colored solution, some amount of light, proportional to the substance concentration, is absorbed. Colorless or weakly colored substances, can assumes strong colors due to the reaction with special reagents (for example in the analysis of nitrate, in the commonly used method, nitric-nitrogen ($N\text{-}NO_3^-$) reacts with sodium salicylate in acid environment and that leads to the formation of nitrosalicylate acid, which under alkaline conditions, gives rise to its ionized form, characterized by yellow color). Errors of spectrophotometric analysis originate from many sources: turbidity, dilutions, chemical interferences, temperature or pH variations. When moreover the monochromatic light ray invests the cell containing the sample, several phenomena occur: reflection, refraction, absorption by the cell walls, by the solvents and by all the reagent added to form the colored compound, and obviously by the substances under examination. The absorbance actually measured is subject therefore to many factors not connected to the concentration of the substance under investigation and that produce interferences, leading to errors in the determination of the concentration of the latter.

Ion chromatography is a technique, which allows to separate the ionic components of a mixture exploiting the different reaction characteristics of the analysis searched for. A small amount of the sample to analyze is carried out by an eluent through some ion exchangers, polymeric resins on whose molecular skeleton are attached functional group with electric charge, which can exchange ions reversibly with the solution of the mixture under examination. Migration and separation of the different ionic components are due to the distribution of each between two phases, the mobile one (in general, a buffering solution) and the stationary one (the ion exchange resin). The behavior of the ions during the separation depends therefore by the equilibrium, them establish with the active groups (charged) of the resin. Any ionic species exit from the column at different and know times (retention time), detected by a conductivity meter. The chromatographic gives in ouput a chromatogram in which the peaks for each ionic species are visible. Even this type of analysis may suffer from interferences, which occur whenever a substance has the same residence time of the anions being analyzed. Another type of interference may occur when a ion is present in a concentration so high, to compromise the resolution of the other substances. This interferences can be greatly reduced by simply diluting the samples (Jackson, 2000).

Table 5 – *Test results on the leachate samples collected on July 23th, 2013. Here are reported the values detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

Columns	Date	Sulphates concentration recorder in LISA lab (mg/l)	Sulphates concentration recorder in EUROFINS lab (mg/l)	Nitrates concentration recorder in LISA lab (mg/l)	Nitrates concentration recorder in EUROFINS lab (mg/l)	Sulphates difference (mg/l)	Nitrates difference (mg/l)
R1	23/07	1762	1698	705	714	64	-9
R2	23/07	1445	1620	165	242	-175	-77
R3	23/07	1945	2284	1622	1673	-339	-51
R4	23/07	1859	1665	919	921	194	-2
R5	23/07	2804	2680	1,3	13,7	124	-12,4
R6	23/07	1762	1472	1577	1160	290	417

Table 6 – *Test results on the leachate samples collected on July 23th, 2013. Here are reported the values detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

Columns	Date	Sulphates concentration recorder in LISA lab (mg/l)	Sulphates concentration recorder in EUROFINS lab (mg/l)	Nitrates concentration recorder in LISA lab (mg/l)	Nitrates concentration recorder in EUROFINS lab (mg/l)	Sulphates difference (mg/l)	Nitrates difference (mg/l)
R1	29/07	1612	1766	729	742	-154	-13
R2	29/07	1527	1855	268	270	-328	-2
R3	29/07	2186	2351	1534	1705	-165	-171
R4	29/07	1662	1677	907	913	-15	-6
R5	29/07	2753	2637	0,6	8,6	116	-8
R6	29/07	1643	1555	1100	1232	88	-132

Table 7 – *Test results on the leachate samples collected on July 23th, 2013. Here are reported the values detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

Columns	Date	Sulphates concentration recorder in LISA lab (mg/l)	Sulphates concentration recorder in EUROFINS lab (mg/l)	Nitrates concentration recorder in LISA lab (mg/l)	Nitrates concentration recorder in EUROFINS lab (mg/l)	Sulphates difference (mg/l)	Nitrates difference (mg/l)
R1	05/08	1697	1357	710	597	340	113
R2	05/08	1355	1386	144	137	-31	7
R3	05/08	2107	1936	1394	1352	171	42
R4	05/08	1538	1251	700	747	287	-47
R5	05/08	1696	2189	0,4	4,5	-220	-4,1
R6	05/08	1532	1148	1077	946	384	131

Considering the literature, the most accurate method for the determination of the anions, appear to be the ion chromatographic. Nevertheless, the results of the EUROFINS laboratory of the sulphates, which realized the analysis by ion chromatography, resulted very similar to the results of our laboratory, determined with spectrophotometric method. As regards the nitrates, the results of the two laboratories are always very similar. The only significant difference regarding the nitrates, is observed in reactor 5, where the difference is of an order of magnitude. The reasons for this difference is attributable to the observance calibration range of the analysis methods. The observance calibration range of the spectrophotometric method adopted in the LISA laboratory is 0,125 – 1,240, and the observance of the nitrate concentration in reactors 5 are much lower of the lower limit of the range, because of the concentration of nitrates in this column is practically zero. Therefore, by the spectrophotometric method is not possible to evaluate the concentration of nitrate and a sensitive error is generate in the estimation of the nitrate observance.

The following graphs, regarding the columns 2,3 and 4, are presented by way of example, in order to show that the results obtained on the analysis of sulphates and nitrates performed in dates July 23, July 29 and August 5 in the two laboratory are almost the same.

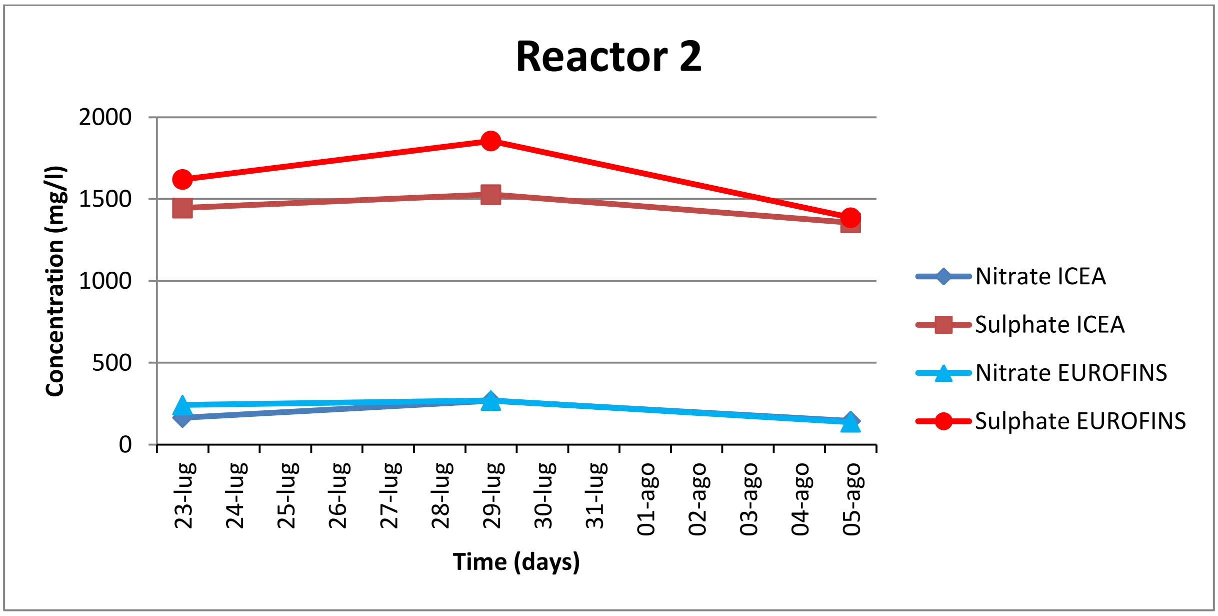

Figure 7 – *Comparison of the concentration values of sulphates and nitrates of reactor 2 detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

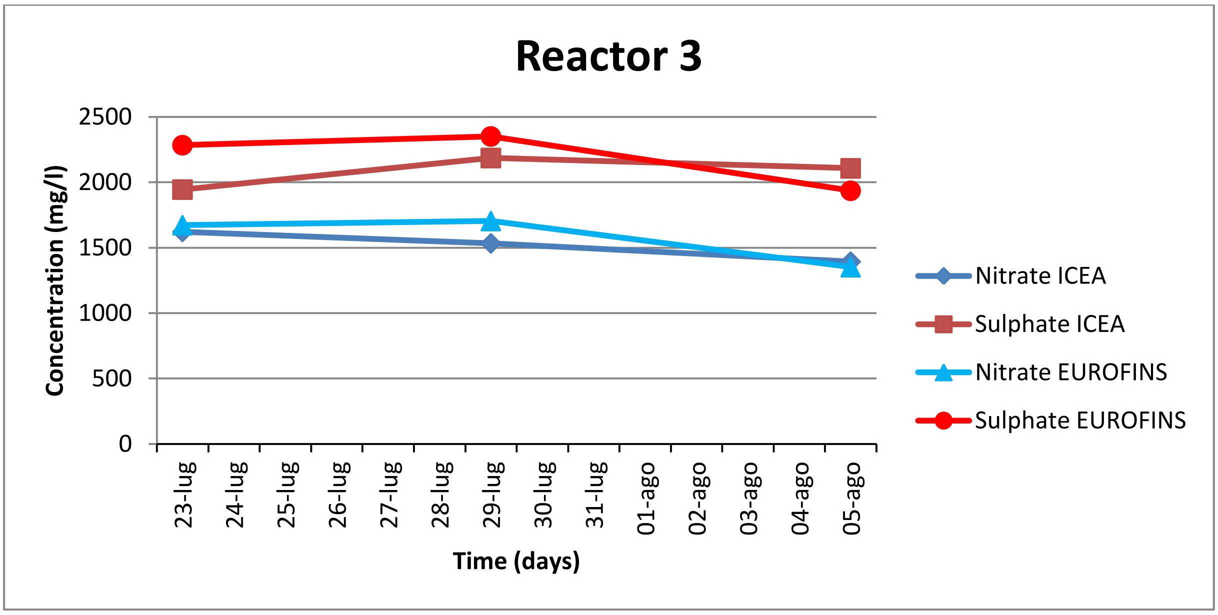

Figure 8 – *Comparison of the concentration values of sulphates and nitrates of reactor 3 detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

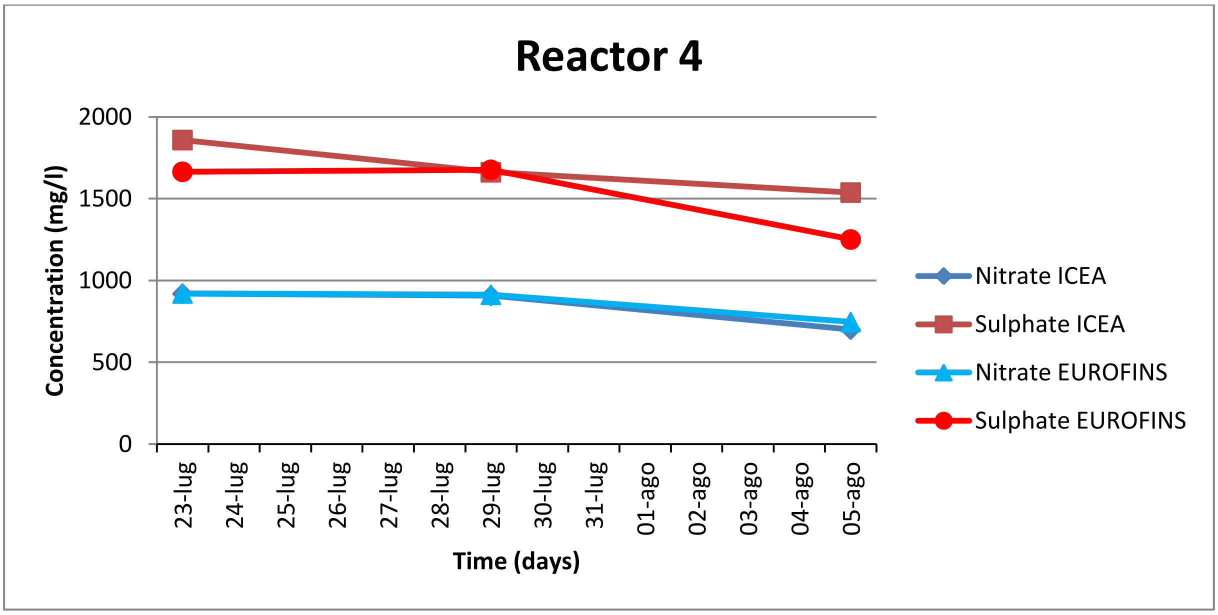

Figure 9 – *Comparison of the concentration values of sulphates and nitrates of reactor 4 detected on the same samples by the internal laboratory LISA by spectrophotometric analysis and by the external laboratory EUROFINS by ion chromatography method*

Another problem relevant to mention in this part of elaborate is related to a leak of the leachate from all the columns. For a period of two weeks during the month of August, the LISA Laboratory was closed. During this period occurred a damage to the pipes of the leachate recirculation, which was cutted in the portion of pipes that crossed the pumps, probably due to the attrition. The pipes were immediately replaced and the recirculation was switched off in order to collect in the bottle connected with the drain valve all the leachate remained in the columns. It was assess that about all the leachate contained in the columns was lost, and no leachate sample could be taken for analysis. It was so decided to replace the water in the columns, and 2 l of distilled water was added to each reactors. No analysis was performed for a week and the recirculation was kept active in order to stabilize the leachate in the columns. It was added only 2 l of distilled water to avoid an excessive dilution of the leachate. Before the restarting of the analysis, the recirculation was switched off to collect again all the leachate in the bottle in order to evaluate the quantity of liquid in the columns. The table 9 shows the liquid volume inside the container after the reintroduction of the water in the columns.

Table 8 – *Liquid volume inside the container after the reintroduction of the water in the columns*

Reactors	Liquid volume inside the container (l)
R1	1,6
R2	1,5
R3	2
R4	1,7
R5	1,5
R6	1,8

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PART II

Scientific Article

Abstract

Bioreactor landfill are an improvement to normal sanitary landfills, because the waste is stabilised faster and the landfill gas is produced in a shorter period of time in a controlled way. Recent studies proved how in situ nitrogen removal techniques by air injections, which allows the contemporary occurring of nitrification and denitrification, are advantageous and effective; however, they are lacking the data required to enable adequate implementation at field scale bioreactor landfills. The factors determining nitrogen removal processes in aerated landfill were investigated in this study, with six column reactors (R1, R2, R3, R4, R5, R6) filled with stabilized waste coming from an old landfill. The research objectives are (1) to analyze the effects of the aerobic conditions on the emissions of leachate and biogas and on the biological stability of waste and (2) to better understand the autotrophic and heterotrophic denitrification modalities in old waste aerated environment. Results demonstrate that in situ nitrification is feasible in an aerated solid waste environment and that the potential for simultaneous nitrification and denitrification in field scale bioreactor landfills is significant.

1. Introduction

There is general agreement that leachate from municipal solid waste landfill, characterized as having a high content of oxygen-consuming organics (COD) and ammonia, should be dealt with specifically in order not to be a pollution source of water body or environment (Kjeldsen et al., 2003).

An important parameter to consider the safe closure of landfill sites is the NH_4^+ content of the leachate (Barlaz et al., 2002; Burton and Watson-Craik, 1998). NH_4^+ tends to accumulate since there are no removal mechanisms under strict anaerobic conditions, especially in landfills with leachate recirculation (Onay and Pohland, 1998; Price et al., 2003). NH_4^+ can be removed from the leachate of landfills via methods such as nitrification/denitrification, precipitation and even irrigation schemes (Jokela et al., 2002; Li and Zhao, 2003; Ohlinger et al.,1998). However, these approaches are likely to produce NO_x and N_2O, which are significant pollutants for their contribution to climate change (Price et al., 2003).

A bioreactor landfill for municipal solid waste (MSW), with leachate recirculation in landfill layers as a kernel operation, could accelerate the stabilization of organics in landfilled waste, enhance the production of landfill gas and promote simultaneously the degradation of pollutants present in the leachate, so that the process has been highlighted in the past decades (Barlaz et al., 1990; Reinhart et al., 2002).

There are many advantages to the operation of landfills as bioreactors including: (1) settlement before placement of the final cover which decreases the risk of damage to the final cover, (2) increased effective refuse density and landfill capacity, (3) in situ leachate treatment, (4) increased rates of gas production which may make energy recovery more favorable, (5) the potential for additional revenue for commercial liquid waste disposal and (6) acceleration of refuse decomposition which may shorten the regulated post-closure monitoring period and thereby reduce the overall cost of the landfill (Reinhart et al., 2002; Barlaz et al., 1990).

Recently, landfill owners and regulators have begun to consider in more detail strategies for the long-term management of landfills after closure and one consideration is leachate quality (Barlaz et al., 2002). The biological oxygen demand (BOD) and chemical oxygen demand (COD) of landfill leachate typically decrease substantially as refuse decomposes, and ultimately the remaining organic matter in leachate from well decomposed refuse is largely humic matter (Kjeldsen et al., 2003). However, MSW has been estimated to contain about 4% protein and therefore, ammonia (NH_3–N) is produced during the decomposition of organic nitrogen (Barlaz et al., 1990).

Because ammonia–nitrogen has been implicated as one of the most significant long-term pollution problems in landfills, it is likely that its presence will determine when the landfill is biologically stable and when postclosure monitoring may end or be reduced (Kjeldsen et al. 2003).

Thus, high concentrations of ammonia persist long after the BOD and COD have decreased to concentrations representative of well-decomposed refuse, and the treatment of leachate to remove ammonia is an important aspect of long-term landfill management (Barlaz et al., 2002).

Researchers have successfully demonstrated the efficacy of both nitrification (Berge et al. 2006; Hanashima 1999; Jokela et al., 2002; Onay and Pohland 1998; Youcai et al. 2002) and denitrification (Burton and Watson-Craik 1998; Price et al. 2003) in solid waste environments at the laboratory scale. More recently, leachate from a field-scale aerated landfill (Mertoglu et al. 2006) and aerated waste (He and Shen 2006) were shown to contain nitrifying microbial populations. Specifically, the leachate analyzed contained populations of both *Nitrosonomas*-like ammonia oxidizers and *Nitrospira*-related nitrite oxidizers (Mertoglu et al. 2006), suggesting nitrification processes do occur within aerated landfills.

