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CLEANLEACH

Treatment of nursery leachates by combining slow sand filtration and horizontal subsurface flow constructed wetlands

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Project Coordinator: Institut de Recerca i Tecnologia Agroalimentàries (IRTA)

D3.2. Performance checking and monitoring of the replicate in the Spanish Nursery

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1. Introduction

The purpose of the works performed after the construction of the Sala Graupera replicate (nursery located in Sant Andreu de Llavaneres, Maresme county, Catalonia-Spain) that was described in the Deliverable D3.1 were:

- To analyze the technical management of the nursery (irrigation schedule, fertilization practices) that would determine the performance of the CLEANLEACH system
- To identify possible troubles regarding the initial design of the replicate
- To amend the possible mentioned troubles
- To monitor the performance of the system, once the modifications have been carried out
- To optimize the setup of the system, considering not only the case study of the Sala Graupera nursery management, but also other possible conditions held in other nurseries.
- To measure the greenhouse gases emissions of the two techniques of the CLEANLEACH system (slow sand filter and constructed wetland)
- To perform a microbiological assessment of the mentioned techniques



2. Definitive replicate

2.1 Description of the specific conditions of the Sala Graupera nursery

Plants grown in this nursery are watered using micro sprinklers, being the scheduled frequency low because of the typology of the cultivated species. With this purpose, a reservoir was installed to collect leachates from the container area. A hydraulic low-pressure pump was installed in the tank. The mentioned pump was plugged into a timer to control the constructed wetland inflow. In this way, the inflow was discontinuous but the frequency of the influent entrance was enough -1 impulse each two hours- to achieve a semi-continuous flow.

The fertilization management of the nursery plants consists of the use of controlled release fertilizers (Osmocote Plus[®]) at a dose of $2 \text{ kg} \cdot \text{m}^{-3}$. When compared to other fertilization practices in the area, this dosage is relatively low because of the low nutrient requirements of the cultivated plants in Sala Graupera nursery. This is the reason why the measured leachate nutrient content, and particularly the nitrate concentration, was relatively low (below $100 \text{ mg NO}_3^- \cdot \text{L}^{-1}$).



Figure 1. Outdoor cultivated area where the horizontal sand filter was installed (under the *hors-sol* mat). Plants grown in containers have been distributed over the sand filter and micro sprinklers were also installed.

2.2 Troubles of the designed replicate and its performance. Modifications carried out.

2.2.1 Modification of the slope of the constructed wetland (CW)

The first action undertaken was the modification of the level of the wetland. Initially, the wetland level was the same than the corresponding to the aisle adjacent to the constructed wetland (CW), which was much higher than the projected slope (5‰) (Figure 2). For this reason, the water level in the constructed wetland was below 40 cm. In other words, water flowed through the wetland but the wetland lacked the required subsurface water level (> 40

cm), which is necessary to achieve anaerobic conditions to allow the action of denitrifying microorganisms. In addition, the macrophyte plants installed in the constructed wetland could not grow up because of the water unavailability. These are the reasons why on 4 September 2014 we reduced the tilt to achieve a soft slope (5%), more suitable to achieve a flat water level and to allow plant grow (Figure 3). Macrophytes had to be re-planted.

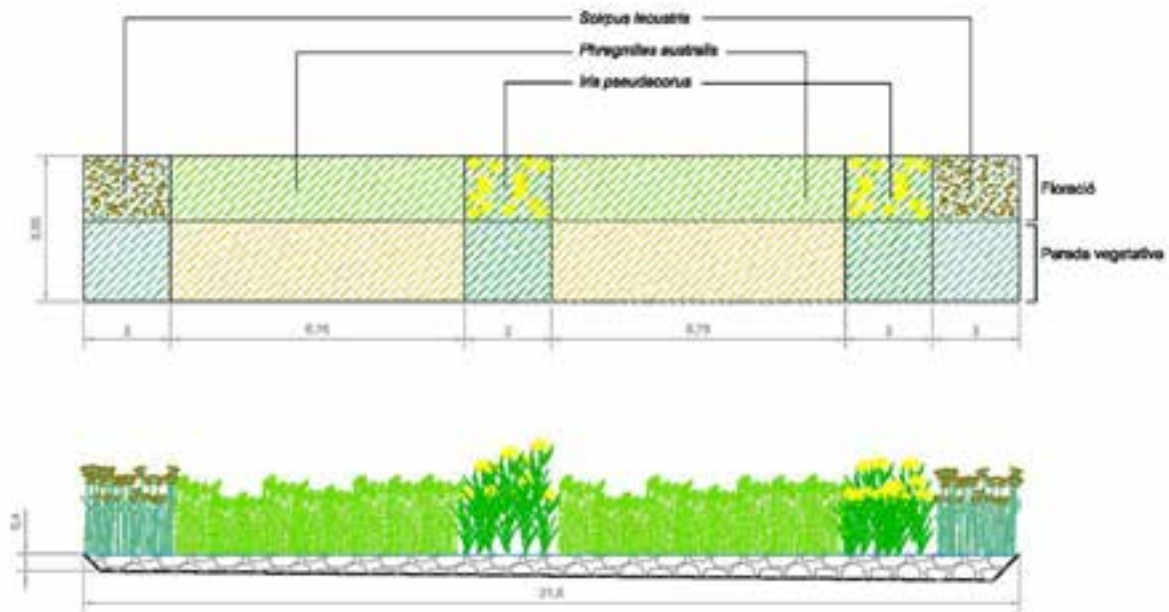


Figure 2. View of the constructed wetland before the slope modification; the scarce development of macrophyte plants could be checked



Figure 3. View of the constructed wetland after the lift modification

2.2.2 Low nitrate concentration in the inflow. Nitrate addition to the leachates (reservoir).

From 16/09/2014 onwards, the constructed wetland (inflow and outflow) was monitored for the following parameters: pH, electrical conductivity (EC), nitrate concentration (NO_3^-) and chemical oxygen demand (COD). As stated above, the initial nitrate concentration in the inflow (leachates) was low (up to 100 mg L^{-1}). In order to achieve a nitrate higher level in the inflow, and thus to assess the denitrifying potential of the full-scale constructed wetland, diluted nitrate salts (80% KNO_3 and 20% NH_4NO_3) were poured into the reservoir (from 13/11/2014 onwards).