Biological ammonia removal takes place in two stages: the first is the aerobic nitrification of ammonia to nitrite and to nitrate, and the second is the anoxic denitrification of nitrates to gaseous nitrogen. The overall nitrification reaction, by autotrophic bacteria *Nitrosomonas* and *Nitrobacter*, can be described by the following stoichiometric expression that takes into account for both the bacterial synthesis and ammonia oxidation (Crites and Tchobanoglous, 1998):

$$NH_4^+ + 1{,}863\ O_2 + 0{,}098\ CO_2 \longrightarrow 0{,}0196\ C_5H_7NO_2 + 0{,}0941\ H_2O + 0{,}98\ NO_3^- + 1{,}98\ H^+$$

In landfill, take place both autotrophic denitrification as well as heterotrophic one (Onay and Pohland, 2001). Denitrification is an anoxic process that reduces nitrate to nitrite, nitric oxide, nitrous oxide, and finally nitrogen gas, as shown in the reactions:

$$\begin{aligned} NO_3^- + 2e^- + 2H^+ &\longrightarrow NO_2^- + H_2O \\ NO_2^- + e^- + 2H^+ &\longrightarrow NO + H_2O \\ 2NO + 2e^- + 2H^+ &\longrightarrow N_2O + H_2O \\ N_2O + 2e^- + 2H^+ &\longrightarrow N_2(g) + H_2O \end{aligned}$$

Tipically, denitrifying bacteria are heterotrophic, facoltative aerobes, which use nitrate as an electron acceptor when oxygen is absent or limiting. A potential advantage of heterotrophic denitrification is the simultaneous carbon and nitrate destruction without requiring oxygen input (Berge et al. 2005).

Autotrophic denitrification is instead carried out primarily by bacteria such as *Thiobacillus denitrificans*. This bacterium use an inorganic sulpur source (i.e. H_2S, S,

SO_3^{2-}) rather than an organic carbon source when reducing nitrate to nitrogen gas according to the following reaction:

$$2NO_3^- + 1{,}25HS^- + O{,}75H^+ \longrightarrow N_2 + 1{,}25SO_4^{2-} + H_2O$$

This nitrate removal mechanism produce sulfate. At low carbon to nitrogen ratios this removal mechanism is favored over heterotrophic denitrification. Autotrophic denitrification may occur in landfill, expecially in order landfills or older portion of landfills where the carbon to nitrogen ratio may be low. The increased sulfate concentration may have an adverse effect on methane production rates by limiting the amount of organic carbon available to the methanogens due to competition with sulfidogens (Berge et al. 2005).

This article discusses the nitrogen transformation and removal processes that may occur in aerated landfills. This experiment was conducted with laboratory-scale simulated landfill units, to evaluate the ammonia and nitrates removal rates in old waste, with the following objectives:

- Analyze the effects of the aerobic conditions on the emissions of leachate and biogas and on the biological stability of waste;
- Evaluate the development capacity of the processes of nitrification and denitrification during the aeration of waste,
- Compare the autotrophic and heterotrophic denitrication processes focusing on the distinctive aspects, since they take part simultaneously to the process of nitrate removal in landfill.

2. Materials and Method

2.1 Analytical Instruments

For the experiment have been used six column reactors (R1, R2, R3, R4, R5, R6) made of Plexiglas, with an inner diameter of 24 cm and a height of 106 cm.

Each column is closed at the top and the bottom by means of bolted flanges, provided with double rubber seals, to ensure a perfect seal. In the upper flange are positioned four valves in stainless steel, thanks to which it is possible to insufflate air into the waste, to carry out sampling of gas, to replace water and to recirculate leachate. The lower flange is equipped with a drain valve that allows the leachate to flow by gravity into a collection container. From it leachate is recirculated to the top of the column by a peristaltic pump Heidolph PD 5001, controlled by a timer wich is set up to pump only 15 minutes at 6.00 h, 12.00 h and 18.00 h every day.

The waste aeration take places place overhead, with humidified air, through a perforated, vertical, PVC pipe, placed at the center of the waste mass and fed by a compressor, controlled by means of a flow meter. The air was saturated prior the introduction of each reactor to replenish any water lost due to evaporation and was added continuously throughout the duration of the experiment.

The gas exiting from the reactor passes through an acid scrubber, in order to highlight and quantify the possible presence of ammonia in gaseous phase in output from the system. The acid scrubber consists of a glass bottle containing 500 ml of boric acid and dye solutions (methylene blue, methyl red). The gas exiting from the columns is bubbled from below upward within the solution. Ammonia possibly present in the gas, come back in liquid phase in acid environment and accumulates within the solution, which changes color going from purple to green. By titration with sulfuric acid it is therefore possible quantify the amount of ammonia exiting the system in the gas phase.

The temperature of each reactor was set at 35 °C. It is possible to monitor the temperature of the reactors through an armored insulating resistance, adjustable by a thermostat. The armor completely wraps the entire cylinder of the column, ensuring a dual function: heat the reactor through the presence of electrical resistances arranged in a serpentine line on the inner surface of the shell, and at the same time insulate the column itself by the presence of insulating materials on the external surface of the shell. This dual function ensures an homogeneous heating of the entire reactor, without significant thermal differences that could inhibit the biological processes.

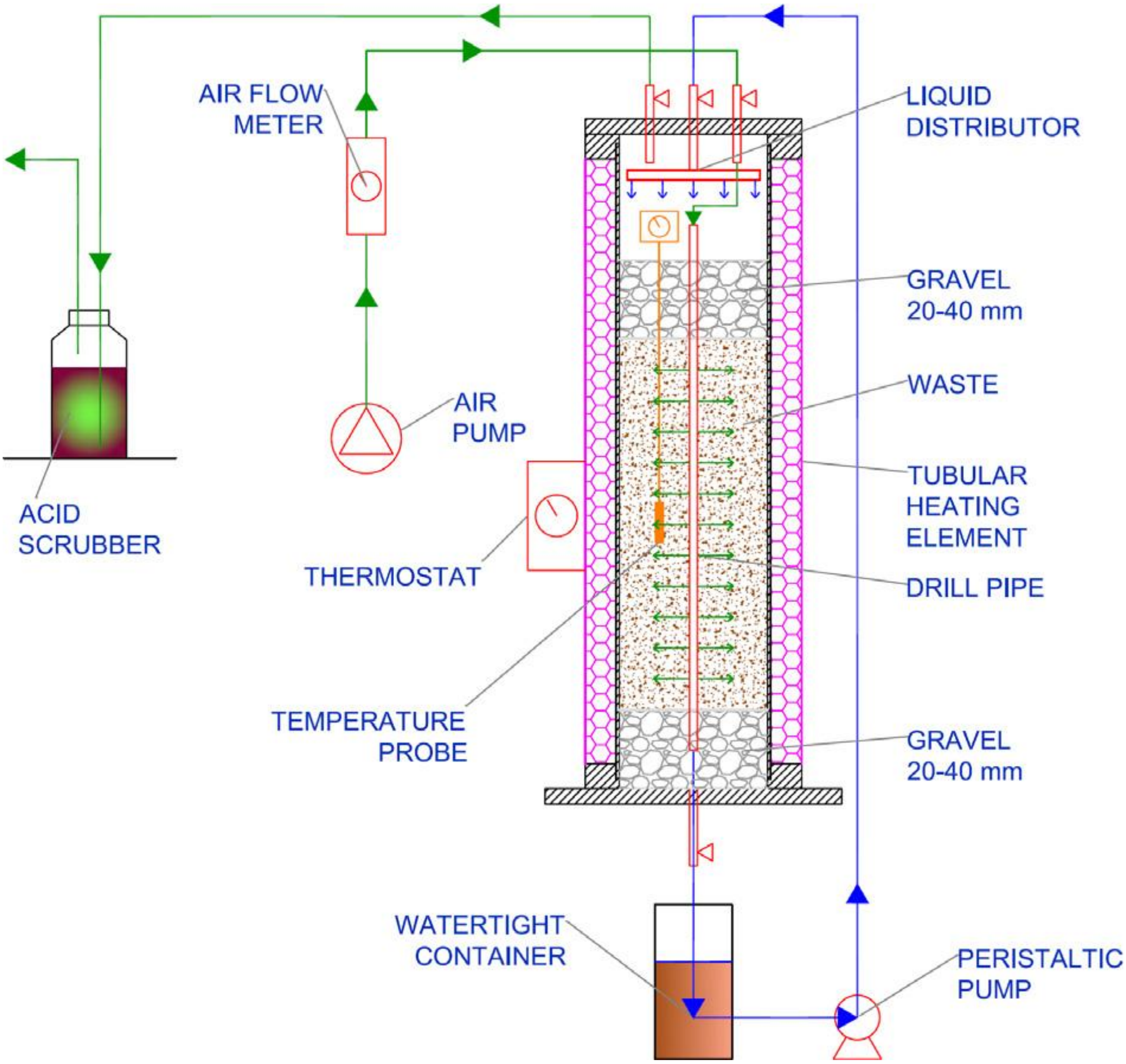

Figure 1 – *Sketch of the test reactor under aerobic conditions*

2.2 Municipal Solid Waste Characteristics

The waste used for this experiment come from an old landfill, next to Aosta, in operation since 1989. The waste were collected by drilling in the landfill body, and on each of the excavated waste sample was performed the grain size distribution analysis. In order to increase the homogeneity of the samples and to ensure a good air distribution within the waste body, has been decided to fill the reactors with the undersieve 20 mm, where the major part of the putrescibles are supposed to be present, and plastic having a size 20-50 mm, deputed to provide the mixture with proper porosity. The mixing was realized so that the plastic/undersieve weight ratio was equal for all the reactors (table 1).

Inside each column, at the time of the experimentation were present approximately 30 kg of waste. It is assumed that the old waste constitute the most suitable environment for nitrogen removal processes. In fact, high concentrations of organic carbon associated with newly placed waste determine an unfavorable habitat for nitrifying organisms, due to the competition with heterotrophic bacteria for the available oxygen (Berge et al, 2005). It has been demonstrated, also through experiments of field scale, that high organic carbon concentrations inhibit the nitrification processes (He et al, 2006). Furthermore, operating in environments with old waste, reduces aeration costs associated to oxygen demand related to waste rich in organic substances.

Table 1 – *Characteristics of the waste present in each reactors*

Reactor	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
Plastic-undersieve mixture (Kg)	27,90	30,80	30,10	31,30	31,00	29,20
Plastic/undersieve ratio (Kg/Kg)	0,16	0,16	0,16	0,16	0,16	0,16
Mixture density (t/m^3)	0,95	0,98	0,97	0,99	0,98	0,97

2.3 Analytical Techniques

On the leachate extracted from the reactors, were analyzed two times a week the following parameters: ammonia-nitrogen content ($N\text{-}NH_4^+$), nitrates ($N\text{-}NO_3^-$) and sulphates (SO_4^{2-}) by UV-vis spectrophotometric method (method IRSA-CNR 29/2003 vol. 2 n° 4030 A2, method IRSA-CNR 29/2003 vol. 2 n° 4040 A1, method IRSA-CNR 29/2003 vol. 2 n° 4140 B).

The pH was measured with a pH-meter (method IRSA-CNR 29/2003 vol. 1 n° 2060), and alkalinity by titration (method IRSA-CNR 29/2003 vol.1 n° 2010 B).

The oxygen (O_2), carbon dioxide (CO_2) and methane (CH_4) content were determined via IR-analyzer (model LFG20 of Eco-Control). It was not possible to detect the nitrogen content of gas and thus made complete nitrogen mass balance could not be implemented.

Table 2 – *Parameters analyzed on leachate samples. The units of measure and the analytical methodology used are reported*

Parameter	U.M.	Analytical Techniques
$N\text{-}NH_4^+$	mg $N\text{-}NH_4^+$/l	IRSA-CNR 29/2003 vol. 2 n° 4030 A2
$N\text{-}NO_3^-$	mg$N\text{-}NO_3^-$/ l	IRSA-CNR 29/2003 vol. 2 n° 4040 A1
SO_4^{2-}	mgSO_4^{2-}/ l	IRSA-CNR 29/2003 vol. 2 n° 4140 B
Alkalinity	mg$CaCO_3$/l	IRSA-CNR 29/2003 vol.1 n° 2010 B
pH	-	IRSA-CNR 29/2003 vol. 1 n° 2060

2.4 Methodology

During all the experiment, all six reactors were operated under aerobic conditions by means of air injection at a flow rate of 2 Nl/h for 24 h/d. Before the start of the experiment, all the systems of the columns were turned on, and so the air valve and the leachate valve were open.

The purpose of the experiment was to understand the autotrophic and the heterotrophic denitrification mechanisms in old waste environment subject to aeration, focusing on the distinction and quantification of them, since they occur at the same time in landfill.

The first phase was a characterization phase, which consists in the recirculation of the remaining leachate in the columns, in order to achieve the field capacity of the waste mass in all the reactors. It was not added new water because the original characteristics of the leachate wanted to be maintained. However, in order to conserve the same quantity of water inside the columns, the water taken for the sampling analysis was always replaced. The sampling consist in about 100 ml of leachate collected from the valve in the bottom of the columns. The water replacing was performed with the injections of the solution or adding only distilled water when no injections were programmed.

After the characterization phase, the injection phase started, and ammonia-based and nitrate-based solutions were added to the columns in order to asses the nitrification and denitrification processes.

Reactor 1 was subjected to injections of potassium nitrate (KNO_3) on days 36 and 65 from the start of the experiment, which raise the concentration of nitrate bringing it back to 1000 mgN-NO_3^-/l. This compound was selected because is a source of nitrate-nitrogen and in this way it was purposed to encourage the autotrophic denitrification process in order to be able to focus on it. Also a buffer solution of sodium bicarbonate ($NaHCO_3$) was added on day 38 due to prevent the pH drop caused by the autotrophic denitrification.

Also in reactor 2 was injected potassium nitrate, which raise the concentration of nitrate bringing it back to 1000 mgN-NO_3^-/l. This reactor was choose to study the nitrate removal with predominant heterotrophic denitrification, and so it was also added sodium acetate (CH_3COONa) because the biodegradable carbon in the waste was low. The first injection of both the compounds was performed on day 64. On days 77 and 84 additional sodium acetate injections were performed in order to provide other carbon source, consumed during the heterotrophic denitrification process.

Reactor 3 was chosen as a control reactor due to the highest levels of nitrate present inside the waste mass. On day 35 was performed an injection of buffer solution in order to asses if the lack of alkalinity was the reason whereby the denitrification didn’t happen. On the day 70 was performed an addition of a sulphur source. The solution injected was the sodium sulfide (Na_2S), and this injection has had the aim of studying the behavior of autotrophic denitrifying bacteria under optimal environmental conditions, that without the sulfur may become a limiting factor for the process. The purpose was also to understand if the addition of a further sulfur source, compared to that already present in the waste, favors the activity of the autotrophic denitrifying population. On day 76 a buffer solution of sodium bicarbonate ($NaHCO_3$) was added to prevent the pH drop caused by the autotrophic denitrification.

In reactor 4 was injected ammonium chloride (NH_4Cl) on day 64, which raises the ammonia concentration bringing it back to 1000 mgN-NH_4^+/l. This compound was selected because is a source of ammonia-nitrogen, but not of carbonaceous substrate; in this way it was purposed to encourage the autotrophic denitrification process. In the same day and also on day 70 it was also injected a buffer solution of sodium bicarbonate ($NaHCO_3$) to prevent the pH drop.

Reactor 5 was prepared to simulate heterotrophic denitrification because of the high carbon content in the mass waste; therefore a solution of ammonium acetate (CH_3COONH_4) was injected on day 36 and 65 in order to study the nitrification and the heterotrophic denitrification processes.

Finally, reactor 6 was chosen as a control reactor due to the average levels of the parameters subjected to analysis such as NH_4^+, NO_3^- and SO_4^{2-}. In this reactor no injection was performed, in order to maintain its function of control reactor.

Table 3 – *Summary of the injected compounds and objectives for each reactors*

Reactors	System Environment	Injected compound	Objective
R1	Autotrophic	KNO_3	Evaluate autotrophic denitrification
R2	Heterotrophic	KNO_3 + CH_3COONa	Evaluate heterotrophic denitrification
R3	Control	Na_2S	Verify if sulphur was a limiting element for nitrate depletion
R4	Autotrophic	NH_4Cl	Evaluate nitrification and autotrophic denitrification
R5	Heterotrophic	CH_3COONH_4	Evaluate nitrification and heterotrophic denitrification
R6	Control	-	-

3. Results and Discussion

3.1 Analytical Results on the Gas

Column gas content of oxygen (O_2), carbon dioxine (CO_2) and methane (CH_4), was detected during all the duration of the experiment by means of a portable analyzer of Eco-Control model LFG20.

The columns were maintained aerated for the entire duration of the experiment, to simulate an in situ aeration intervention. The selected flow rate was equal to 2 Nl/h for 24 h/d, value that is sufficient for the preservation of aerobic conditions inside the reactors.

By way of example, in figure 2 (a) and (b) is shown the gas percentage volumetric composition in reactors R2 and R4.

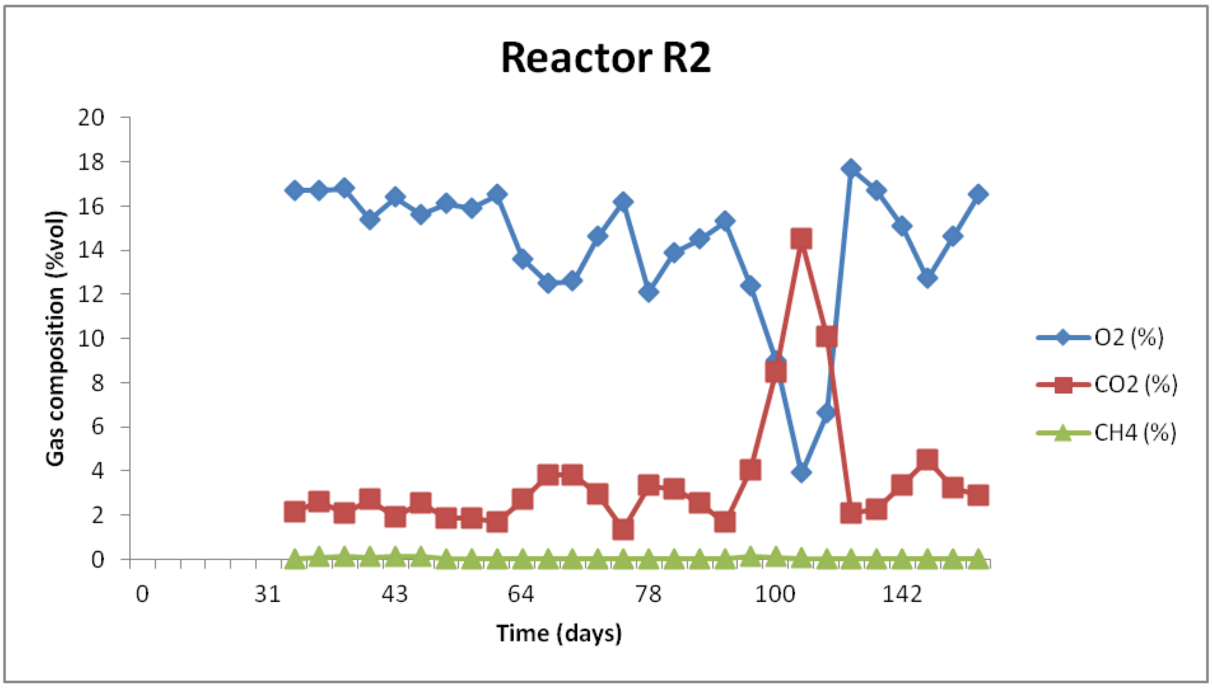

Figure 2 – *Gas percentage volumetric composition from reactor R2, detected with portable analyzer of Eco-Control model LFG20*

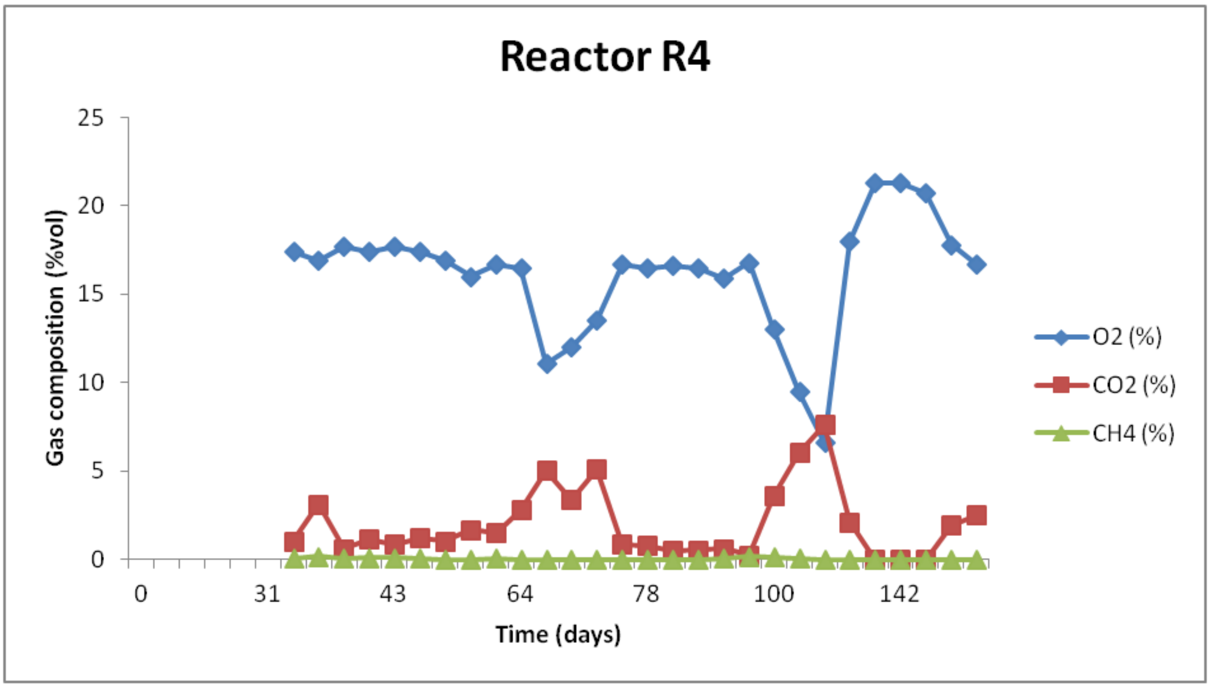

Figure 3 - *Gas percentage volumetric composition from reactor R4, detected with portable analyzer of Eco-Control model LFG20*

As we can see from the graphs, oxygen concentration was subject to a decrease during the first days the experiment, while specularly carbon dioxine increased. Reached a minimum, oxygen begins to grow again and the percentage of carbon dioxine to decrease. This behavior is observed also after subsequent injections, performed on day 64, 77 and 84. Oxygen consumption is clearly due to ammonia oxidation by nitrifying organisms, which being aerobic, are fully active during the aeration. The oxygen consumption is greater for high ammonia concentrations because it is used in the nitrification process. On the contrary, when the concentration of ammonia decreases, the oxygen level is restored.

During this aerobic experiment, the concentration of methane is equal to zero, the concentration of carbon dioxine stabilized around values between 2-4 %, and the oxygen concentration has averaged 11-15 %, which is considered to be a sufficient concentration for the going of the nitrification process.

3.2 Carbon Balance

Before starting with the injections in the columns, a characterization of the carbon in the leachate was performed. In table 4 are represented the values of TOC, COD and BOD expressed in mg/l measured in the leachate sample of the reactors, taken on May, 16, on the 23th day of the experiment, and the values of TOC expressed in mg/l measured in the leachate sample of the reactors, taken day 76.

Table 4 – *Initial and final leachate carbon content characterization*

	Initial leachate parameters (16/03/2013)			**Final leachate parameters (08/07/2013)**
Columns	**TOC (mg/l)**	**COD (mg/l)**	**BOD (mg/l)**	**TOC (mg/l)**
R1	841	1822	1,6	381
R2	784	2008	0,5	396
R3	748	1027	0,8	563
R4	1107	1588	2,2	687
R5	2934	388	10,1	620
R6	621	420	0,8	448

The initial leachate parameter results reveal a low biodegradable carbon content characteristic of a well decomposed waste.

The 76th day, on July, 8, another sample was taken in order to assess the TOC level and to understand if the carbon content had decrease. The results shows a decrease of the TOC level in all the columns. It was observed a percentage reduction of 55% in reactor 1, 49% in reactor 2, 25% in reactor 3, 38% in reactor 4, 79% in reactor 5 and 28% in reactor 6.

The lower reduction of the TOC level occurs in the control reactors (R3 and R6), and this is why no nitrogen source were injected and so the carbon was less degraded by the microorganisms for the nitrogen removal processes. In the reactors R1 and R5 were performed two injections of nitrogen source during the experiment, instead of one in the reactors R2 and R4, and this explain why the removal was higher. Also in reactor R2 the carbon removal was high. In this reactor and in reactor R5 were performed injections of acetate together with the nitrogen source, in order to stimulate the heterotrophic denitrification. The occurs of the heterotrophic denitrification process could be an explanation of the high carbon removal, since the bacteria use organic carbon source to reduce the nitrate. Furthermore, despite the acetate injected was enough to promote total nitrate denitrification, it appears that denitrifying bacteria were able to use as well the hardly biodegradable carbon released by the aeration and recirculation in the columns.

3.3 Nitrogen and Sulphates Fate in the Reactors

The changes in concentrations of ammonia-nitrogen, nitrous-nitrogen and sulphates in the output leachate from various columns during the experiment are hereafter discussed.

The masses of ammonia and nitrate measured were never as high as stoichiometry would suggest, confirming other processes, in addition to nitrification and denirification processes, were contributing to nitrogen removal. Denitrification process clearly contributed to nitrate removal, while they were produced by the nitrification process. During the experimentation, sulphates production was recorderd in all the columns, suggesting a portions of nitrate removal may be attributed to autotrophic denitrification. During the sulphates spikes, more nitrate disappeared; thus, it is possible that nitrate removal may also be attributed to heterotrophic denitrification, resulting in a conversion of nitrates to nitrogen gas which could not be measured.

To calculate the rate of nitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From this concentrations, know the volume of water present in each column, was calculated the mass of ammonia, nitrates and sulphates present from time to time in the columns. Then were calculate all the variations, and from them the cumulative variations. It was also calculated for each concentration of ammonia and nitrate, the ammonia removal rate and the nitrates removal rate. From the difference between the nitrates was calculated the nitrated removed, and from the difference between the sulphates was calculated the sulphates produced. From the nitrates removed was calculated the theoretical sulphates produced assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of SO_4^{2-} per mg of $N\text{-}NO_3^-$ reduced to N_2. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction was then calculated the heterotrophic fraction of the denitrification process.

The calculation done to estimate the autotrophic and heterotrophic denitrification percentages are performed considering range of time inside which the concentration trends of nitrates and sulphate are linear. This means that the percentages obtained are meaningful only within this limited period, and that they cannot be considered valid for all the duration of the experiment. Calculating the same percentages considering different values, would be obtained results completely different. It is thus important to clarify that this percentages represent only an example of the autotrophic and heterotrophic denitrification ratio and they don’t represent the process in the entire experiment.

In reactor 1 were realized potassium nitrate (KNO_3) additions on days 36 and 65, which aimed to bring $N\text{-}NO_3^-$ concentration around 1000 mg/l, in order to make nitrous-nitrogen concentration more readily appreciable. A buffer solution of sodium bicarbonate ($NaHCO_3$) was also added to prevent the pH drop.

Before the first injection the parameters were stable and the average concentrations of nitrate, alkalinity and sulphate were 273 $mgN\text{-}NO_3^-/l$, 218 $mgCaCO_3/l$ and 1417 $mgSO_4^{2-}/l$ respectively.

After the first injection of day 36, 49% of nitrate removal was achieved, and after the second injection of day 65, 58% of nitrate removal was achieved. Thus in any injection period denitrification was complete.

Considering the highest nitrate concentration and the lowest nitrate concentration registered in the first injection period, respectively 852 $mgN\text{-}NO_3^-/l$ on day 43 and 458 $mgN\text{-}NO_3^-/l$ on day 58, it was possible to calculate the nitrate removal rate equal to 235 $mgN\text{-}NO_3^-/d$. Regarding the sulphates, which were expecting to increase due to the autotrophic denitrification, had only a pick observed on day 36. From the stoichiometric calculations in this injection period and taking into account the assumptions made it was possible to state that 29% of nitrate was removed by autotrophic denitrification, 71% by heterotrophic one.

Because the system have a low biodegradable carbon content with initial leachate BOD of 1,6 mg O_2/l autotrophic denitrification was expected to occur, however factors like the carbon released from the waste by the action of recirculation and the consequent competition with heterotrophic bacteria for the nitrate available could limit autotrophic denitrification.

In the second injection, denitrification occurs with a nitrate removal rate of 208 mgN-NO_3^-/d, considering the highest nitrate concentration of 1397 mgN-NO_3^-/l on day 65 and the lowest nitrate concentration of 657 mgN-NO_3^-/l on day 100. In this phase the sulphate levels maintained a constant trend from day 78 to day 107 with an average concentration of 1724 mgSO_4^{2-}/l, that was a lower sulphate production than expected considering the stoichiometric amount. Calculating a direct relation with the stoichiometric ratio (4,64 mg of SO_4^{2-} per mg of N-NO_3^- reduced to N_2), denitrification outcome 33% of autotrophic reactions and 67% of heterotrophic reactions.

Alkalinity levels according to the literature need to be between 1000 and 5000 mg$CaCO_3$/l (Metcalf and Eddy, 2001). However is unlikely to be the reason why denitrification is limited in this case, because denitrification occurs and alkalinity didn´t decrease sharply, instead it´s available for the reaction. Additionally, heterotrophic denitrification produces alkalinity (3,57 mg $CaCO_3$/ mg N-NO_3^- reduced) (Oh et al., 2001) and if heterotrophic denitrification appears to be the dominant nitrate removal reaction alkalinity is not likely to be the limitative factor.

In the final phase of the experiment, after the losses of leachate, both the nitrate and sulphates concentrations tend to decrease, reaching concentration values similar to the values founded in the first phase of the test.

With regard to reactor 2, potassium nitrate (KNO_3) and sodium acetate (CH_3COONa) injections was performed performed in order to simulate denitrification with an available carbon source. Potassium nitrate injection was made on day 64. Sodium acetate had to be injected three times due to the carbon natural removal, which makes stoichiometric carbon amount insufficient, the first acetate injection was made together with nitrate source, the second acetate injection was made on day 76, the third acetate injection was made on day 83.

During the characterization phase, before the potassium nitrate injection, the average concentrations of sulphate, nitrate and alkalinity were respectively 1413 mgSO_4^{2-}/l, 845 mgN-NO_3^-/l and 192 mg$CaCO_3$/l.

On the basis of the assumption previously exposed, the percentage of autotrophic denitrification following the nitrate injection on day 64 was 33%, while the heterotrophic denitrification percentage was 67%. As in column R1, also in column R2 the heterotrophic denitrification result to be predominant respect to the autotrophic one.

Denitrification after the injection on day 64 occurs with a nitrate removal rate of 227 mgN-NO_3^-/d, and 90.5% of nitrate removal was achieved. However the nitrate removal rate was lower than what expected, probably due to the extra carbon source addition. Extra carbon additions were added due to the natural conversion of acetate to CO_2, which make the initial stoichiometric amount of acetate insufficient to complete the denitrification.

Sulphate variation was consistent during the experiment, however its value was never higher than the average values recorded in characterization phase. This could mean that denitrification occurs by heterotrophic pathway exclusively. As expected the higher concentration of sulphate was coincident with the final phase of denitrification due to the slower development of autotrophic bacteria.

Alkalinity increase from 164 mg$CaCO_3$/l to a final concentration of 507 mg$CaCO_3$/l. Alkalinity in heterotrophic denitrification is produced in an approximate rate of 3,5 mg$CaCO_3$/mgN-NO_3 reduced (Oh et al., 2001). Alkalinity increasing is another evidence of prevalence of heterotrophic denitrification.

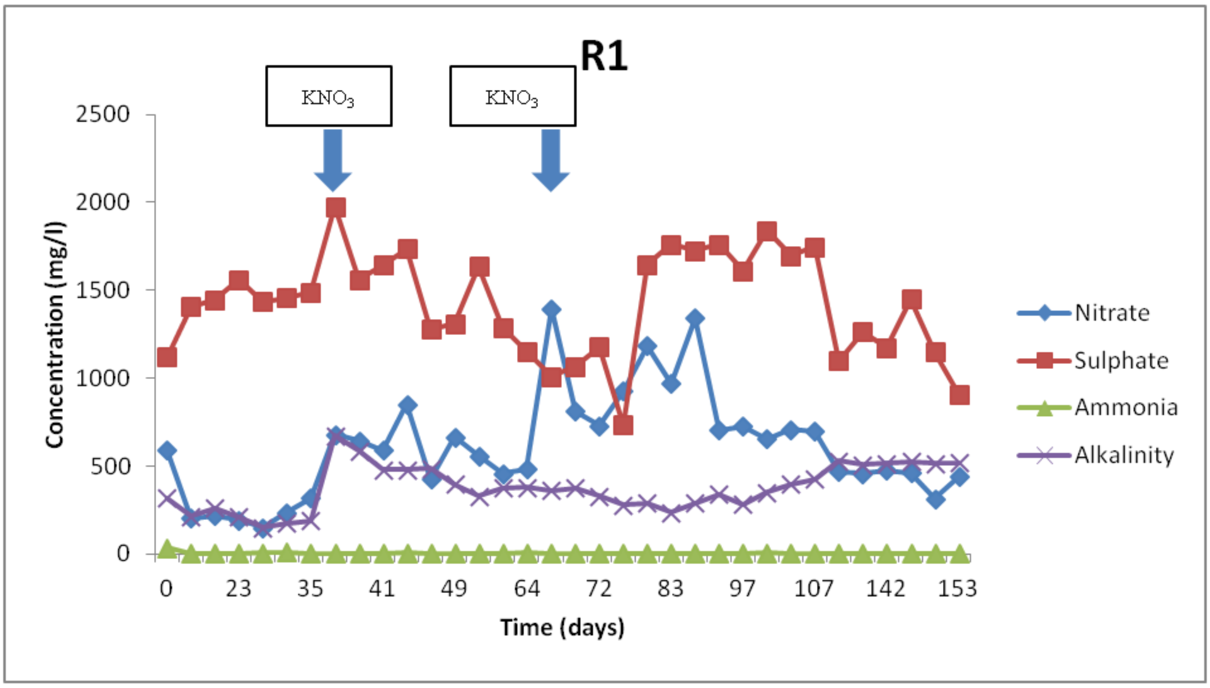

Figure 4 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R1*

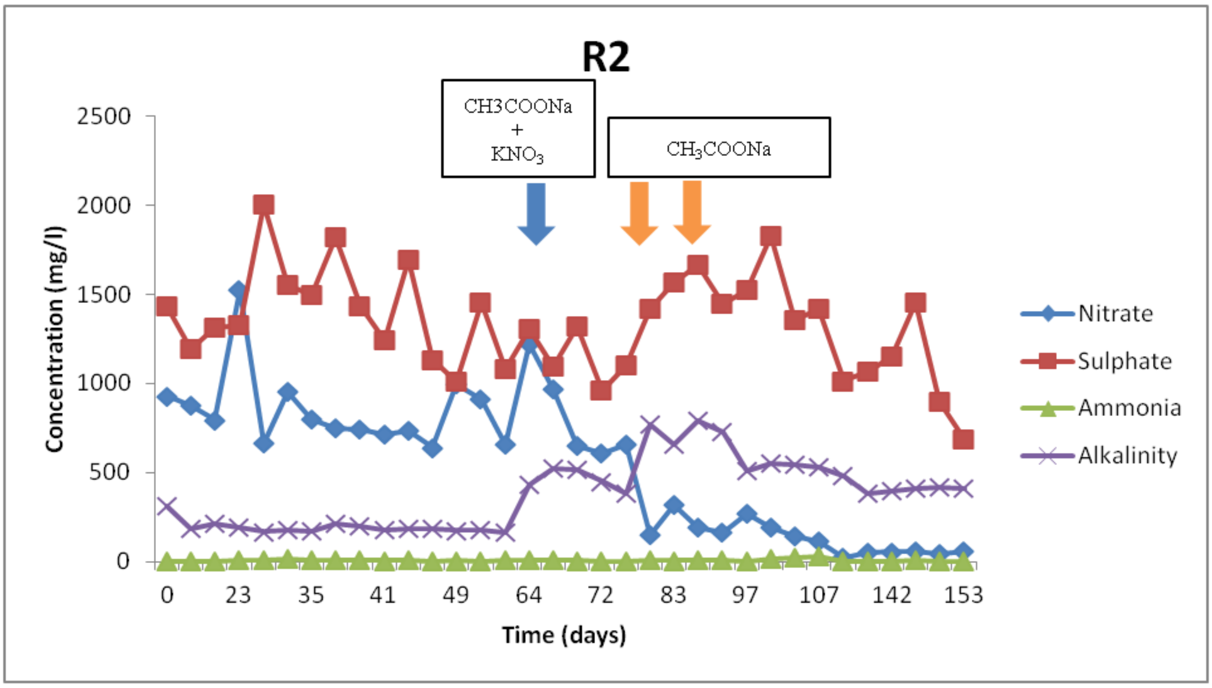

Figure 5 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R2*

Speaking about reactor 4, ammonium chloride (NH_4Cl) and sodium bicarbonate ($NaHCO_3$) injections were performed. Two injections of sodium bicarbonate were performed on day 63 and 70 to prevent the pH drop. The ammonium chloride injection, which aimed to bring $N\text{-}NH_4^+$ concentration around 1000 mg/l, was performed on day 63 in order to asses the nitrification rate of column R4.

On the characterization phase reactor 4 leachate had stable values in alkalinity, ammonium, and nitrate, with average concentration respectively equal to 134 $mgCaCO_3/l$, 7 $mgN\text{-}NH_4^+/l$, 683 $mgN\text{-}NO_3^-/l$. In other hand sulphate values were not so stable and it was unlikely due to denitrification, most probably it was due to sulphate reduction spots and subsequent oxidation of that reduced forms.

Nitrification occurs in a range of 99% with an ammonium nitrogen removal rate of 270 $mgN\text{-}NH_4^+/day$. Nitrous-nitrogen pick appear days after nitrification. From the amounts measured in terms of nitrate produced and from the amount of ammonium injected, it is indicated that the sorption or volatilization processes were negligible in ammonium transformation.