2.2.3 Carbon source tank installation

Normally, nursery leachates are high in nitrates, but the organic matter content (measured in terms of COD, for instance) is low. Therefore, denitrification process should be enhanced by the addition of an appropriate carbon source with easily degradable carbohydrates.

The effluent from brewery industry which was used in experiments carried out at IRTA (see Deliverable D2.2) was also used in the full-scale replicate. This effluent needs to be diluted in water before its application.

This is the reason why a 200-L tank was installed. This piece of equipment was raised, 2 meters above the wetland level using a stainless structure, and was placed in the inlet area of the constructed wetland (Figure 4). A pipe connected the mentioned tank to the mixing chamber, where leachates and diluted carbon source were mixed. A motorized valve controlled the amount of the diluted carbon to be poured into the mixing chamber (Figure 4 and Figure 5).

On 27th of November of 2014 we started the addition of the carbon source to the system.



Figure 4. 200-L carbon source tank and motorized valve to dose the diluted carbon source



Figure 5. Mixing chamber, where leachates and diluted carbon source (CS) are mixed

2.2.4 Improvement of the inflow characteristics of the constructed wetland

Analytical results regarding nitrate concentration in the inflow (early December 2014-January 2015) showed that the influent composition was not homogenized enough. In order to address this issue a low-power pump was installed in the leachate tank to homogenize the CW influent concentration (Figure 4).

2.2.5 Increasing the water depth of the subsurface constructed wetland.

After having poured the appropriate carbon source to the leachate stream, the analytical results showed that the denitrification was not achieved, yet. This lead us to analyze the conditions of the CW; we realized that the water level achieved in the constructed wetland was below 40 cm. In order to address this issue, at the end of February 2015 the constructed wetland had to be modified, once again (Figure 6, 7 and 8). Then, macrophytes had to be replanted (Figure 9). This was the definitive plantation because from this moment onwards, plants grew correctly (Figure 10).



Figure 6. Modification of the constructed wetland to achieve a 40-cm water level



Figure 7. View of the outlet chamber of the constructed wetland (CW). To a certain extent, showed pipe could raise the water level of the CW



Figure 8. View of the constructed wetland, once the modification of the wetland to achieve 40-cm depth was performed



Figure 9. View of the constructed wetland, where finally macrophyte plants were growing up in optimal conditions

2.2.6 Solar panel

In order to achieve the electric energy to run the two mentioned pumps and the motorized valve, a solar panel –and all items required to run this panel- were installed on 19th March 2015 (see Deliverable D2.3). The items were: solar collector, the converter (continuous to alternate) and battery (Figure 4). The installation of the solar panel avoids the connection of the system to the electric grid.



Figure 10. View of the constructed wetland, once the modifications were carried out and plants developed in optimal environmental conditions

3. Monitoring of the performance of the constructed wetland

Figures show the time course of the following parameters: pH, EC, NO_3^- , and COD (Figures 11, 12, 13 and 14). These parameters were determined in both, influent and effluent.

3.1 Denitrification monitoring: nitrate and COD in the influent and effluent

Regarding the nitrate content, two stages could be distinguished. The first one corresponds to the period from October 2014 to early April 2015. This period corresponds to the different modifications carried out that have been stated in the section 2.2. During this phase, the nitrate content in the influent and effluent fluctuated considerably. These ups and downs were due to the lack of homogenization of the diluted nitrates in the influent. But this issue was correctly addressed after having installed a small pump that would have facilitated the mixture of the liquid contained in the leachate tank (see section 2.2.4).

Moreover, results show that, after the carbon source application, denitrification does not occur. This was because anaerobic conditions were not reached yet (see section 2.2.5).

However, after the two issues were addressed (homogenization of the liquid in the leachate reservoir and the achievement of the targeted water level), the denitrification process was triggered (Figure 11).

From early April 2015 to the end of monitored period (13/10/2015) a complete denitrification was achieved, being the removal rates close to 100%.

Two C/N ratios were tested: 1.45 (initial months) and 1.14 (from June 2015 onwards). In both cases, the COD of the effluent was close to zero and, as stated above, a high denitrification rate was achieved (Figure 12).

3.2 Monitoring of pH and electrical conductivity (EC) in the influent and effluent

pH of the effluent was lower than the pH of the influent due to the denitrification process held in the wetland, being the pH of the effluent close to 7.5 (Figure 13). This pH could favor the phosphorous precipitation within the constructed wetland, as it will be showed later on.

During the period in which constructed wetland were monitored, except for the period in which the increase of nitrates in the effluent was linked to the rise in the EC, the EC of the influent and effluent trend series were parallel (Figure 14).

Results achieved show that optimal design and operation are needed to achieve optimal removal denitrification rates of the leachates. It should be highlighted that results were achieved using as carbon source a liquid waste from the brewery industry.

Therefore, the results obtained at intermediate pilot scale have been confirmed at full-scale operation of the CLEANLEACH system (Deliverable 2.2).

Figure 11. Time course of the nitrate content in the inflow and the outflow of the constructed wetland