Regarding the denitrification process, following the injection of day 63, 17% of nitrate was removed by autotrophic denitrification, 83% by heterotrophic one. Nitrate removal didn´t occur totally, thus only 46,6 % of nitrate nitrogen was removed with a removal rate of 130 mg $N\text{-}NO_3^-/day$. Denitrification occur but not completely because it was observed that nitrates levels remain higher than before the injection. The nitrate average concentration before the injection was 603 $mgN\text{-}NO_3^-/l$, and after the injection was 803 $mgN\text{-}NO_3^-/l$. However due to the carbon content, present in low quantity, denitrification was expected to accur.

Comparing with reactor 1 which was run under similar conditions, it obtains a faster nitrate nitrogen removal rates and a higher percentage of removal as well. However the estimated percentage of autotrophic denitrification was lower than in reactor 4, which indicates a slower removal as bigger is the percentage of autotrophic bacteria. In both cases, reactor 1 and 4, nitrate wasn´t removed totally which indicates some limitation in autotrophic denitrification process. A limitative factor could be the alkalinity because alkalinity optimal range is between 1000 and 5000 mg/l, and average alkalinity after the injections in reactor 1 are respectively 466 $mgCaCO_3/l$ and 395 $mgCaCO_3/l$, either reactor 4 had an average alkalinity after the injection of 188 $mgCaCO_3/l$. Another limitative factor could be the electron donor problem. In both cases readily biodegradable carbon source was not available and autotrophic denitrification needed reduced sulphur sources to occur. Thus, because sulphate levels were high in both reactors, a reduction to sulphide for the availableness of reduced sulphur forms was a critical reaction. Sulphate reduction kinetics are slow and the migration of sulphate in the leachate could limit the reduction in the anaerobic pockets.

In reactor 5 two injections of ammonia acetate (CH_3COONH_4) was performed an day 35 and 65 with the target concentration of 100 mgN-NH_4^+/l in order to assess nitrification process and ammonium nitrogen removal rates, as well as denitrification process.

The average concentration of alkalinity, ammonia, nitrate and sulphate in the characterization phase was respectively 922 mg$CaCO_3$/l, 12 mgN-NH_4^+/l, 11 mgN-NO_3^-/l and 842 mgSO_4^{2-}/l.

During the first injection nitrification occurred successfully since 99.7% of ammonium-nitrogen was completely removed, with an ammonium-nitrogen removal rate of 464 mgN-NH_4^+/d. In the same injection period, i.e. before day 65, nitrate didn´t increase which could be due to simultaneous nitrification and denitrification, due to the presence of anoxic pockets. However two nitrate picks were measured on days 37 and 49 with nitrous-nitrogen concentrations of 21 mgN-NO_3^-/l and 34 mgN-NO_3^-/l respectively. Those two picks coincided with the two sulphate picks, in which sulphate concentration values were 1168 mgSO_4^{2-}/l and 1403 mgSO_4^{2-}/l. The mean of this fact could be that autotrophic denitrification occurs even with a carbon source available. Calculating a direct relation with the stoichiometric ratio, denitrification outcome 68% of autotrophic reactions and 32% of heterotrophic reactions.

In the second injection another amount of ammonium acetate was added to the reactor and again nitrification was successfully with 99,2% of ammonium nitrogen removal, with an ammonium nitrogen removal rate was 380 mgN-NH_4^+/d. Like in the first injection, nitrate nitrogen didn´t accumulate which means that denitrification occurs. A low pick of nitrate was recorded on day 78 of 33 mg N-NO_3^-/l.

The sulphate concentration in this second injection phase sharply increases until a concentration of 3361 mgSO_4^{2-}/l. The pick of sulphate could be due to the occurrence of effective autotrophic denitrification, and this could be confirmed by the denitrification percentages equal to 80% for the autotrophic one and 20% for the heterotrophic one. The evident increase on sulphates concentration could be explained also by the available oxygen on the system. Indeed, the oxygen concentration increase on this specific period probably due to the end of carbon oxidation, thus the oxygen available starts to oxidize the sulphur sources of the waste.

Alkalinity was always kept constant, the only two peaks were registered in correspondence with the ammonia acetate injections. The alkalinity in column R5 was higher than the other reactors due to the heterotrophic denitrification, as expected. As it is known heterotrophic denitrification produces 3,5 mg$CaCO_3$/l, which ensure that the consumption of alkalinity by nitrification process (7,07 mg $CaCO_3$/l) do not affect pH in the system.

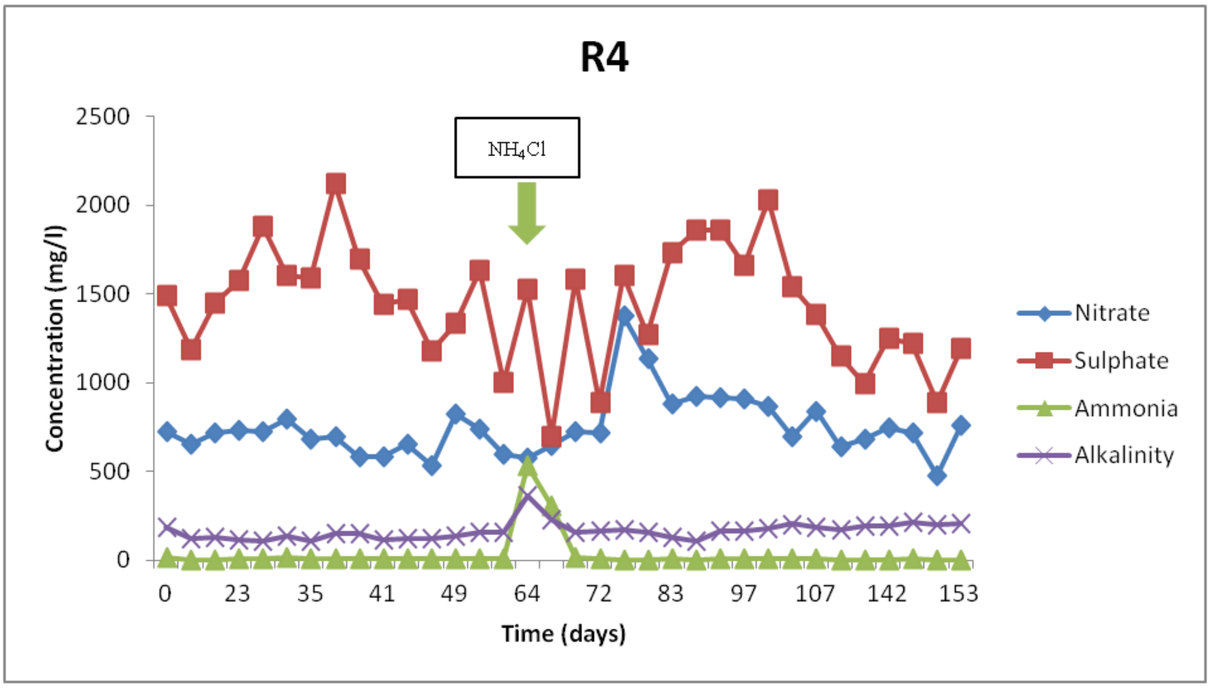

Figure 6 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R4*

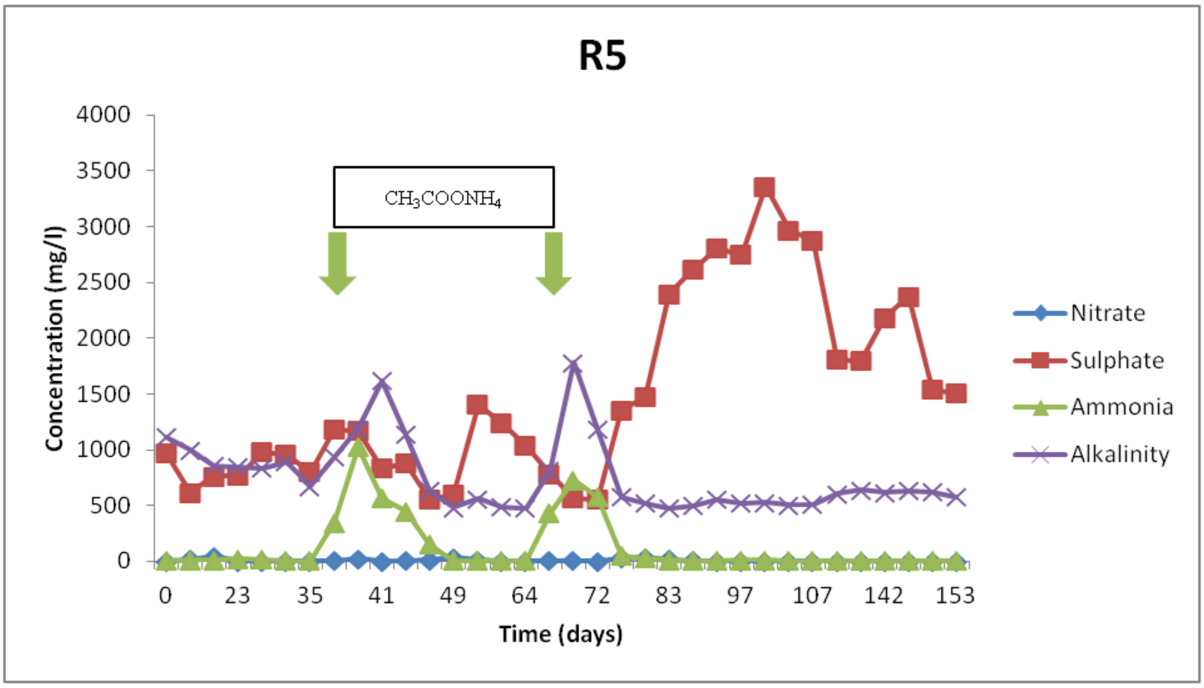

Figure 7 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R5*

Reactor 3 and reactor 6 were chosen as control reactors. In reactor 3 was observed the higher concentrations of $N\text{-}NO_3^-$ and so it was kept as control reactor in order to understand if nitrate concentration starts to decrease with the recirculation action. Autotrophic denitrification was expected to occur due to the leachate low biodegradable carbon initial content, with a BOD concentration of 0,8 mg O_2/l.

However nitrate didn´t decrease and an eventual lack of sulphur source in the waste mass was hypothesized, because reduced forms of sulphur are used as electron donors by the autotrophic bacteria.

For this reason, on day 70, an injection of sodium sulfide was performed in the waste mass in order to increase the sulphide concentration and to asses if the sulphur was the limited factor to the autotrophic denitrification. The average sulphate and nitrate concentrations before the sulphur addition was respectively 12916 mgSO_4^{2-}/l and 1797 mgNO_3^-/l; the average sulphate and nitrate concentrations after the sulphur addition was respectively 11367 mgSO_4^{2-}/l and 1539 mgNO_3^-/l

It was detected an immediate increase in the sulphate concentration after the injection, however nitrate get stable on about 1500 mgNO_3^-/l instead of decrease. The increasing of sulphate concentration suggests the oxidation of sulphur because the system had been aerated for 70 days. In fact air results suggest this immediate oxidation because before sulphur source addition average oxygen percentage on reactor 3 was 17,2% and drop to 16,0% after the addition, recording a minimum value of 15,4 %, showing a consumption of oxygen.

Reactor 6 was kept as a control reactor due to the average levels of the parameters subjected to analysis such as NH_4^+, NO_3^- and SO_4^{2-} and no injections were performed. The average concentration of alkalinity, ammonia, nitrate and sulphate during the experiment was respectively 104 $mgCaCO_3/l$, 5.3 $mgN\text{-}NH_4^+/l$, 1080 $mgN\text{-}NO_3^-/l$ and 1426 $mgSO_4^{2-}/l$.

In this reactor no ammonia pick was reported from the leachate analysis, however desorption of ammonia and instantaneous nitrification could possibly occur. By the nitrate values which maintain a constant behavior during the experiment time, it is possible to state that no denitrification has taken place in this column.

Because denitrification has not occurred, sulphate curve variation has not coincidence with denitrification process. Therefore, sulphate increasing is unlikely to be a by-product due to autotrophic denitrification. Instead it could be a chemical reaction of sulphur composts oxidation.

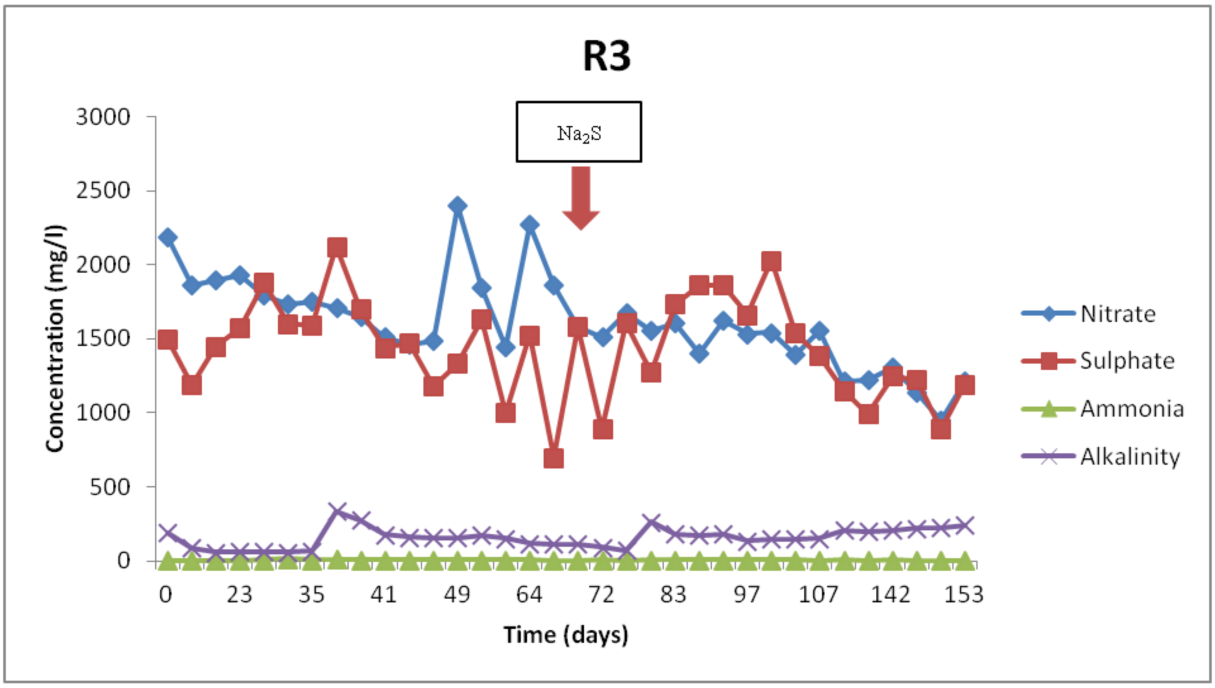

Figure 8 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R3*

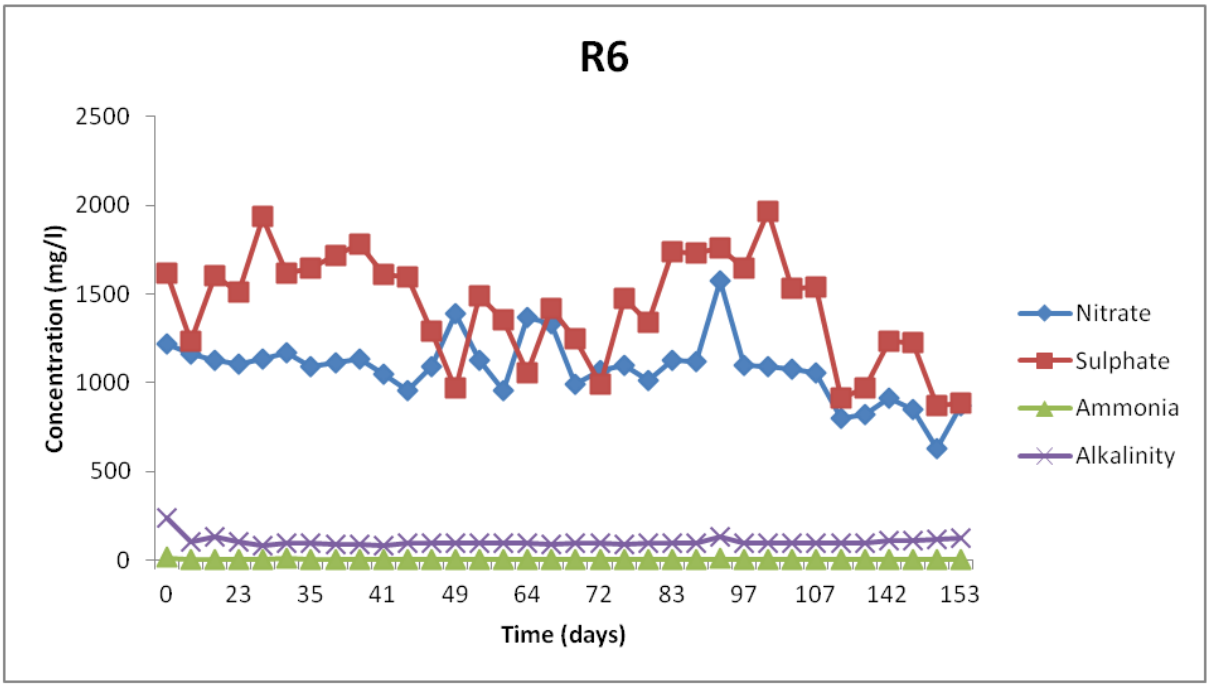

Figure 9 – *Ammonia-nitrogen, nitrous-nitrogen, sulphates and alkalinity concentrations from reactor R6*

3.4 Nitrogen Balance

It was realized the nitrogen mass balance for each column. The nitrogen balance was calculated and reported in Table 5. The initial total amount of nitrogen in the leachate was calculated adding the amount of ammonia-nitrogen with the amount of nitrous-nitrogen recorded at the start of the experiment and immediately after the injection days. Then was calculated the amount of ammonia-nitrogen and nitrous-nitrogen removed from the leachate realizing the cumulative sum of the differences between each concentration value and its previous one. From the addition of these two contribution was obtained the total nitrogen removed from the leachate. Finally the nitrogen that remains in the leachate was calculated by the difference between the initial amount of nitrogen and the nitrogen removed.

This nitrogen balance is not completed. The reason is that it was not possible to take into account all the contributions. First of all, it was not performed a solid sample analysis of the waste present in the columns, and so it was not possible to know the amount of ammonia-nitrogen and nitrous-nitrogen in the solid waste. The mass balance was thus realized taking into account only the contribution of nitrogen present in the leachate. Moreover, the by-products of the processes of nitrification and denitrification, such as nitrogen oxide (NO) and nitrogen dioxide (N_2O) were not measured. In addition, other processes, which were not taken into account, other than nitrification and denitrification, may have contributed to the attenuation of ammonia and nitrates. An example is assimilation, which could be held responsible for a small part of the ammonia and nitrates disappearance. Also the abiotic conversion of nitrates may have given a contribution, and probably a portion of nitrogen has been lost because of experimental difficulties. Ammonification is another process which could give a contribution to the balance since the production of ammonia-nitrogen and its subsequent dissolution in the leachate that occurring when this reaction is present. Additionally, sorption of some of the nitrogen species could have contributed to the low recovery of nitrogen over time.

During all the period of the experiment, distilled water was periodically added to the columns because of the compensation of the liquid volume that is extracted for the sample analysis. This dilution is not considered to affect the nitrogen concentration of the leachate, because when operating the landfill as a bioreactor, leachate is very recycled, and hence ammonia-nitrogen is continually reintroduced to the landfill while additional ammonia is solubilized into the leachate. On day 134 the amount of water added to the columns was consistent due to the loss of leachate, therefore the analysis performed in the sample extracted in the final phase of the experiment could be not very significant because of the high dilution to which was subject the leachate. Nevertheless also the concentration values of ammonia-nitrogen and nitrous-nitrogen obtained from the sample analysis of the final period of the experiment were considered in the nitrogen mass balance.

Finally, another consideration that it was done regards the evolution of nitrogen in the period elapsing the samples of the leachate. It was make the assumption that the variation of nitrogen was linear, because of the needed of simplification.

Table 5 –*Nitrogen balance at the end of the study*

Columns		$N\text{-}NH_4^+$ initial (mg)	$N\text{-}NO_3^-$ initial (mg)	N initial total (mg)	$N\text{-}NH_4^+$ removed (mg)	$N\text{-}NO_3^-$ removed (mg)	N removed total (mg)	N remained (mg)
R1	**Injection 1**	40	5975	6015	1	2119	2120	3896
	Injection 2	24	12573	12597	20	11998	12018	579
	total	64	18548	18612	21	14118	14138	4474
R2	**Injection 1**	76	7258	7334	36	2149	2185	5148
	Injection 2	45	10771	10816	42	10703	10744	72
	total	121	18029	18150	78	12852	12930	5220
R3		78	11944	12022	73	9877	9949	2073
R4	**Injection 1**	55	3811	3866	29	1051	1080	2785
	Injection 2	2543	3501	6044	2538	2433	4971	1074
	total	2598	7313	9910	2567	3484	6051	3859
R5	**Injection 1**	2227	18	2245	2194	9	2203	42
	Injection 2	2869	31	2901	2864	31	2895	5
	total	5096	50	5146	5058	40	5098	48
R6		53	8190	8243	49	6881	6930	1314

3.5 pH and Alkalinity Variation

The reaction of nitrification and denitrification have an important influence on the pattern of alkalinity. Nitrification produces a market effects on alkalinity: 7.07 grams of alkalinity (expressed as $CaCO_3$) are consumed for each gram of oxidized $N\text{-}NH_4^+$. Subsequent denitrification occurring in anoxic spots would result in the recovery of approximately half of the alkalinity used for denitrification (Berge et al., 2007). Heterotrophic denitrification reactions have the production of 3.57 grams of alkalinity for each gram of $N\text{-}NO_3^-$ reduced. Even the autotrophic denitrification process has influence on alkalinity, but contrary to the heterotrophic denitrification, the autotrophic one consume alkalinity: in particular theoretical bicarbonate alkalinity consumption is 4.57 grams of $CaCO_3$ per gram of $N\text{-}NO_3^-$ reduced to nitrogen gas (Oh et al., 2001).

At the start of the experiment alkalinity was around 300 mg$CaCO_3$/l for column R1 and R2, was lower in columns R3, R4, R6, respectively equal to 190, 184 and 238 mg$CaCO_3$/l and much higher in column R5, around 1000 mg$CaCO_3$/l. The concentrations of alkalinity increase in columns R1 and R4 because of the injection of sodium bicarbonate ($NaHCO_3$) performed respectively on day 38 and on days 64 and 70, in columns R2 and R3 due to the injections of sodium acetate (CH_3COONa) performed respectively on days 64, 77, 84 and on day 77, and finally in column R5 due to the injection of ammonium acetate (CH_3COONH_4) performed on days 36 and 65. At the end of the test column R1 and R5 reached a value of alkalinity around 550 mg$CaCO_3$/l, columns R3 and R4 around 200 mg$CaCO_3$/l, column R2 around 400 mg$CaCO_3$/l, and column R6 reached the minimum value around 100 mg$CaCO_3$/l. The leachate decrease in alkalinity over time is due both to the washout, and to the destruction by nitrification and by autotrophic denitrification.

Similarly to alkalinity, decrease the pH too, in all reactors except than in R2, in which the sodium acetate offset the alkalinity destroyed by the process of nitrification. All reactors, at the start of the experiment, had a pH around 7.5, the lower was observed in reactor R3, equal to 6.43, the higher in the reactor R5, equal to 7.18. During the first phase of the experiment, the pH in all reactors tend to settle around 7; at the end of the test all the columns reached a pH around 6.7. The final lower pH was detected in column R4, equal to 6.63, the highest in column R2, equal to 6.85. Nitrification is very sensitive to pH, at a pH of 6.5, the rate is 35% lower than when the pH is 7.5. Below a pH of 6.5, the rate decreases by approximately 5% for each 0.1 drop in pH (Berge et al., 2007). On the other hand it is know that the optimum pH range, for the denitrification processes is between 7-7.5, pH lower than 6 determine a rapid decrease in the activity of denitrification (Christensen, 2001).

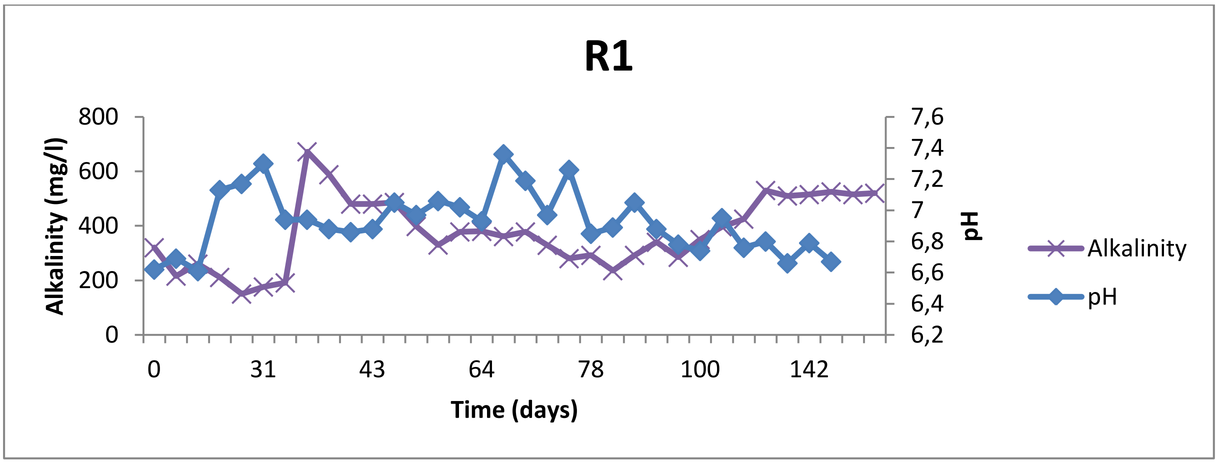

Figure 10 – *pH and alkalinity trends from column R1*

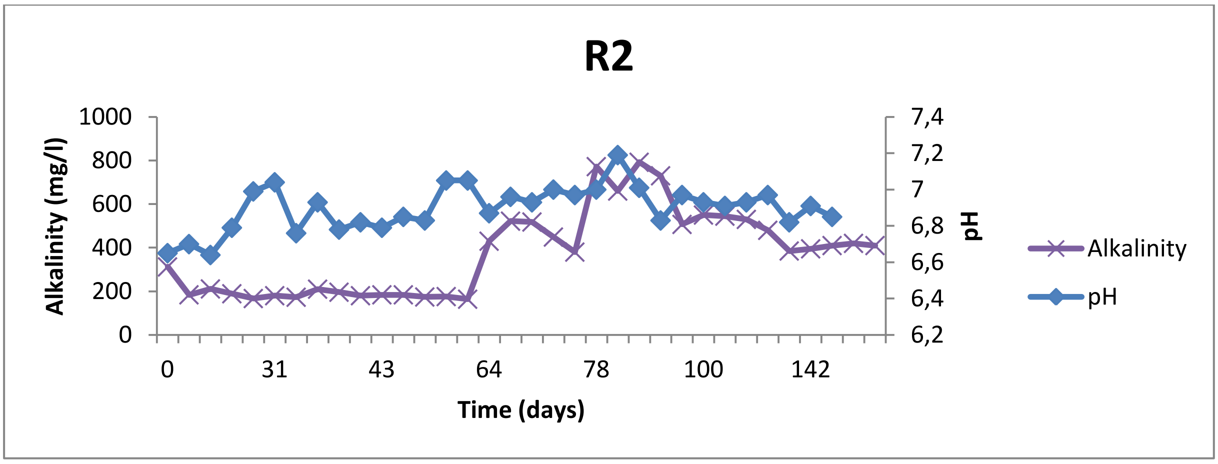

Figure 11 – *pH and alkalinity trends from column R2*

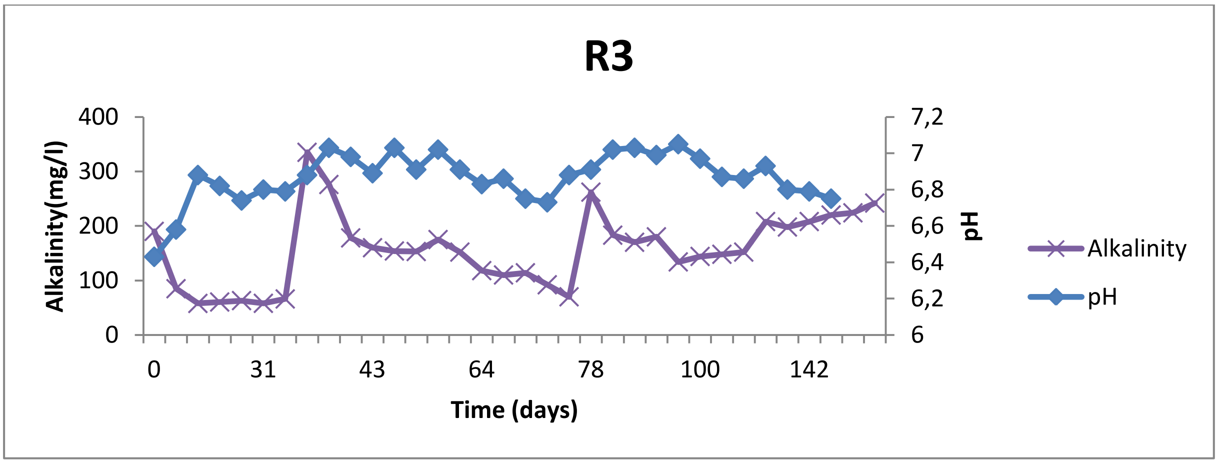

Figure 12 – *pH and alkalinity trends from column R3*

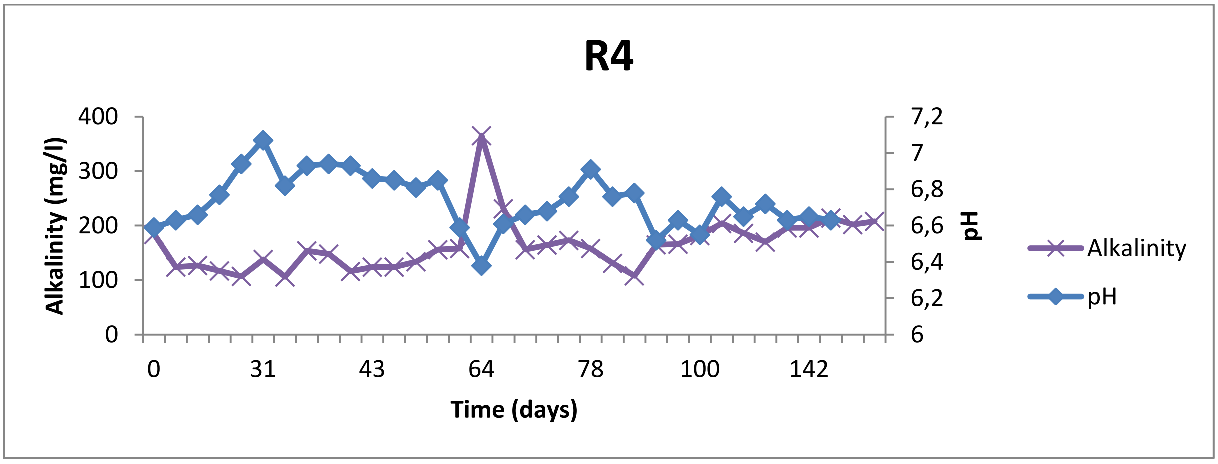

Figure 13 – *pH and alkalinity trends from column R4*

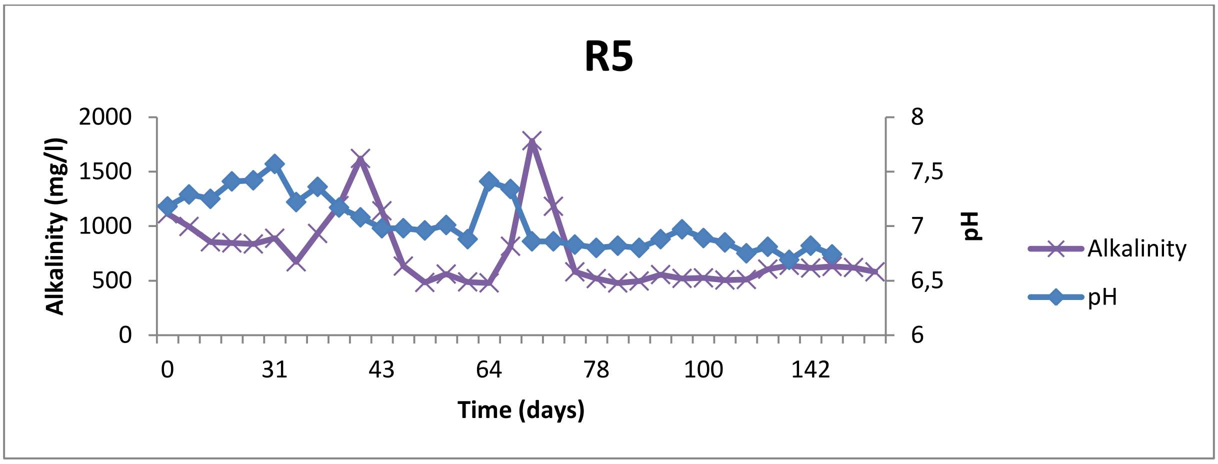

Figure 14 – *pH and alkalinity trends from column R5*

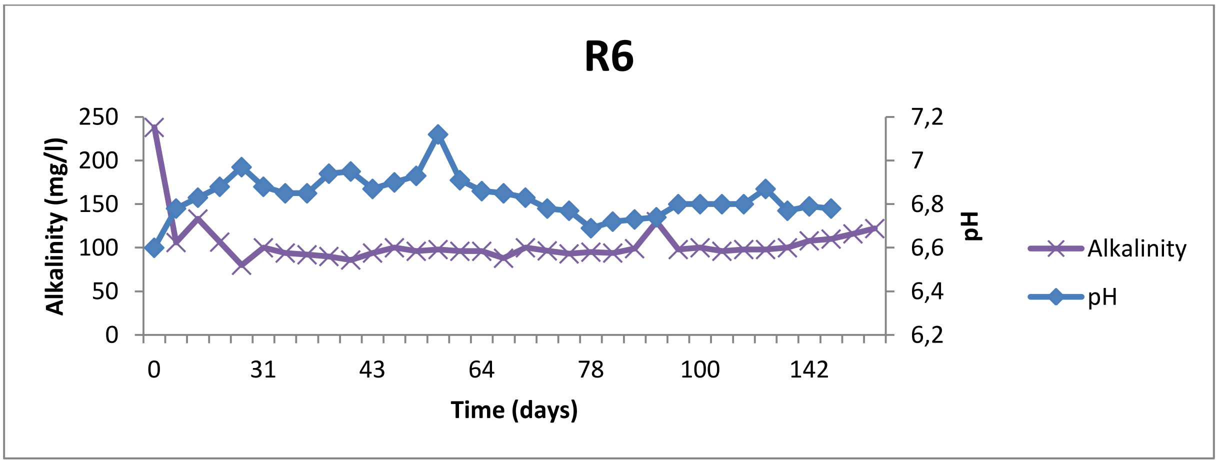

Figure 15 – *pH and alkalinity trends from column R6*

4. Conclusion

Based on the data obtained in this study, the following guidelines can be provided for the implementation of in situ ammonia removal on field-scale. As already mentioned, in situ nitrogen removal should be a final treatment in the life of a landfill. It is, indeed, an effective treatment on the long term pollutants, which persist even when the organic strength of leachate has been already reduced for a long time.

The results show that ammonia removal via nitrification and denitrification is feasible in bioreactor landfills, and that nitrification and denitrification processes may occur simultaneously in one aerobic landfill cell, rather than requiring two separate cells containing two different in situ environments.

Temperature is an influence parameter for the process of in situ nitrogen removal and temperatures in landfills assume variable values, they can also become very high, with a maximum between 55 to 66 °C (Berge et al., 2005). The nitrification process has been shown to proceed better at 35 °C, than for high temperature, at 45 and 55 °C it result inhibited. For nitrification rates, the maximum occur at 35 °C.

Another important parameter for in situ nitrogen removal was found to be pH. Nitrification is very sensitive to pH, as well as the process of denitrification. When the leachate is characterized by high concentration of ammonia, such as 1000 $mgN\text{-}NH_4^+/l$, has a low initial alkalinity and expecially at elevated temperatures, the pH may decrease a lot, producing the necessity to add a buffering agents to prevent the rapid inhibition of the nitrogen removal processes.

On this study ammonium removal was achieved from 99,0 to 99,7 % and the removal rates were from 270 $mgN\text{-}NH_4^+/d$ to 464 $mgN\text{-}NH_4^+/day$, suggesting a fast reduction of ammonium nitrogen accumulated in landfill leachate.

Nitrate removal was also achieved, however only with available carbon sources it was reduced efficiently with removal from 85% to 90%. In environments with well degraded waste, with low biodegradable carbon available, nitrate removal only was achieved from 46,6% to 57,9%. The nitrate removal rate were from 108 $mgN\text{-}NO_3^-/d$ to 235 $mgN\text{-}NO_3^-/d$. This results could suggest that an addition of an external carbon source could be useful to the denitrification process when the leachate presents a high concentration of nitrate to denitrify. However, the continuous recirculation could lead to an accumulation of nitrate in leachate if any carbon source is available, due to the low efficiency of nitrate removal by the autotrophic denitrification, as evidenced in reactor 1.

The attempt to assess the denitrification origin it’s a tricky point due to the oxidation of the reduced sulphur sources. In other hand when the sulphur source is exhausted and only sulphate remains in the leachate it is difficult to quantify the sulphate produced by autotrophic bacteria, because sulphate is being reduced at the same time it is used to denitrify.

Sulphate measurements reveal to be not effective on the autotrophic assessment due to the chemical oxidation of sulphur compounds, instead of biological use by autotrophic bacteria. Thus the origin of denitrification process became inconclusive about the role of autotrophic bacteria in well decomposed waste leachate denitrification.

Nevertheless, in this experiment it was possible to asses that autotrophic denitrification could represents from 17% to 28% in denitrification process, whereas the heterotrophic denitrification could represents from 72% to 88% in denitrification process.