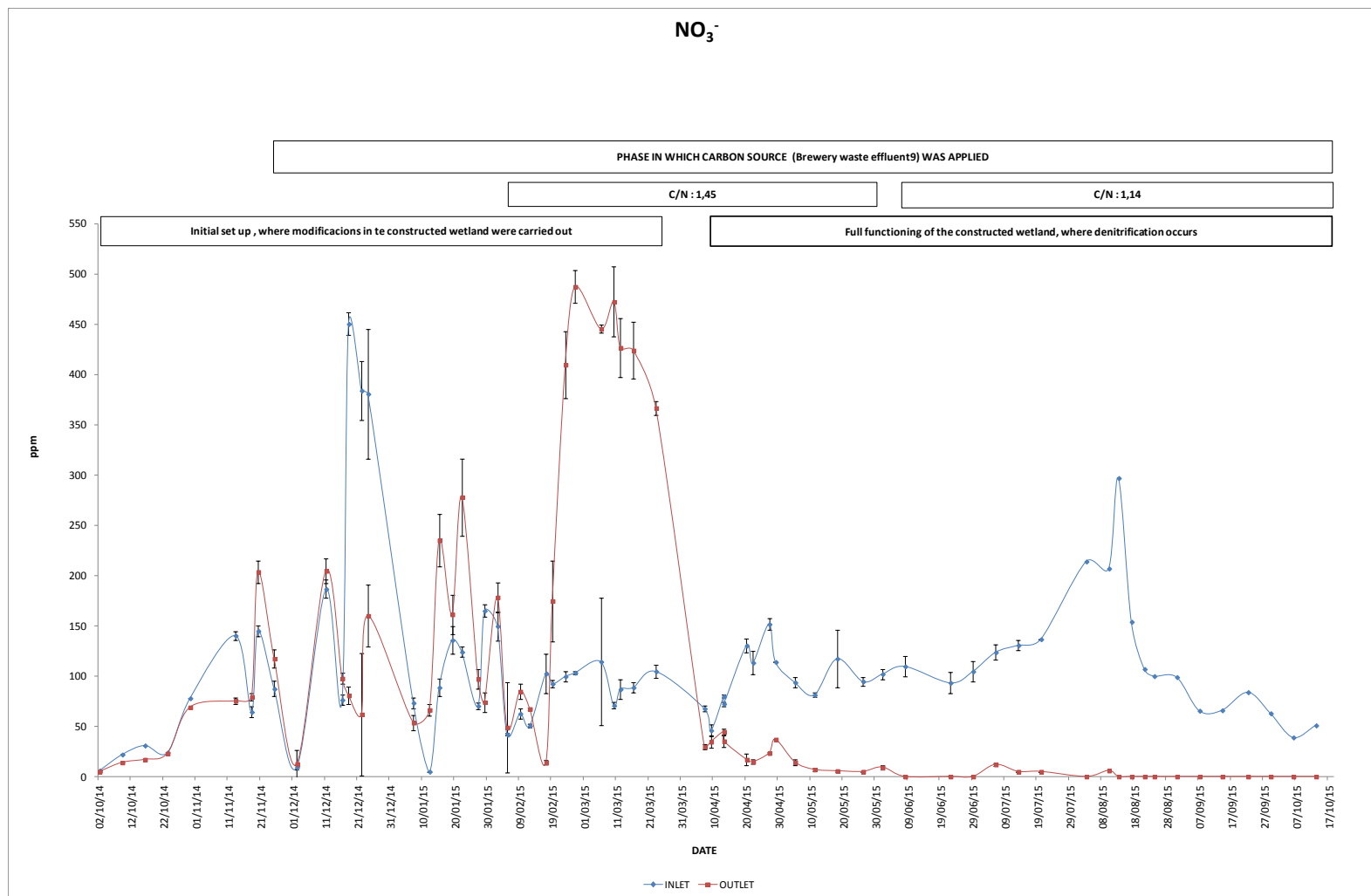


Figure 12. Time course of the chemical oxygen demand (COD) in the inflow and the outflow of the constructed wetland

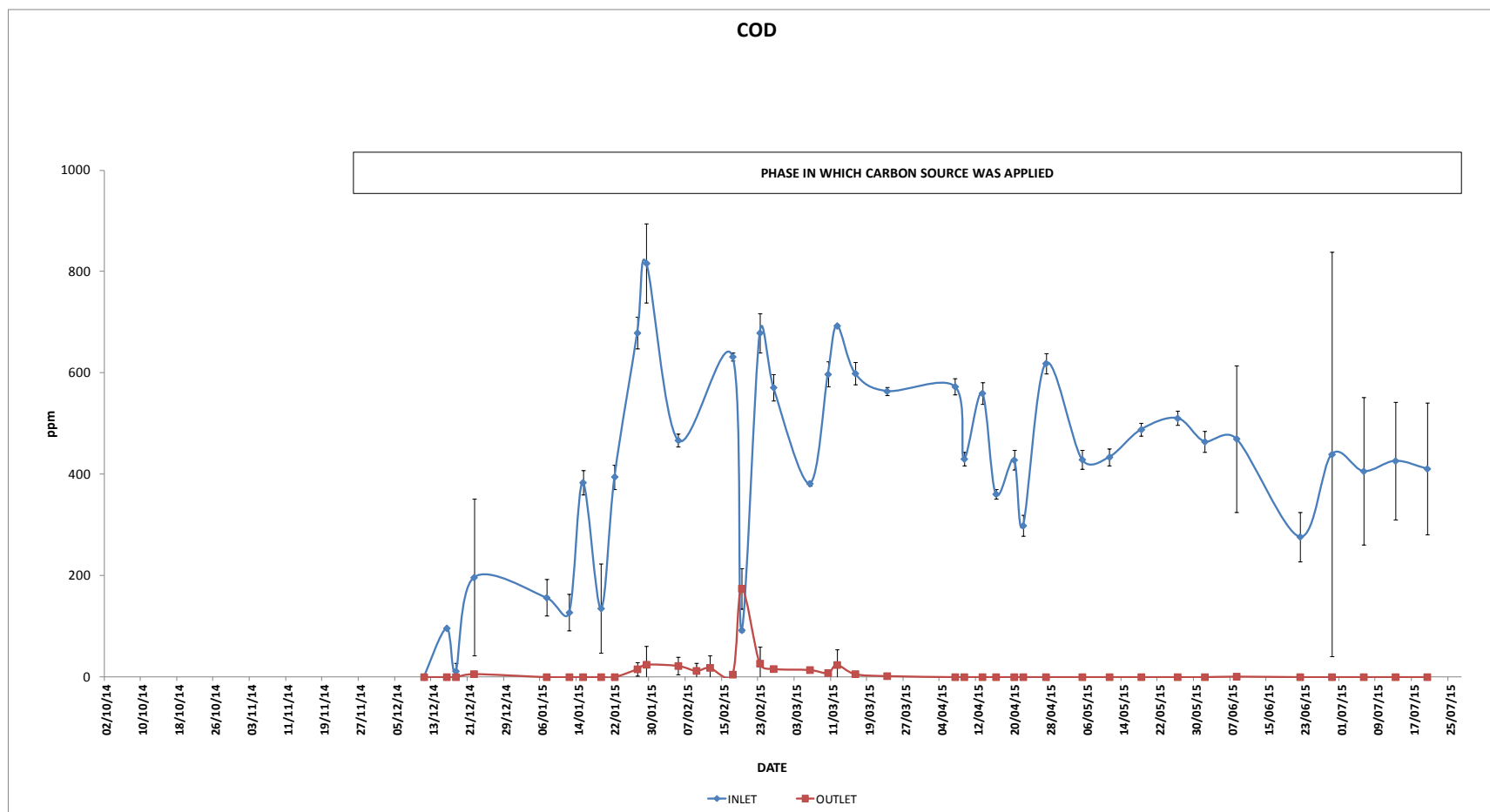


Figure 13. Time course of the pH in the inflow and the outflow of the constructed wetland