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PART III

Documents Attached

ANNEX A

Analytical Results

Table 1 – *Analytical results expressed in mg/l on the leachate extracted from* ***reactor R1*** *during the experimental period*

REACTOR 1						
Days	**Date**	**pH**	**Alkalinity ($mgCaCO_3/l$)**	**$N\text{-}NH_4^+$ (mg/l)**	**$N\text{-}NO_3^-$ (mg/l)**	**SO_4^{2-} (mg/l)**
0	23-apr	6,62	320	35,4	591	1121
14	07-mag	6,69	216	2,1	204	1406
20	13-mag	6,61	260	2,0	220	1446
23	16-mag	7,13	211	3,5	193	1562
27	20-mag	7,17	150	6,9	151	1435
31	24-mag	7,30	176	9,2	235	1458
35	28-mag	7,20	191	5,4	320	1488
36	29-mag	7,00	672	5,9	679	1976
37	30-mag	6,94	588	3,7	644	1560
41	03-giu	6,94	480	5,3	591	1645
43	05-giu	6,88	480	6,2	852	1736
45	07-giu	6,86	486	3,9	428	1277
49	11-giu	6,88	398	3,9	667	1310
55	17-giu	7,05	330	3,0	560	1638
58	20-giu	6,97	378	5,5	458	1290
64	26-giu	7,06	380	6,4	482	1152
65	27-giu	7,02	361	3,4	1397	1010
70	02-lug	6,93	378	3,6	814	1067
72	04-lug	7,36	329	3,9	730	1176
76	08-lug	7,19	280	3,0	928	734
78	10-lug	6,97	292	3,4	1185	1647
83	15-lug	7,26	236	4,6	969	1761
86	18-lug	6,85	292	2,9	1342	1726
90	22-lug	6,89	340	4,2	705	1762
97	29-lug	7,05	285	3,2	729	1612
100	01-ago	6,88	350	6,2	657	1839
104	05-ago	6,78	397	4,7	710	1697
107	08-ago	6,74	425	5,4	697	1748
134	04-set	6,95	530	3,9	471	1101
139	09-set	6,76	510	3,4	457	1265
142	12-set	6,80	515	5,7	476	1174
146	16-set	6,66	525	3,8	460	1450
149	19-set	6,79	515	3,9	316	1151
153	23-set	6,67	520	4,2	442	908

Table 2 – *Analytical results expressed in mg/l on the leachate extracted from reactor* ***R2*** *during the experimental period*

REACTOR 2						
Days	**Date**	**pH**	**Alkalinity ($mgCaCO_3/l$)**	**N-NH_4^+ (mg/l)**	**N-NO_3^- (mg/l)**	**SO_4^{2-} (mg/l)**
0	23-apr	6,65	312	3,0	928	1431
14	07-mag	6,70	184	3,0	878	1193
20	13-mag	6,64	211	2,0	795	1316
23	16-mag	6,79	189	6,1	1522	1328
27	20-mag	6,99	167	6,9	667	2003
31	24-mag	7,04	180	12,8	955	1555
35	28-mag	6,90	173	9,9	798	1499
36	29-mag	6,90	210	5,5	747	1822
37	30-mag	6,76	196	5,9	743	1432
41	03-giu	6,93	180	4,9	716	1240
43	05-giu	6,78	184	6,7	734	1694
45	07-giu	6,82	184	4,3	640	1133
49	11-giu	6,79	174	4,5	993	1011
55	17-giu	6,85	176	3,8	908	1455
58	20-giu	6,83	164	6,5	655	1078
64	26-giu	7,05	430	6,6	1224	1305
65	27-giu	7,05	522	5,6	965	1095
70	02-lug	6,87	518	4,5	648	1322
72	04-lug	6,96	449	4,2	610	958
76	08-lug	6,93	380	3,1	660	1104
78	10-lug	7,00	772	5,9	149	1421
83	15-lug	6,97	660	4,6	320	1569
86	18-lug	7,00	792	6,4	191	1663
90	22-lug	7,19	730	5,5	165	1445
97	29-lug	7,01	507	4,1	268	1527
100	01-ago	6,83	550	12,6	191	1828
104	05-ago	6,97	545	23,7	144	1355
107	08-ago	6,93	530	27,5	111	1417
134	04-set	6,91	480	2,9	20	1013
139	09-set	6,93	385	3,9	52	1064
142	12-set	6,97	395	4,1	54	1151
146	16-set	6,82	410	5,1	56	1455
149	19-set	6,91	420	3,7	42	896
153	23-set	6,85	410	3,9	57	686

Table 3 – *Analytical results expressed in mg/l on the leachate extracted from reactor* ***R3*** *during the experimental period*

REACTOR 3						
Days	**Date**	**pH**	**Alkalinity ($mgCaCO_3/l$)**	**N-NH_4^+ (mg/l)**	**N-NO_3^- (mg/l)**	**SO_4^{2-} (mg/l)**
0	23-apr	6,43	190	2,3	2187	1286
14	07-mag	6,58	84,8	3,0	1860	1146
20	13-mag	6,88	57,8	2,0	1894	1346
23	16-mag	6,82	60,4	3,0	1927	1312
27	20-mag	6,74	63	6,9	1795	1386
31	24-mag	6,80	58	14,6	1731	1337
35	28-mag	6,80	66	7,0	1752	1449
36	29-mag	6,80	335	7,5	1706	1893
37	30-mag	6,79	276	6,2	1650	1502
41	03-giu	6,88	178	6,4	1514	1346
43	05-giu	7,03	160	7,0	1460	1623
45	07-giu	6,98	154	6,4	1484	1308
49	11-giu	6,89	153	6,6	2397	1224
55	17-giu	7,03	175	5,9	1842	1415
58	20-giu	6,91	152	6,8	1441	1206
64	26-giu	7,02	118	6,0	2270	1203
65	27-giu	6,91	110	4,5	1859	1517
70	02-lug	6,83	114	4,0	1575	1001
72	04-lug	6,86	92	6,0	1512	1315
76	08-lug	6,75	70	4,0	1675	1520
78	10-lug	6,73	262	5,8	1552	1570
83	15-lug	6,88	183	7,0	1606	2182
86	18-lug	6,91	170	5,5	1402	2475
90	22-lug	7,02	180	6,6	1622	1945
97	29-lug	7,03	134	6,5	1534	2186
100	01-ago	6,99	144	5,6	1542	2598
104	05-ago	7,05	148	6,2	1394	2107
107	08-ago	6,97	152	4,4	1552	2098
134	04-set	6,87	208	5,3	1215	1224
139	09-set	6,86	198	3,2	1220	1297
142	12-set	6,93	208	5,7	1305	1445
146	16-set	6,80	220	4,9	1139	1641
149	19-set	6,79	224	4,7	946	1406
153	23-set	6,75	242	4,3	1216	1385

Table 4 – *Analytical results expressed in mg/l on the leachate extracted from reactor* ***R4*** *during the experimental period*

REACTOR 4						
Days	**Date**	**pH**	**Alkalinity (mgCaCO$_3$/l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**
0	23-apr	6,59	184	15,1	726	1494
14	07-mag	6,63	124	2,6	652	1188
20	13-mag	6,66	127	2,0	721	1448
23	16-mag	6,77	117	5,8	734	1573
27	20-mag	6,94	107	6,9	726	1878
31	24-mag	7,07	138	14,6	794	1602
35	28-mag	7,00	106	7,0	684	1587
36	29-mag	6,90	154	6,7	699	2122
37	30-mag	6,82	148	6,2	580	1699
41	03-giu	6,93	116	6,4	582	1440
43	05-giu	6,94	124	7,0	657	1472
45	07-giu	6,93	124	6,4	533	1181
49	11-giu	6,86	134	6,6	823,3	1333
55	17-giu	6,85	156	5,9	736	1631
58	20-giu	6,81	158	7,1	600	1002
64	26-giu	6,85	365	536,0	574	1524
65	27-giu	6,59	231	306,0	650	698
70	02-lug	6,38	156	17,9	725	1583
72	04-lug	6,61	164,5	9,1	715	891
76	08-lug	6,66	173	5,0	1377	1603
78	10-lug	6,68	158	5,3	1139	1272
83	15-lug	6,76	131	7,2	882	1731
86	18-lug	6,91	108	4,5	921	1860
90	22-lug	6,76	165	7,1	919	1859
97	29-lug	6,78	166	8,4	907	1662
100	01-ago	6,52	182	7,1	865	2028
104	05-ago	6,63	204	6,8	700	1538
107	08-ago	6,55	186	7,8	842	1384
134	04-set	6,76	170	4,7	643	1149
139	09-set	6,65	196	4,3	683	992
142	12-set	6,72	196	4,7	749	1249
146	16-set	6,63	214	5,6	721	1223
149	19-set	6,65	202	4,5	477	887
153	23-set	6,63	208	5,0	763	1193

Table 5 – *Analytical results expressed in mg/l on the leachate extracted from reactor* ***R5*** *during the experimental period*

REACTOR 5						
Days	**Date**	**pH**	**Alkalinity (mgCaCO$_3$/l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**
0	23-apr	7,18	1113	6,6	1,25	966
14	07-mag	7,29	996	10,5	16,70	614
20	13-mag	7,25	855	10,5	45,00	763
23	16-mag	7,41	845,5	22,2	0,00	768
27	20-mag	7,42	836	17,2	0,56	983
31	24-mag	7,57	888	7,8	0,68	961
35	28-mag	7,50	672	3,4	0,07	799
36	29-mag	7,40	932	345,0	2,18	1185
37	30-mag	7,22	1188	1021,4	20,70	1168
41	03-giu	7,36	1620	567,1	0,73	836
43	05-giu	7,17	1140	446,8	3,16	881
45	07-giu	7,08	633	151,4	11,10	557
49	11-giu	6,98	483	7,9	34,30	600
55	17-giu	6,98	560	3,6	12,00	1403
58	20-giu	6,96	488	3,5	0,97	1235
64	26-giu	7,01	479	5,7	1,20	1033
65	27-giu	6,88	815	434,0	3,70	783
70	02-lug	7,41	1783	729,0	1,90	568
72	04-lug	7,34	1181,5	573,0	0,65	552
76	08-lug	6,86	580	48,0	24,10	1357
78	10-lug	6,86	518	24,0	33,00	1474
83	15-lug	6,83	478	9,9	16,00	2396
86	18-lug	6,80	496	6,3	5,20	2617
90	22-lug	6,82	555	8,4	1,30	2804
97	29-lug	6,80	520	9,1	0,60	2753
100	01-ago	6,88	525	11,7	0,40	3361
104	05-ago	6,97	505	6,1	0,40	2969
107	08-ago	6,89	510	6,4	0,50	2873
134	04-set	6,85	605	6,9	0,80	1806
139	09-set	6,75	640	4,1	0,40	1797
142	12-set	6,81	615	8,2	0,30	2185
146	16-set	6,69	630	7,1	0,40	2375
149	19-set	6,82	620	4,9	0,30	1540
153	23-set	6,74	580	4,5	0,30	1505

Table 6 – *Analytical results expressed in mg/l on the leachate extracted from reactor* **R6** *during the experimental period*

REACTOR 6						
Days	**Date**	**pH**	**Alkalinity (mgCaCO$_3$/l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**
0	23-apr	6,60	238	15,1	1222	1619
14	07-mag	6,78	106	3,0	1165	1231
20	13-mag	6,83	133	2,0	1127	1604
23	16-mag	6,88	106,5	3,0	1106	1510
27	20-mag	6,97	80	6,3	1136	1933
31	24-mag	6,88	100	9,8	1170	1619
35	28-mag	6,90	94	7,3	1092	1649
36	29-mag	7,00	92	5,7	1113	1713
37	30-mag	6,85	90	4,2	1133	1778
41	03-giu	6,85	86	4,9	1047	1610
43	05-giu	6,94	94	5,6	959	1594
45	07-giu	6,95	100	4,5	1091	1292
49	11-giu	6,87	96	5,3	1387	967
55	17-giu	6,90	98	4,1	1128	1486
58	20-giu	6,93	96	4,7	956	1353
64	26-giu	7,12	96	6,5	1367	1058
65	27-giu	6,91	88	4,7	1332	1415
70	02-lug	6,86	100	3,8	992	1250
72	04-lug	6,85	96,5	5,1	1073	990
76	08-lug	6,83	93	2,9	1101	1474
78	10-lug	6,78	95	5,1	1010	1338
83	15-lug	6,77	94	6,1	1124	1737
86	18-lug	6,69	99	5,1	1119	1729
90	22-lug	6,72	130	8,1	1577	1762
97	29-lug	6,73	98	6,6	1100	1643
100	01-ago	6,74	100	5,6	1089	1967
104	05-ago	6,80	96	5,2	1077	1532
107	08-ago	6,80	98	5,6	1053	1538
134	04-set	6,80	98	3,4	799	913
139	09-set	6,80	100	3,6	823	972
142	12-set	6,87	108	4,3	912	1231
146	16-set	6,77	110	4,5	850	1228
149	19-set	6,79	116	3,7	630	873
153	23-set	6,78	122	3,7	873	885

Table 7 – *Gas volumetric percentage composition (O_2, CO_2, CH_4) analyzed in output from the reactors* ***R1, R2*** *and* ***R3***

Days	**Date**	**R1**			**R2**			**R3**		
		O_2 (%)	**CO_2 (%)**	**CH_4 (%)**	**O_2 (%)**	**CO_2 (%)**	**CH_4 (%)**	**O_2 (%)**	**CO_2 (%)**	**CH_4 (%)**
35	28-mag	16,6	3,45	0,01	16,7	2,13	0,05	17,3	1,60	0,14
36	29-mag	15,7	6,81	0,10	16,7	2,60	0,10	17,3	1,41	0,10
37	30-mag	16,1	4,90	0,15	16,8	2,09	0,10	17,0	2,91	0,05
41	03-giu	14,0	5,25	0,10	15,4	2,70	0,10	17,2	1,57	0,10
42	04-giu	14,5	4,46	0,12	16,4	1,90	0,09	17,3	1,57	0,13
44	06-giu	13,5	5,50	0,12	15,6	2,54	0,20	17,2	1,57	0,09
48	10-lug	12,2	4,90	0,00	15,9	2,20	0,10	16,8	1,05	0,00
49	11-giu	15,9	2,69	0,00	16,1	1,85	0,00	16,9	1,09	0,01
55	17-giu	16,7	1,81	0,00	15,9	1,85	0,01	16,4	1,57	0,05
58	20-giu	15,4	2,82	0,01	16,5	1,69	0,03	17,5	1,01	0,05
59	21-giu	15,0	3,13	0,01	16,5	1,85	0,08	17,7	0,81	0,01
63	25-giu	14,8	3,29	0,01	15,7	2,01	0,01	17,5	0,81	0,02
64	26-giu	14,6	3,74	0,01	13,6	2,69	0,01	17,5	0,81	0,05
66	28-giu	13,5	3,89	0,00	12,5	3,82	0,00	17,2	0,61	0,01
70	02-lug	12,3	4,21	0,00	12,6	3,82	0,00	17,0	0,53	0,01
71	03-lug	15,6	2,17	0,00	14,4	2,97	0,00	16,9	0,53	0,01
72	04-lug	16,7	1,33	0,00	14,6	2,94	0,00	15,5	0,00	0,00
76	08-lug	16,8	0,93	0,00	16,2	1,33	0,00	15,9	4,37	0,00
78	10-lug	15,8	1,25	0,00	12,1	3,33	0,00	16,4	1,77	0,00
83	15-lug	16,2	0,85	0,00	13,9	3,17	0,00	16,2	1,05	0,01
86	18-lug	15,9	1,05	0,00	14,5	2,53	0,00	15,9	1,05	0,05
90	22-lug	16,0	0,85	0,01	15,3	1,65	0,01	15,9	0,93	0,05
97	29-lug	16,4	0,36	0,12	12,4	4,05	0,18	15,9	1,29	0,22
100	01-ago	16,5	0,28	0,10	9,0	8,46	0,05	16,2	0,93	0,18
104	05-ago	15,2	1,05	0,05	3,9	14,50	0,01	16,1	0,97	0,18
107	08-ago	14,5	1,01	0,00	6,6	10,10	0,00	15,4	0,85	0,00
134	04-set	17,6	2,85	0,00	17,7	2,09	0,00	18,7	1,53	0,00
139	09-set	19,9	0,74	0,00	16,7	2,25	0,00	17,8	1,81	0,00
142	12-set	21,2	0,00	0,00	15,1	3,37	0,00	21,0	0,01	0,00
146	16-set	16,6	2,56	0,00	12,7	4,49	0,00	17,3	2,25	0,00
149	19-set	15,3	3,69	0,00	14,6	3,25	0,00	16,9	2,19	0,00
153	23-set	16,7	3,33	0,00	16,5	2,89	0,00	15,6	2,81	0,00

Table 8 – *Gas volumetric percentage composition (O_2, CO_2, CH_4) analyzed in output from the reactors* ***R4, R5*** *and* ***R6***

Days	**Date**	**R4**			**R5**			**R6**		
		O_2 (%)	**CO_2 (%)**	**CH_4 (%)**	**O_2 (%)**	**CO_2 (%)**	**CH_4 (%)**	**O_2 (%)**	**CO_2 (%)**	**CH_4 (%)**
35	28-mag	17,4	1,05	0,05	11,0	3,53	0,05	17,4	0,93	0,01
36	29-mag	16,9	3,13	0,18	10,7	4,18	0,15	17,2	0,89	0,06
37	30-mag	17,7	0,60	0,05	8,2	6,01	0,14	17,3	0,89	0,06
41	03-giu	17,4	1,17	0,10	10,4	7,60	0,10	17,6	0,77	0,09
42	04-giu	17,7	0,85	0,11	11,6	6,90	0,14	17,5	1,05	0,05
44	06-giu	17,4	1,21	0,06	12,5	5,80	0,10	17,7	0,85	0,14
48	10-lug	17,0	0,97	0,00	14,1	3,45	0,00	17,6	0,45	0,00
49	11-giu	16,9	1,05	0,00	10,1	5,66	0,00	17,5	0,57	0,00
55	17-giu	16,0	1,69	0,00	8,5	7,50	0,02	17,6	0,69	0,00
58	20-giu	16,7	1,50	0,05	13,7	4,54	0,05	17,4	0,89	0,02
59	21-giu	17,2	1,13	0,01	13,6	4,42	0,05	17,5	0,81	0,03
63	25-giu	17,1	1,05	0,01	13,7	4,22	0,01	17,4	0,69	0,01
64	26-giu	16,5	2,81	0,02	13,6	4,29	0,01	17,5	0,54	0,01
66	28-giu	11,1	5,06	0,00	6,7	7,42	0,01	16,9	0,57	0,01
70	02-lug	12,0	3,37	0,00	9,1	6,90	0,01	17,0	0,25	0,00
71	03-lug	13,5	5,10	0,00	11,0	5,50	0,00	16,0	0,45	0,00
72	04-lug	14,8	3,85	0,00	12,0	4,66	0,00	16,7	0,57	0,00
76	08-lug	16,7	0,85	0,00	12,5	4,10	0,00	16,9	0,49	0,00
78	10-lug	16,5	0,77	0,00	12,4	3,91	0,00	16,6	0,53	0,00
83	15-lug	16,6	0,49	0,00	12,7	3,85	0,00	16,6	0,49	0,00
86	18-lug	16,5	0,53	0,01	12,4	4,09	0,00	16,7	0,24	0,00
90	22-lug	15,9	0,61	0,05	12,5	3,93	0,01	16,4	0,24	0,01
97	29-lug	16,8	0,25	0,18	12,6	4,58	0,15	16,8	0,24	0,05
100	01-ago	13,0	3,58	0,13	12,0	4,98	0,10	16,5	0,41	0,01
104	05-ago	9,5	6,02	0,05	16,9	0,24	0,09	16,5	0,69	0,05
107	08-ago	6,6	7,60	0,01	15,9	0,24	0,00	15,9	0,32	0,00
134	04-set	18,0	2,13	0,00	19,8	0,38	0,00	20,7	0,26	0,00
139	09-set	21,3	0,01	0,00	11,2	6,36	0,00	20,5	0,33	0,00
142	12-set	21,3	0,01	0,00	21,3	0,01	0,00	20,3	0,33	0,00
146	16-set	20,7	0,01	0,00	10,9	7,24	0,00	19,9	0,58	0,00
149	19-set	17,8	1,93	0,00	11,8	6,55	0,00	19,6	0,58	0,00
153	23-set	16,7	2,53	0,00	11,4	6,43	0,00	19,5	0,62	0,00

ANNEX B

Calculation Procedures and Tables for the Estimation of Nitrification and Denitrification (Autotrophic and Heterotrophic) Activities

The calculation procedures for the estimation of nitrification and denitrification (autotrophic and heterotrophic) activities are hereafter explained.