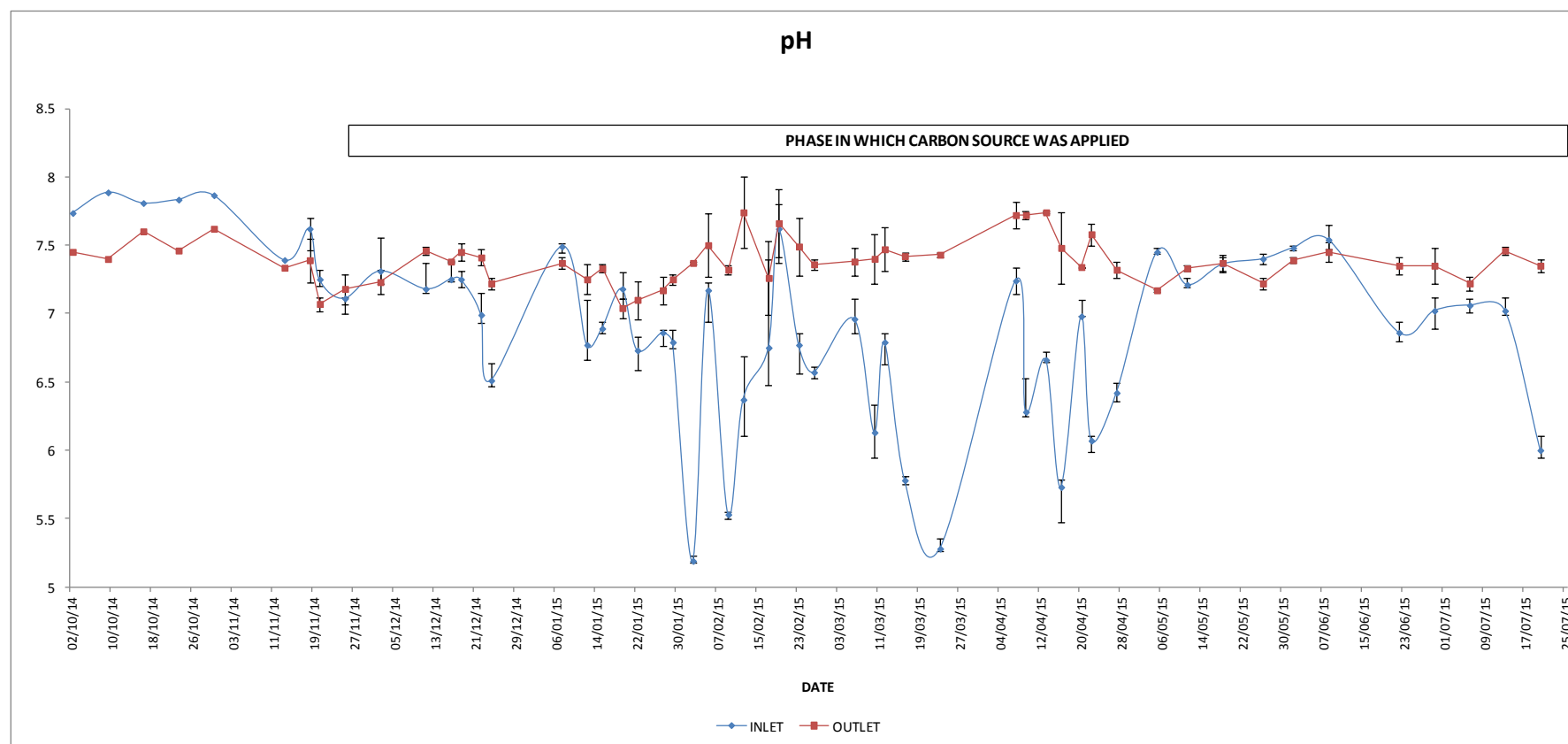
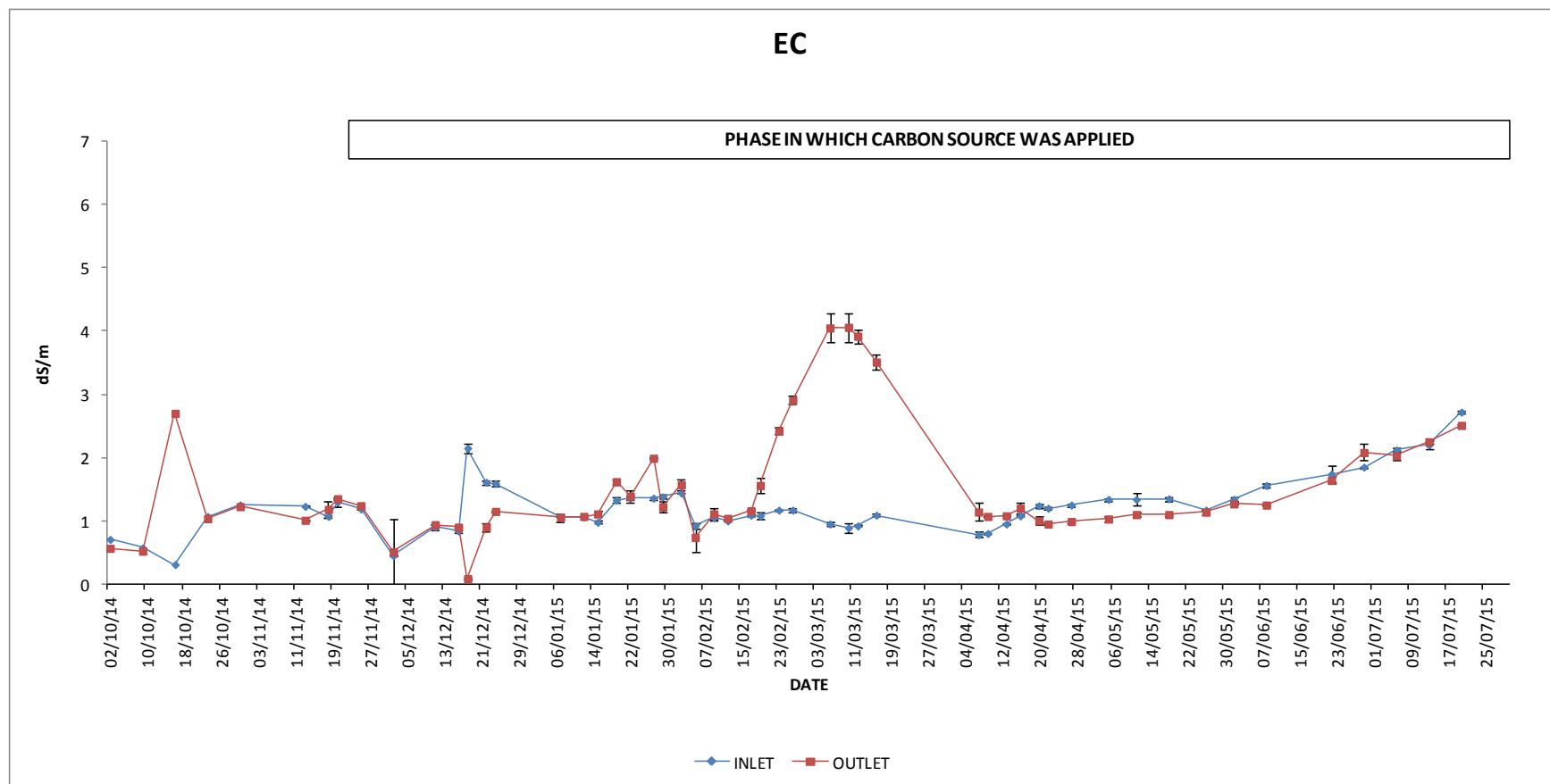


Figure 14. Time course of the electrical conductivity (EC) in the inflow and the outflow of the constructed wetland



3.3 Phosphorous removal

The soluble forms of P measured in the influent were low because of the fertilization practices described above (see section 2.1) (Table 1). The phosphorous measured in the effluent was low enough to consider that the elimination rate of the constructed wetland was close to 100%. This high removal rate should be attributed to both, the mentioned pH slightly alkaline and the nutrient uptake carried out by macrophytes (Table 1 and Figure 15).

Table 1. Soluble phosphorous content ($\text{mg}\cdot\text{L}^{-1}$) in the influent and in the effluent during the monitored period

	Inflow		Outflow		% removal
	P	P	P	P	
Data	mean	error	mean	error	
18/11/2014	0.01	0.00	0.01	0.00	
12/01/2015	0.01	0.00	0.01	0.00	
12/03/2015	2.2	0.14	0.01	0	99.55
15/04/2015	1.67	0.08	0.01	0.00	99.40
08/06/2015	1.90	0.37	0.04	0.07	97.89
06/07/2015	4.17	0.08	0.01	0.00	99.76

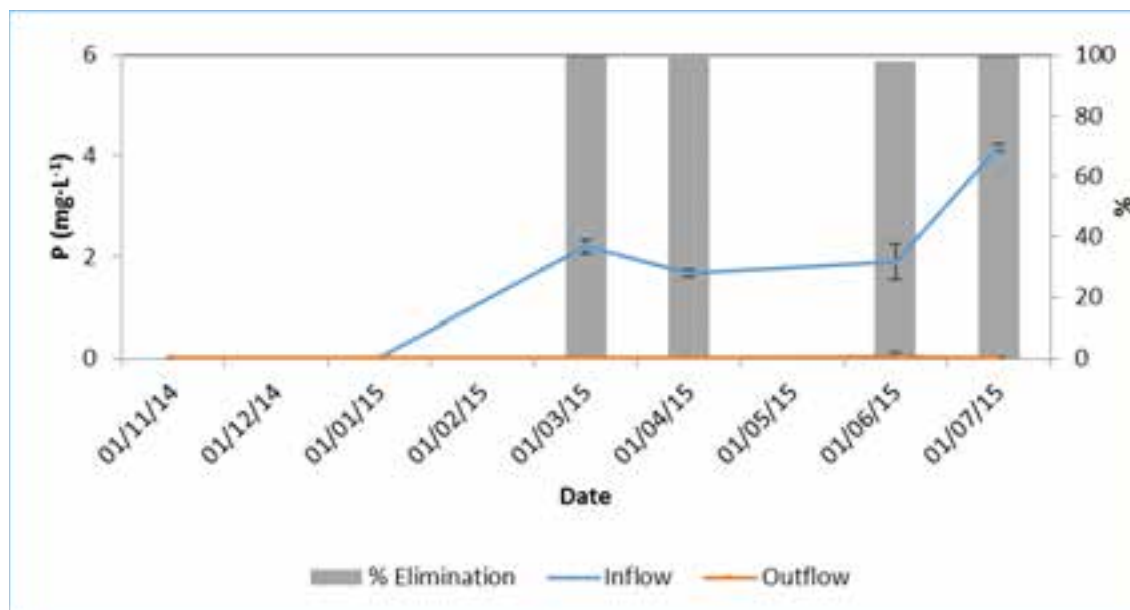


Figure 15. Phosphorous concentration in the influent and effluent of the constructed wetland and the corresponding removal rate (%)

4. Characterization of the system (horizontal sand filter and constructed wetlands). Monitoring of greenhouse gases emission and microbiological assessment.

This section aims at gaining insight in better understand microbial community dynamics that occurs in the bed of the constructed wetland (water inflows/outflows and gravels) and slow sand filter (SSF) (water inflows/outflows and sand material in different zones), trying to link denitrification and potential greenhouse gases emission (GHG) processes to the predominance of certain operational conditions, microbial populations and the abundance of functional genes (Task 3.1.3). A DNA-based diversified approach by qPCR of 16S rRNA gene (total Eubacteria), ITS1 rRNA region (Fungi) and functional genes linked to denitrification process (nosZ genes) was performed.