For each columns two calculation tables are created. In the first of these two tables the nitrification and denitrification activities are estimated. The calculation started from the concentrations of ammonia, nitrates and sulphates detected during the experiment.. From this concentrations, known the volume of water present in each column, was calculated the mass of ammonia, nitrates and sulphates present from time to time in the columns.

$$\text{Mass (mg)} = \text{Concentration (mg/l)} \times \text{Liquid Volume (l)}$$

Then all the variations between a value and its previous were calculated, and from them the cumulative variations. It was also calculated for each mass (M) of ammonia and nitrate, the ammonia removal rate (R_R) and the nitrates removal rate using a central difference method of analyses:

$$R_R = \frac{M_{t-1} - M_{t+1}}{t_{t+1} - t_{t-1}}$$

The rows of the tables highlighted represents the calculation done in the injection day.

In the second of these two tables the autotrophic and heterotrophic denitrification percentages are estimated. First the mass of nitrated (mg) and the mass of sulphates produced (mg) was calculated from the difference respectively between two mass values of $N\text{-}NO_3^-$ and two mass of SO_4^{2-} chosen in a range of time inside which the concentrations trend is linear:

$$\text{Nitrate removed} = N\text{-}NO_3\ (t_1) - N\text{-}NO_3\ (t_2)$$
$$\text{Sulphate produced} = SO_4^{2-}\ (t_1) - SO_4^{2-}\ (t_2)$$

From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of SO_4^{2-} per mg of $N\text{-}NO_3^-$ reduced to N_2.

$$\text{Theoretical sulphate produced (mg)} = \text{Nitrate removed (mg)} \times \frac{\text{Mol }(SO_4^{2-})}{\text{Mol }(NO_3^{2-})}$$

Then the autotrophic and heterotrophic denitrification percentages was calculated. The autotrophic fraction of the denitrification process was calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction was then calculated the heterotrophic fraction of the denitrification process.

$$\%\ \text{Autotrophic} = \frac{\text{Sulphate produced (mg)}}{\text{Theoretical sulphate produced (mg)}} \times 100$$

% Heterotrophic = 100 – % Autotrophic

The mass values of nitrates and sulphates considered to perform the calculation above explained are the ones in red in the first table of the two ones for each column.

Regarding the column R5, the calculation done for the nitrates was different, because denitrification occurs simultaneously with nitrification in this column, and thus it is not possible to know the real amount of nitrate that was denitrified.. It was thus estimated the stoichiometric nitrates produced in order to obtain the autotrophic and heterotrophic denitrification percentages of reactor 5. From the mass of ammonia the amount (mg) of nitrates produced was calculated assuming a molecular weight (MW) ratio between nitrogen and ammonia of 1,29 and a stoichiometric ratio between the produced nitrates and the reduced ammonia of 0,98 mg of NO_3^- per mg of $N\text{-}NH_4^+$ reduced, according with the following stoichimetric expression:

$$NH_4^+ + 1{,}863\ O_2 + 0{,}098\ CO_2 \longrightarrow 0{,}0196\ C_5H_7NO_2 + 0{,}0941\ H_2O + 0{,}98\ NO_3^- + 1{,}98\ H^+$$

Then the amount (mg) of nitrous-nitrogen produced was calculated assuming a molecular weight (MW) ratio between nitrates and nitrogen of 0,23. Finally, from the ratio between the nitrous-nitrogen produced and the volume of liquid in the column was calculated the nitrous-nitrogen produced expressed in mg/l.

$$\text{Stoichiometric } NO_3^- \text{ produced (mg)} = \text{mass of } NH_4^+ \text{ (mg)} \times \frac{\text{MW (N)}}{\text{MW }(NH_4^+)} \times 0{,}98$$

$$\text{Stoichiometric N-NO}_3^- \text{ produced (mg)} = \text{Stoichiometric NO}_3^- \text{ produced (mg)} \times \frac{\text{MW (NO}_3^-\text{)}}{\text{MW (N)}}$$

$$\text{Stoichiometric N-NO}_3^- \text{ produced (mg/l)} = \frac{\text{Stoichiometric N-NO}_3^- \text{ produced (mg)}}{\text{Column leachate (l)}}$$

The calculation done to estimate the nitrate removed in the second table was performed making the difference between the stoichiometric N-NO_3^- produced expressed in mg, considering an appropriate range of time.

Table 1 – *Nitrification and denitrification activities estimation from reactor* ***R1****. To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 1															
Days	**Column leachate (l)**	**$N\text{-}NH_4^+$ (mg/l)**	**$N\text{-}NO_3^-$ (mg/l)**	**SO_4^{2-} (mg/l)**	**$N\text{-}NH_4^+$ (mg)**	**$N\text{-}NO_3^-$ (mg)**	**SO_4^{2-} (mg)**	**$\Delta N\text{-}NH_4^+$ (mg)**	**$\Delta N\text{-}NO_3^-$ (mg)**	**ΔSO_4^{2-} (mg)**	**$\Delta N\text{-}NH_4^+$ cumulative (mg)**	**$\Delta N\text{-}NO_3^-$ cumulative (mg)**	**ΔSO_4^{2-} cumulative (mg)**	**Ammonia R_R (mgN-NH_4^+/d)**	**Nitrate R_R (mgN-NO_3^-/d)**
0	7,0	35,4	591	1121	247	4139	7845	247	4139	7845	0	0	0	0,0	0,0
14	7,0	2,1	204	1406	15	1425	9842	-233	-2715	1997	0	0	1997	16,6	193,9
20	7,0	2,0	220	1446	14	1537	10122	-1	112	280	0	112	2277	0,1	-18,7
23	7,0	3,5	193	1562	25	1350	10934	11	-187	812	11	0	3089	-3,5	62,3
27	7,0	6,9	151	1435	48	1054	10045	24	-296	-889	34	0	2200	-5,9	74,0
31	6,9	9,2	235	1458	64	1623	10057	16	570	12	50	570	2212	-3,9	-142,4
35	6,8	5,4	320	1488	37	2176	10118	-27	553	61	23	1123	2273	6,8	-138,2
36	8,8	5,9	679	1976	52	5975	17385	0	0	0	0	0	0	-15,0	-3799,2
37	8,7	3,7	644	1560	32	5603	13572	-20	-372	-3813	0	0	0	19,8	372,4
41	8,6	5,3	591	1645	45	5083	14147	14	-520	575	14	0	575	-3,4	130,1
43	8,5	6,2	852	1736	53	7238	14753	7	2155	606	21	2155	1181	-3,6	-1077,6
45	8,4	3,9	428	1277	33	3592	10727	-20	-3646	-4026	1	0	0	9,8	1823,0
49	8,3	3,9	667	1310	32	5536	10873	-1	1944	146	1	1944	146	0,2	-486,1
55	8,2	3,0	560	1638	25	4592	13432	-8	-944	2559	0	1000	2705	1,3	157,4
58	8,1	5,5	458	1290	45	3710	10449	20	-882	-2983	20	118	0	-6,7	294,1
64	8,0	6,4	482	1152	51	3856	9216	7	146	-1233	27	264	0	-1,1	-24,4
65	9,0	3,4	1397	1010	31	12573	9090	0	0	0	27	0	0	20,6	-8717,0
70	8,9	3,6	814	1067	32	7245	9496	1	-5328	406	28	0	406	-0,3	1065,7
72	8,8	3,9	730	1176	34	6424	10349	2	-821	853	30	0	1259	-1,1	410,3
76	8,7	3,0	928	734	26	8074	6386	-8	1650	-3963	22	1650	0	2,1	-412,4
78	8,6	3,4	1185	1647	29	10191	14164	3	2117	7778	25	3767	7778	-1,6	-1058,7

83	8,5	4,6	969	1761	39	8237	14969	10	-1955	804	35	1813	8583	-2,0	390,9
86	8,4	2,9	1342	1726	24	11273	14498	-15	3036	-470	20	4849	8113	4,9	-1012,1
90	8,3	4,2	705	1762	35	5852	14625	11	-5421	126	31	0	8239	-2,6	1355,3
97	8,2	3,2	729	1612	26	5941	13138	-9	90	-1487	22	90	6752	1,3	-12,8
100	8,1	6,2	657	1839	50	5289	14804	24	-653	1666	46	0	8418	-7,9	217,5
104	8,2	4,7	710	1697	38	5787	13831	-12	498	-973	34	498	7445	2,9	-124,4
107	8,1	5,4	697	1748	43	5611	14071	5	-176	241	39	322	7686	-1,7	58,5
134	1,6	3,9	471	1101	6	754	1762	-37	-4857	-12310	2	0	0	1,4	179,9
139	1,5	3,4	457	1265	5	686	1898	-1	-68	136	1	0	136	0,2	13,6
142	1,6	5,7	476	1174	9	762	1878	4	76	-19	5	76	117	-1,3	-25,4
146	1,5	3,8	460	1450	6	690	2175	-3	-72	297	2	4	413	0,9	17,9
149	1,4	3,9	316	1151	5	442	1611	0	-248	-564	1	0	0	0,1	82,5
153	1,3	4,2	442	908	5	575	1180	0	132	-431	1	132	0	0,0	-33,1

Table 2 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R1****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of N-*NO_3^- *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
KNO_3 injection 1	893	4,64	4142	1181	29	71
KNO_3 injection 2	829	4,64	3847	1259	33	67

Table 3 – *Nitrification and denitrification activities estimation from reactor* ***R2****. To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 2															
Days	**Column leachate (l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**	**N-NH$_4^+$ (mg)**	**N-NO$_3^-$ (mg)**	**SO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ (mg)**	**ΔN-NO$_3^-$ (mg)**	**ΔSO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ cumulative (mg)**	**ΔN-NO$_3^-$ cumulative (mg)**	**ΔSO$_4^{2-}$ cumulative (mg)**	**Ammonia R_R (mgN-NH$_4^+$/d)**	**Nitrate R_R (mgN-NO$_3^-$/d)**
0	7,6	3,0	928	1431	23	7055	10877	23	7055	10877	0	0	0	0,0	0,0
14	7,6	3,0	878	1193	23	6673	9067	0	-382	-1810	0	0	0	1,3	27,3
20	7,6	2,0	795	1316	15	6039	9998	-8	-634	931	0	0	931	-10,4	105,6
23	7,6	6,1	1522	1328	46	11567	10093	31	5528	95	31	5528	1026	-1,4	-1842,7
27	7,6	6,9	667	2003	52	5069	15223	6	-6498	5130	37	0	6156	-11,3	1624,5
31	7,6	12,8	955	1555	97	7258	11815	45	2189	-3408	82	2189	2748	5,4	-547,2
35	7,6	9,9	798	1499	76	6065	11390	-22	-1193	-425	60	996	2323	31,4	298,3
36	8,1	5,5	747	1822	44	6051	14755	-31	-14	0	0	0	0	-3,0	14,1
37	8,0	5,9	743	1432	47	5944	11456	3	-107	-3299	3	0	0	2,0	106,7
41	7,9	4,9	716	1240	39	5656	9794	-8	-288	-1662	0	0	0	-6,6	71,9
43	7,8	6,7	734	1694	52	5724	13215	13	68	3421	13	68	3421	9,6	-34,0
45	7,7	4,3	640	1133	33	4925	8724	-19	-799	-4491	0	0	0	-0,3	399,8
49	7,6	4,5	993	1011	34	7547	7684	1	2622	-1041	1	2622	0	0,7	-655,5
55	7,9	3,8	908	1455	30	7173	11495	-4	-374	3811	0	2248	3811	-6,9	62,3
58	7,8	6,5	655	1078	51	5109	8408	21	-2064	-3086	21	184	725	-1,2	688,1
64	8,8	6,6	1224	1305	58	10771	11484	0	0	0	0	0	0	9,4	-943,7
65	8,7	5,6	965	1095	49	8396	9527	-9	-2376	-1958	0	0	0	2,0	2375,7
70	8,6	4,5	648	1322	39	5573	11369	-10	-2823	1843	0	0	1843	1,5	564,5
72	8,5	4,2	610	958	36	5185	8143	-3	-388	-3226	0	0	0	2,4	193,9
76	8,4	3,1	660	1104	26	5544	9274	-10	359	1131	0	359	1131	-14,7	-89,8
78	9,4	5,9	149	1421	55	1401	13357	29	-4143	4084	29	0	5214	2,1	2071,7

83	9,8	4,6	320	1569	45	3136	15376	-10	1735	2019	19	1735	7233	-5,7	-347,1
86	9,7	6,4	191	1663	62	1853	16131	17	-1283	755	36	452	7988	2,3	427,8
90	9,6	5,5	165	1445	53	1584	13872	-9	-269	-2259	27	183	5729	2,0	67,2
97	9,5	4,1	268	1527	39	2546	14507	-14	962	635	13	1145	6364	-26,5	-137,4
100	9,4	12,6	191	1828	118	1795	17183	79	-751	2677	92	395	9040	-25,5	250,2
104	9,3	23,7	144	1355	220	1339	12602	102	-456	-4582	194	0	4459	-10,9	114,1
107	9,2	27,5	111	1417	253	1021	13036	33	-318	435	227	0	4893	9,2	106,0
134	1,5	2,9	20	1013	4	30	1520	-249	-991	-11517	0	0	0	-0,2	36,7
139	1,4	3,9	52	1064	5	73	1490	1	43	-30	1	43	0	-0,2	-8,6
142	1,5	4,1	54	1151	6	81	1727	1	8	237	2	51	237	-0,2	-2,7
146	1,4	5,1	56	1455	7	78	2037	1	-3	311	3	48	547	0,8	0,7
149	1,3	3,7	42	896	5	55	1165	-2	-24	-872	0	25	0	0,0	7,9
153	1,2	3,9	57	686	5	68	823	0	14	-342	0	38	0	0,0	-3,5

Table 4 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R2****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of N-*NO_3^- *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
KNO3 + CH_3COONH_4 injection 1	5227	4,64	24254	7988	33	67
CH_3COONH_4 injection 2	1552	4,64	7201	3311	46	54

Table 5 – *Nitrification and denitrification activities estimation from reactor* ***R3****. To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 3															
Days	**Column leachate (l)**	**$N\text{-}NH_4^+$ (mg/l)**	**$N\text{-}NO_3^-$ (mg/l)**	**SO_4^{2-} (mg/l)**	**$N\text{-}NH_4^+$ (mg)**	**$N\text{-}NO_3^-$ (mg)**	**SO_4^{2-} (mg)**	**$\Delta N\text{-}NH_4^+$ (mg)**	**$\Delta N\text{-}NO_3^-$ (mg)**	**ΔSO_4^{2-} (mg)**	**$\Delta N\text{-}NH_4^+$ cumulative (mg)**	**$\Delta N\text{-}NO_3^-$ cumulative (mg)**	**ΔSO_4^{2-} cumulative (mg)**	**Ammonia R_R (mgN-NH_4^+/d)**	**Nitrate R_R (mgN-NO_3^-/d)**
0	6,9	2,3	2187	1286	16	15088	8870	16	15088	8870	0	0	0	0,0	0,0
14	6,9	3,0	1860	1146	21	12834	7907	5	-2254	-963	5	0	0	-0,3	161,0
20	6,9	2,0	1894	1346	14	13065	9287	-7	231	1380	0	231	1380	1,2	-38,5
23	6,9	3,0	1927	1312	21	13296	9053	7	231	-235	7	462	1145	-2,3	-77,0
27	6,9	6,9	1795	1386	47	12386	9563	27	-911	511	34	0	1656	-6,7	227,7
31	6,9	14,6	1731	1337	101	11944	9222	53	-442	-341	87	0	1315	-13,4	110,4
35	6,9	7,0	1752	1449	48	12089	9996	-52	145	773	35	145	2088	13,1	-36,2
36	7,9	7,5	1706	1893	60	13477	14954	11	1389	4958	0	0	0	-11,2	-1388,6
37	7,8	6,2	1650	1502	49	12870	11716	-11	-607	-3238	0	0	0	10,9	607,4
41	7,7	6,4	1514	1346	49	11658	10361	0	-1212	-1354	0	0	0	-0,1	303,1
43	7,6	7,0	1460	1623	54	11096	12333	4	-562	1971	5	0	1971	-2,2	280,9
45	7,5	6,4	1484	1308	48	11130	9810	-6	34	-2523	0	34	0	2,8	-17,0
49	7,4	6,6	2397	1224	49	17738	9058	1	6608	-752	1	6642	0	-0,2	-1652,0
55	7,3	5,9	1842	1415	43	13447	10330	-6	-4291	1272	0	2351	1272	1,0	715,2
58	7,2	6,8	1441	1206	49	10375	8683	6	-3071	-1646	6	0	0	-2,0	1023,8
64	7,1	6,0	2270	1203	43	16117	8541	-6	5742	-142	0	5742	0	1,1	-957,0
65	7,0	4,5	1859	1517	32	13013	10619	-11	-3104	2078	0	2638	2078	11,1	3104,0
70	6,9	4,0	1575	1001	28	10868	6907	0	0	0	0	0	0	0,8	429,1
72	6,8	6,0	1512	1315	41	10282	8942	13	-586	2035	13	0	2035	-6,6	293,0
76	7,8	4,0	1675	1520	31	13065	11856	-10	2783	2914	4	2783	4949	2,4	-695,9
78	7,7	5,8	1552	1570	45	11950	12089	13	-1115	233	17	1669	5182	-6,7	557,3