Spanish commercial-scale prototype at Sant Andreu de Llavaneres (Sala Graupera nursery), containing (SSF and CW unit) has been improved and characterised through: i) setting up the container crop/equipments; ii) defining the sampling in different conditions: irrigation with water in September/October 2014 (irrigation with well water (SSF), nor carbon source amendment in the CW), well water irrigation (SSF) and carbon source amendment in CW in February 2015 (T0) without successful denitrification; and well water irrigation (SSF) and additional carbon source with the occurrence of a successful denitrification process in the CW (June 2015). A detailed list of the sampling events for GHG and microbiological assessment is presented in table 2.

Table 2. List of assays and sampling events linked to deliverable 3.2 for GHG and microbiological assessment

Description	Date	Time event	Task	Microbiology assessment	GHG assessment
Commercial Plant CW and SSF (Sant Andreu de Llavaneres): Well water irrigation in the SSF, without carbon source in CW	09/26/2014	T-1	3.1.3		X ¹
Commercial Plant CW and SSF (Sant Andreu de Llavaneres): Well water Irrigation (SSF) + Brewery waste in CW (no denitrification occurred)	02/26/2015	T0	3.1.3	X	X
Commercial Plant CW and SSF (Sant Andreu de Llavaneres): Well water irrigation (SSF)+ Brewery waste in CW (good denitrification process)	06/15/2015	T1	3.1.3	X	X

4.1 Microbial community assessment in Slow Sand Filter system and Constructed Wetland

A global quantitative view of the microbial community dynamics (total eubacteria, fungi and potential denitrifiers) throughout the period t0 (February 2015)-t1 (June 2015) in SSF and CW is shown in Figure 16, Figure 17 and Tables A1-A2 (Annex).

In both periods (Feb-June 2015), SSF sand samples (Figure 16), contained higher eubacterial and fungal population (10^7 16S rRNA gene copies \cdot g⁻¹ sand; 10^5 - 10^7 ITS1 rRNA gene copies \cdot g⁻¹ sand, respectively) than water inflows (irrigation water) (10^5 16S rRNA and 10^3 ITS1 rRNA gene copies \cdot mL⁻¹ water) and outflows (leachates generated by plants) (10^5 - 10^6 16S rRNA and 10^3 - 10^4 ITS1 rRNA gene copies \cdot mL⁻¹ water). Even though, it was not possible to determine the efficiency of biomass retention capacity in SSF, both due to the low abundance of fungal biomass in the inflow water in T0 and T1 (10^3 – 0 ITS1 rRNA gene copies respectively).

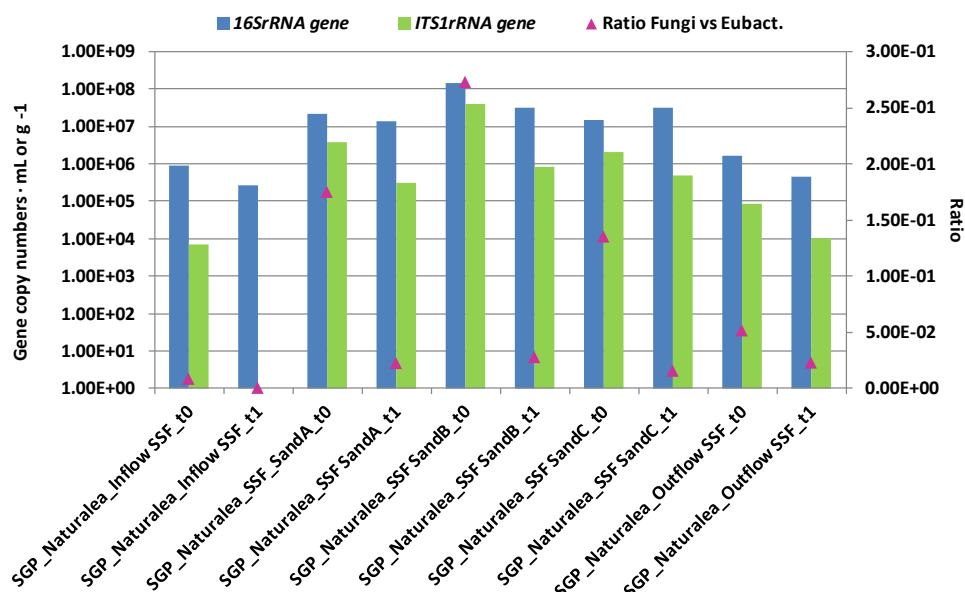


Figure 16. Quantification of total *16S rRNA* (total eubacteria) and *ITS1 rRNA* (*Fungi*) genes from Slow Sand Filter (SF) samples at t0 (Feb-2015) and t1 (Sept-2015) periods

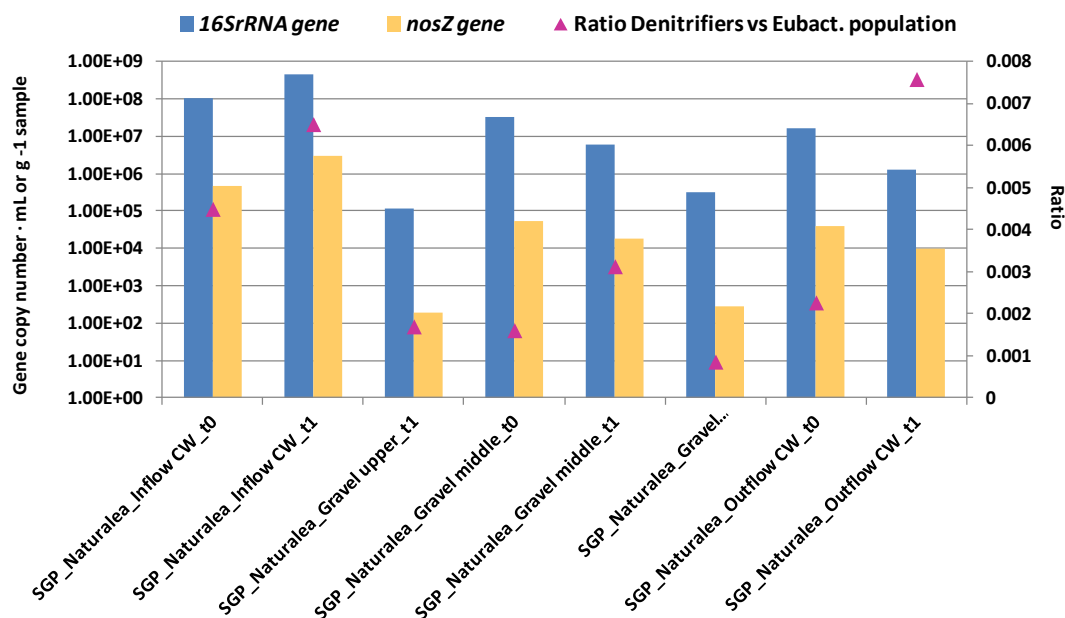


Figure 17. Quantification of total eubacterial population (*16S rRNA* gene) and potential denitrifying populations (*nosZ* gene) in Constructed Wetland (CW) samples at t0 (Feb-2015) and t1 (June-2015) periods

Fungal populations detected in the SSF outflow were low (10^4 *ITS1 rRNA* gene copies · mL⁻¹), in the same range to that described on the SSF pilot plant settled up in Cabrils (see Deliverable D2.1); indeed, it is noteworthy that Cabrils pilot plant has been running during 15 years, whereas



Sala Graupera prototype is newly established. These results confirm that SSF system could operate properly for a long period of time at field scale (15 years).

In figure 17, is remarkable that CW inflow were even enriched by denitrifiers due to the addition of brewery waste as a carbon source (T0 and T1). With DNA-based analysis is possible to see the potential of the microbial population contained at the system as denitrifying microbial community; but, it is not feasible to correlate with the real activity. In fact, denitrifying populations at t0 (February-15) was highly present (10^5 *nosZ* gene copies \cdot mL⁻¹), but denitrifying process did not occur. However at period t1 (June-15) denitrification process was properly established being total denitrifiers one magnitude order below respect t0. Such results revealed that microbial populations in the CW harbor denitrifying potential but needs the optimal operational conditions to activate the process.

Combined RNA and DNA-base studies are necessary to properly study the potential and the activity on denitrifying microbial communities harboured in the gravel biofilm from CW.

4.2 Monitoring greenhouse gases emission rates in SSF and CW in the Spanish replicate (Sala Graupera nursery, Sant Andreu de Llavaneres, Barcelona-Catalonia) under different operational conditions

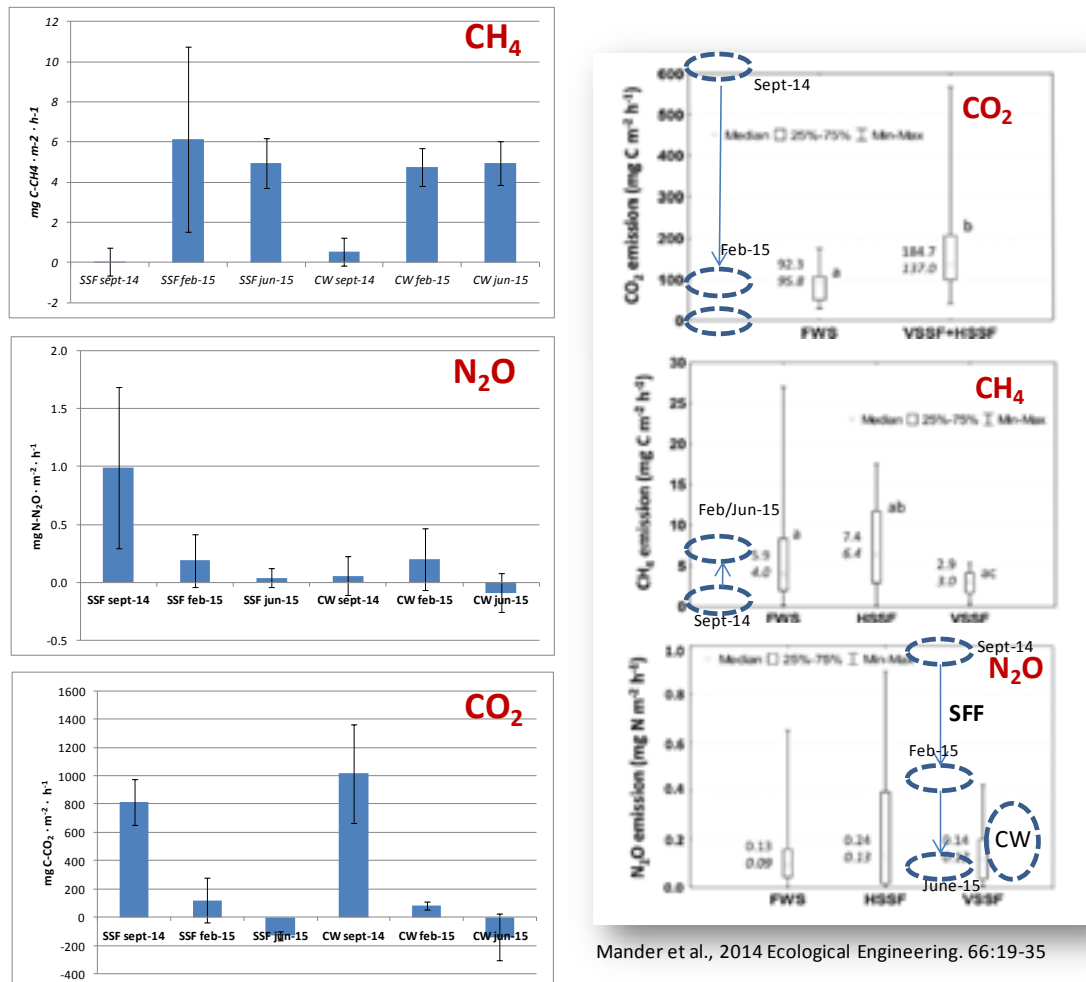
One of the main objectives of the project is to market a zero-emissions system, therefore, it is necessary to verify the emissions, mainly of greenhouse gases, and make the corresponding corrections to achieve the zero-emissions goal. Hence, measurements of emissions of greenhouse gases (CH₄ and N₂O) have been conducted in the constructed wetlands and SSF operated under field conditions.

Gas samples were collected in Tedlar bags from an aerated Lindvall hood, and quickly transported to the lab for the determination of CH₄ and N₂O by gas chromatography (GC-FID for CH₄, and GC-ECD for N₂O and CO₂) (Figure 18).