83	7,6	7,0	1606	2182	53	12206	16583	9	255	4494	26	1924	9676	-1,7	-51,0
86	7,5	5,5	1402	2475	41	10515	18563	-12	-1691	1979	14	233	11656	4,0	563,5
90	7,4	6,6	1622	1945	49	12003	14393	8	1488	-4170	21	1721	7486	-1,9	-372,0
97	7,3	6,5	1534	2186	47	11198	15958	-1	-805	1565	20	917	9051	0,2	114,9
100	7,2	5,6	1542	2598	40	11102	18706	-7	-96	2748	13	821	11799	2,4	31,9
104	7,3	6,2	1394	2107	45	10176	15381	5	-926	-3325	18	0	8474	-1,2	231,6
107	7,2	4,4	1552	2098	32	11174	15106	-14	998	-276	4	998	8199	4,5	-332,7
134	2,0	5,3	1215	1224	11	2430	2448	-21	-8744	-12658	0	0	0	0,8	323,9
139	1,9	3,2	1220	1297	6	2318	2464	-5	-112	16	0	0	16	0,9	22,4
142	2,0	5,7	1305	1445	11	2610	2890	5	292	426	5	292	442	-1,8	-97,3
146	1,9	4,9	1139	1641	9	2164	3118	-2	-446	228	3	0	670	0,5	111,5
149	1,8	4,7	946	1406	8	1703	2531	-1	-461	-587	2	0	83	0,3	153,8
153	1,7	4,3	1216	1385	7	2067	2355	-1	364	-176	1	364	0	0,3	-91,1

Table 6 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R3****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of* $N\text{-}NO_3^-$ *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
Na_2S injection	2550	4,64	11832	9621	81	19

Table 7 – *Nitrification and denitrification activities estimation from reactor **R4**. To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 4															
Days	**Column leachate (l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**	**N-NH$_4^+$ (mg)**	**N-NO$_3^-$ (mg)**	**SO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ (mg)**	**ΔN-NO$_3^-$ (mg)**	**ΔSO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ cumulative (mg)**	**ΔN-NO$_3^-$ cumulative (mg)**	**ΔSO$_4^{2-}$ cumulative (mg)**	**Ammonia R_R (mgN-NH$_4^+$/d)**	**Nitrate R_R (mgN-NO$_3^-$/d)**
0	4,8	15,1	726	1494	73	3487	7170	73	3487	7170	0	0	0	0,0	0,0
14	4,8	2,6	652	1188	12	3130	5702	-60	-357	-1468	0	0	0	4,3	25,5
20	4,8	2,0	721	1448	10	3462	6950	-3	332	1248	0	332	1248	0,5	-55,3
23	4,8	5,8	734	1573	28	3523	7550	18	62	600	18	394	1848	-6,1	-20,6
27	4,8	6,9	726	1878	33	3485	9014	5	-38	1464	23	355	3312	-1,3	9,6
31	4,8	14,6	794	1602	70	3811	7689	37	326	-1325	60	682	1987	-9,3	-81,6
35	4,8	7,0	684	1587	34	3283	7616	-36	-528	-73	24	154	1914	9,1	132,0
36	5,3	6,7	699	2122	36	3705	11248	2	422	3632	26	575	5546	-1,9	-421,5
37	5,2	6,2	580	1699	32	3016	8835	-3	-689	-2414	23	0	3132	3,1	688,7
41	5,1	6,4	582	1440	32	2968	7344	0	-48	-1491	23	0	1642	0,0	11,9
43	5,0	7,0	657	1472	35	3285	7361	3	317	17	26	317	1659	-1,4	-158,4
45	4,9	6,4	533	1181	31	2612	5787	-4	-673	-1574	22	0	85	1,9	336,7
49	4,8	6,6	823	1333	32	3952	6398	0	1340	612	22	1340	696	-0,1	-335,0
55	4,7	5,9	736	1631	28	3459	7666	-4	-493	1267	18	848	1963	0,7	82,1
58	4,6	7,1	600	1002	33	2760	4609	5	-699	-3057	23	148	0	-1,6	233,1
64	6,1	536,0	574	1524	3270	3501	9296	0	0	0	0	0	0	-539,5	-123,6
65	6,0	306,0	650	698	1836	3900	4188	-1434	399	-5108	0	399	0	1433,6	-398,6
70	6,5	17,9	725	1583	116	4713	10290	-1720	813	6102	0	1211	6102	343,9	-162,5
72	6,4	9,1	715	891	58	4576	5702	-58	-137	-4587	0	1075	1514	29,1	68,3
76	6,3	5,0	1377	1603	32	8675	10099	-27	4099	4397	0	5174	5911	6,7	-1024,8
78	6,2	5,3	1139	1272	33	7062	7886	1	-1613	-2213	1	3560	3698	-0,7	806,7

83	6,1	7,2	882	1731	44	5380	10559	11	-1682	2673	12	1879	6371	-2,2	336,3
86	6,0	4,5	921	1860	27	5526	11160	-17	146	601	0	2025	6972	5,6	-48,6
90	5,9	7,1	919	1859	42	5422	10968	15	-104	-192	15	1921	6780	-3,7	26,0
97	5,8	8,4	907	1662	49	5261	9640	7	-162	-1329	22	1759	5452	-1,0	23,1
100	5,7	7,1	865	2028	40	4931	11560	-8	-330	1920	13	1429	7372	2,8	110,0
104	5,6	6,8	700	1538	38	3920	8613	-2	-1011	-2947	11	419	4425	0,6	252,6
107	5,5	7,8	842	1384	43	4631	7612	5	711	-1001	16	1130	3424	-1,6	-237,0
134	1,7	4,7	643	1149	8	1093	1953	-35	-3538	-5659	0	0	0	1,3	131,0
139	1,6	4,3	683	992	7	1093	1587	-1	0	-366	0	0	0	0,2	0,1
142	1,7	4,7	749	1249	8	1273	2123	1	181	536	1	181	536	-0,4	-60,2
146	1,6	5,6	721	1223	9	1154	1957	1	-120	-167	2	61	370	-0,2	29,9
149	1,5	4,5	477	887	7	716	1331	-2	-438	-626	0	0	0	0,7	146,0
153	1,4	5,0	763	1193	7	1068	1670	0	353	340	0	353	340	-0,1	-88,2

Table 8 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R4****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of* $N\text{-}NO_3^-$ *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
NH_4Cl injection	4775	4,64	22064	3673	17	83

Table 9 – *Nitrification and denitrification activities estimation from reactor* **R5**. *To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 5															
Days	**Column leachate (l)**	**N-NH$_4^+$ (mg/l)**	**N-NO$_3^-$ (mg/l)**	**SO$_4^{2-}$ (mg/l)**	**N-NH$_4^+$ (mg)**	**N-NO$_3^-$ (mg)**	**SO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ (mg)**	**ΔN-NO$_3^-$ (mg)**	**ΔSO$_4^{2-}$ (mg)**	**ΔN-NH$_4^+$ cumulative (mg)**	**ΔN-NO$_3^-$ cumulative (mg)**	**ΔSO$_4^{2-}$ cumulative (mg)**	**Ammonia R$_R$ (mgN-NH$_4^+$/d)**	**Nitrate R$_R$ (mgN-NO$_3^-$/d)**
0	7,5	6,6	1	966	50	9	7246	50	9	7246	0	0	0	0,0	0,0
14	7,5	10,5	17	614	79	125	4605	29	116	-2641	29	116	0	-2,1	-8,3
20	7,5	10,5	45	763	79	338	5723	0	212	1118	29	328	1118	0,0	-35,4
23	7,5	22,2	0	768	167	0	5760	88	-338	38	117	0	1155	-29,3	112,5
27	7,5	17,2	1	983	129	4	7373	-38	4	1613	79	4	2768	9,4	-1,1
31	7,4	7,8	1	961	58	5	7112	-71	1	-260	8	5	2507	17,8	-0,2
35	7,3	3,4	0	799	25	1	5833	-33	-5	-1279	0	1	1228	8,1	1,1
36	8,3	345,0	2	1185	2864	18	9836	2838	18	4002	0	0	0	-2838,3	-17,6
37	8,2	1021,4	21	1168	8375	170	9578	5512	152	-258	5512	152	0	-5512,0	-151,6
41	8,1	567,1	1	836	4594	6	6772	-3782	-164	-2806	1730	0	0	945,5	41,0
43	8,0	446,8	3	881	3574	25	7047	-1019	19	276	711	19	276	509,6	-9,7
45	7,9	151,4	11	557	1196	88	4400	-2378	62	-2647	0	82	0	1189,2	-31,2
49	7,8	7,9	34	600	62	268	4680	-1134	180	280	0	262	280	283,6	-45,0
55	7,7	3,6	12	1403	28	92	10803	-34	-175	6123	0	87	6403	5,7	29,2
58	7,6	3,5	1	1235	27	7	9386	-1	-85	-1417	0	1	4986	0,4	28,3
64	7,5	5,7	1	1033	43	9	7748	16	2	-1639	16	3	3347	-2,7	-0,3
65	8,5	434,0	4	783	3689	31	6656	0	0	0	0	0	0	-3646,3	-22,5
70	8,4	729,0	2	568	6124	16	4771	2435	-15	-1884	2435	0	0	-486,9	3,1
72	8,3	573,0	1	552	4756	5	4582	-1368	-11	-190	1067	0	0	683,9	5,3
76	8,2	48,0	24	1357	394	198	11127	-4362	192	6546	0	192	6546	1090,6	-48,1
78	8,1	24,0	33	1474	194	267	11939	-199	70	812	0	262	7358	99,6	-34,8

83	8,0	9,9	16	2396	79	128	19168	-115	-139	7229	0	123	14586	23,0	27,9
86	7,9	6,3	5	2617	50	41	20674	-29	-87	1506	0	36	16093	9,8	29,0
90	7,8	8,4	1	2804	66	10	21871	16	-31	1197	16	5	17290	-3,9	7,7
97	7,7	9,1	1	2753	70	5	21198	5	-6	-673	20	0	16617	-0,6	0,8
100	7,6	11,7	0	3361	89	3	25544	19	-2	4346	39	0	20962	-6,3	0,5
104	7,7	6,1	0	2969	47	3	22861	-42	0	-2682	0	0	18280	10,5	0,0
107	7,6	6,4	1	2873	49	4	21835	2	1	-1027	2	1	17253	-0,6	-0,2
134	7,5	6,9	1	1806	52	6	13545	3	2	-8290	5	3	8963	-0,1	-0,1
139	7,4	4,1	0	1797	30	3	13298	-21	-3	-247	0	0	8716	4,3	0,6
142	1,5	8,2	0	2185	12	0	3278	-18	-3	-10020	0	0	0	6,0	0,8
146	1,5	7,1	0	2375	11	1	3563	-2	0	285	0	0	285	0,4	0,0
149	1,5	4,9	0	1540	7	0	2310	-3	0	-1253	0	0	0	1,1	0,1
153	1,4	4,5	0	1505	6	0	2107	-1	0	-203	0	0	0	0,3	0,0

Table 10 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R5****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of* $N\text{-}NO_3^-$ *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
CH_3COONH_4 injection 1	2043	4,64	9478	6403	68	32
CH_3COONH_4 injection 2	1728	4,64	8018	6376	80	20

Table 11 – *Nitrification and denitrification activities estimation from reactor* ***R6****. To calculate the rate of nitrification and denitrification, it was started from the concentrations of ammonia, nitrates and sulphates. From these concentration, known the volume of leachate present in each column, the mass of ammonia, nitrates and sulphates present from time to time in the columns was calculated. Then all the variations were calculated and from them the cumulative ones. Finally the ammonia removal rate and the nitrate removal rate were calculated. The rows of the table highlighted represent the calculation done in the injection day. The values in red indicate the mass values considered to perform the calculation of the autotrophic and heterotrofic denitrification percentages.*

REACTOR 6															
Days	**Column leachate (l)**	**N-NH_4^+ (mg/l)**	**N-NO_3^- (mg/l)**	**SO_4^{2-} (mg/l)**	**N-NH_4^+ (mg)**	**N-NO_3^- (mg)**	**SO_4^{2-} (mg)**	**ΔN-NH_4^+ (mg)**	**ΔN-NO_3^- (mg)**	**ΔSO_4^{2-} (mg)**	**ΔN-NH_4^+ cumulative (mg)**	**ΔN-NO_3^- cumulative (mg)**	**ΔSO_4^{2-} cumulative (mg)**	**Ammonia R_R (mgN-NH_4^+/d)**	**Nitrate R_R (mgN-NO_3^-/d)**
0	7,0	15,1	1222	1619	106	8554	11333	106	8554	11333	0	0	0	0,0	0,0
14	7,0	3,0	1165	1231	21	8155	8617	-85	-399	-2716	0	0	0	6,1	28,5
20	7,0	2,0	1127	1604	14	7886	11228	-7	-270	2611	0	0	2611	1,2	44,9
23	7,0	3,0	1106	1510	21	7742	10570	7	-144	-658	7	0	1953	-2,3	47,8
27	7,0	6,3	1136	1933	44	7952	13531	23	210	2961	30	210	4914	-5,8	-52,5
31	7,0	9,8	1170	1619	69	8190	11330	24	238	-2201	55	448	2713	-6,1	-59,5
35	7,0	7,3	1092	1649	51	7644	11540	-18	-546	210	37	0	2923	4,5	136,5
36	7,0	5,7	1113	1713	40	7788	11993	-11	144	453	26	144	3376	10,6	-143,5
37	6,9	4,2	1133	1778	29	7818	12268	-11	30	275	15	174	3651	11,0	-30,2
41	6,8	4,9	1047	1610	34	7120	10948	4	-698	-1320	20	0	2331	-1,1	174,5
43	6,7	5,6	959	1594	38	6424	10679	4	-696	-269	24	0	2062	-2,0	347,8
45	6,6	4,5	1091	1292	30	7201	8527	-8	777	-2152	16	777	0	3,9	-388,3
49	6,5	5,3	1387	967	34	9016	6286	5	1815	-2242	20	2592	0	-1,2	-453,7
55	6,4	4,1	1128	1486	26	7219	9510	-8	-1796	3225	12	795	3225	1,4	299,4
58	6,3	4,7	956	1353	30	6023	8524	3	-1196	-987	16	0	2238	-1,1	398,8
64	6,2	6,5	1367	1058	40	8475	6560	11	2453	-1964	26	2453	274	-1,8	-408,8
65	6,1	4,7	1332	1415	29	8125	8632	-12	-350	2072	15	2102	2346	11,6	350,2
70	6,0	3,8	992	1250	23	5952	7500	-6	-2173	-1132	9	0	1215	1,2	434,6
72	5,9	5,1	1073	990	30	6331	5841	7	379	-1659	16	379	0	-3,6	-189,4
76	5,8	2,9	1101	1474	17	6386	8549	-13	55	2708	3	434	2708	3,3	-13,8
78	5,7	5,1	1010	1338	29	5757	7627	12	-629	-923	15	0	1786	-6,1	314,4

83	5,6	6,1	1124	1737	34	6294	9727	5	537	2101	20	537	3886	-1,0	-107,5
86	5,5	5,1	1119	1729	28	6155	9510	-6	-140	-218	14	398	3669	2,0	46,6
90	5,4	8,1	1577	1762	44	8516	9515	16	2361	5	30	2759	3674	-3,9	-590,3
97	5,3	6,6	1100	1643	35	5830	8708	-9	-2686	-807	21	73	2867	1,3	383,7
100	5,2	5,6	1089	1967	29	5663	10228	-6	-167	1521	15	0	4387	2,0	55,7
104	5,1	5,2	1077	1532	27	5493	7813	-3	-170	-2415	13	0	1972	0,6	42,5
107	5,0	5,6	1053	1538	28	5265	7690	1	-228	-123	14	0	1849	-0,5	75,9
134	1,8	3,4	799	913	6	1438	1643	-22	-3827	-6047	0	0	0	0,8	141,7
139	1,7	3,6	823	972	6	1399	1652	0	-39	9	0	0	9	0,0	7,8
142	1,8	4,3	912	1231	8	1642	2216	2	243	563	2	243	572	-0,5	-80,8
146	1,7	4,5	850	1228	8	1445	2088	0	-197	-128	2	46	444	0,0	49,2
149	1,6	3,7	630	873	6	1008	1397	-2	-437	-691	0	0	0	0,6	145,7
153	1,5	3,7	873	885	6	1310	1328	0	302	-69	0	302	0	0,1	-75,4

Table 12 – *Autotrophic and heterotrophic denitrification percentages estimation from column* ***R6****. From the difference between the mass of nitrates, the nitrated removed was calculated, and from the difference between the mass of sulphates, the sulphates produced was calculated. From the nitrates removed the theoretical sulphates produced was calculated assuming a stoichiometric ratio between the produced sulphates and the reduced nitrate of 4.64 mg of* SO_4^{2-} *per mg of* $N\text{-}NO_3^-$ *reduced to* N_2*. The autotrophic fraction of the denitrification process was then calculated from the ratio between the sulphates produced and the theoretical sulphates produced; and from the autotrophic fraction, the heterotrophic fraction of the denitrification process was then calculated.*

	Nitrate removed (mg)	**Sulphate/Nitrate ratio**	**Theoretical sulphate produced**	**Sulphate produced (mg)**	**Autothrophic denitrification %**	**Heterothrophic denitrification %**
Experiment period	1766	4,64	8134	4387	54	46

Table 9 – *Stoichiometric nitrates produced estimation for column* ***R5*** *.From the mass of ammonia the mg of nitrates produced was calculated assuming a molecular weight ratio between nitrogen and ammonia of 1,29 and a stoichiometric ratio between the produced nitrates and the reduced ammonia of 0,98 mg of* NO_3^- *per mg of* $N\text{-}NH_4^+$ *reduced. Then the amount (mg) of nitrous-nitrogen produced was calculated assuming molecular weight ratio between nitrates and nitrogen of 0,23. Finally, from the ratio between the nitrous-nitrogen produced and the volume of liquid in the column was calculated the nitrous-nitrogen produced expressed in mg/l.*

REACTOR 5				
Days	**Column leachate (l)**	**Stoichiometric NO_3 produced (mg)**	**Stoichiometric $N\text{-}NO_3$ produced (mg)**	**Stoichiometric $N\text{-}NO_3$ produced (mg/l)**
0	7,5	62	14	2
14	7,5	99	22	3
20	7,5	99	22	3
23	7,5	210	47	6
27	7,5	163	37	5
31	7,4	73	16	2
35	7,3	32	7	1
36	8,3	3608	815	98
37	8,2	10553	2383	291
41	8,1	5788	1307	161
43	8,0	4504	1017	127
45	7,9	1507	340	43
49	7,8	78	18	2
55	7,7	35	8	1
58	7,6	34	8	1
64	7,5	54	12	2
65	8,5	4648	1050	123
70	8,4	7716	1742	207
72	8,3	5992	1353	163
76	8,2	496	112	14
78	8,1	245	55	7
83	8,0	100	23	3
86	7,9	63	14	2
90	7,8	83	19	2
97	7,7	88	20	3
100	7,6	112	25	3
104	7,7	59	13	2
107	7,6	61	14	2
134	7,5	65	15	2
139	7,4	38	9	1
142	1,5	15	3	2
146	1,5	13	3	2
149	1,5	9	2	1
153	1,4	8	2	1