Figure 18. Lindvall hood system set-up to collect gas-samples from SSF (left) and CW (right) systems at Sala Graupera facilities

A summary of GHG emission rates of CW and SSF systems at Sala Graupera (Spanish prototype) are shown in Figure 19 and Table 3.



Mander et al., 2014 Ecological Engineering, 66:19-35

Figure 19. GHG emission rates of SSF (HSF) and CW systems in Sant Andreu de Llavaneres (Sala Graupera nursery) in the period Sept-2014- June 2015. The results are globally within median levels described in the review carried out by Mander et al., 2014 (right column).

Methane, Nitrous oxide and CO₂ emission rates in CW and SSF were in the low range (Table 3) compared with GHG emission rate described in other CW wastewater treatment plants described in Mander et al., 2014. This paper is a review of 158 papers published in SCI journals from 1994-2013.

Methane and CO₂ emission rates were slightly lower than those measured at Cabrilis (Deliverable D2.1).

Methane measured in CW was within 0.55-4.95 mg C-CH₄ · m⁻² · h⁻¹, N₂O emission rate was also low in the range of 0-0,2 mg N-N₂O · m⁻² · h⁻¹, and higher in SSF (0,04-0,99 mg N-N₂O · m⁻² · h⁻¹). Regarding CO₂ emission rate, this rate was clearly higher at the initial conditions (sept-14) (with values of 813-1015 mg C-CO₂ · m⁻² · h⁻¹ in SSF and CW respectively) respect to the other sampling events.

Table 3. GHG emission rates in CW and SSF systems at Sala Graupera nursery during the period Sept-14-June-15. Data are presented as: $\text{mg C-CH}_4 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$; $\text{mg C-CO}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$; $\text{mg N-N}_2\text{O} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$.

	C-CH₄	N-N₂O	C-CO₂	C-CH₄ desv	C-N₂O desv	C-CO₂ desv
SSF sept-14	0.047	0.990	813.483	0.713	0.699	159.483
SSF feb-15	6.137	0.187	121.230	4.623	0.231	155.218
SSF jun-15	4.950	0.036	-131.249	1.237	0.082	30.215
CW sept-14	0.557	0.053	1015.390	0.696	0.168	348.985
CW feb-15	4.760	0.200	79.627	0.950	0.269	28.113
CW jun-15	4.950	-0.090	-139.498	1.072	0.164	163.816

It is worth mentioning that, although higher C-CH₄ emission were detected under nutrient and carbon supplementation Feb/June-15, negative-close to zero emission values of CO₂ were detected in Feb/June-15 in both systems (-131/-139 $\text{mg C-CO}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). Globally, methane, N₂O and CO₂ emission range were still located in the normal range described for other CW systems (Mander et al., 2015).

5. Conclusions

As a result of the constructive modifications carried out in the constructed wetland (CW) and the key monitored parameters, we conclude that:

- It is essential to check the correct leveling of the CW bed. The slope must not be higher than 5 ‰ in order to ensure a certain water depth along the bed. The initial project design should consider this key point.
- In order to ensure the correct operation management of the constructed wetland, anaerobic conditions in the bed are necessary so as to facilitate the denitrifiers action. This would be achieved if the depth of the water level is, at least, 40 cm.
- Intermittent inflow should be reduced as much as possible; however, a good performance has been demonstrated when one inflow pulse is scheduled each two hours.
- The carbon source could be added using a motorized valve synchronized with the leachate entrance to the system. The mixture of the carbon source plus the leachate could be done in a mixing chamber placed in the inlet area of the constructed wetland.
- The feasibility of using brewery effluent for enhancing the denitrification process in the full-scale constructed wetland has been demonstrated. A C/N ratio interval 1.14-1.4 is suitable but other operation conditions (e.g. flow conditions and nitrate in the inflow) should be taken into account.
- In the operating conditions of nursery, where the leachate phosphorous concentration is low, a high removal rate of soluble forms of phosphates has been achieved.
- The macrophytes that were planted in the constructed wetland (winter –early spring season) were suitable and developed correctly through the surface of the wetland.
- The horizontal sand filter performance during the monitoring period was appropriate, as it has also been checked at pilot plant scale (see Deliverable D2.2, section 5).
- The sizing of the constructed wetland in relation to the container area is appropriate. Further studies should be conducted to check the feasibility to reduce the surface devoted to the leachate depuration process, keeping the nitrate and phosphorous removal rates achieved.

Regarding GHG emission measurements and the microbiological approach, we conclude that:

- According to the measures carried out, both techniques (SSF and CW) implemented in the Sala Graupera nursery release low amount of greenhouse gases.
- Sand from slow sand filter contains higher amount of eubacterial and fungus population than the irrigation water (inflow) and the discharged leachates (outflow).
- Denitrifying potential has been increased when leachates (CW inflow) are mixed with the carbon source (brewery waste).
- The gravels of the CW installed in Sala Graupera nursery harbour denitrifying populations but needs the optimal operational conditions to trigger the process.
- In general terms, the obtained results on gas emissions and microbial communities are in line with the results obtained at the pilot plant (see Deliverable D2.1).

6. References

Mander, Ü; Dotro, G., Ebie, Y., Towprayoon, S., Chiemchaisri, C., Nogueira, S.F., Jamsranjav, B., Kasak, K., Truu, J., Tournebize, J., Mitsch W.J. (2014). Greenhouse gas emission in constructed wetlands for wastewater treatment: A review. *Ecological Engineering* 66: 19-35.

7. Annex I

Table A1. Quantitative evolution of total microbial populations (total eubacteria (16S rRNA gene) and denitrifiers (*nosZ* gene) expressed as gene copies/mL or gene copies/g in CW system. T0 represents Feb-15; T1 represents June-15.

	16S rRNA gene	<i>nosZ</i> gene	ratio
SGP_Naturalea_Inflow CW_t0	1,03E+08	4,63E+05	0,004495
SGP_Naturalea_Inflow CW_t1	4,61E+08	3,00E+06	0,00651
SGP_Naturalea_Gravel upper_t1	1,15E+05	1,96E+02	0,001699
SGP_Naturalea_Gravel middle_t0	3,32E+07	5,33E+04	0,001608
SGP_Naturalea_Gravel middle_t1	5,83E+06	1,82E+04	0,003129
SGP_Naturalea_Gravel bottom_t1	3,29E+05	2,83E+02	0,000861
SGP_Naturalea_Outflow CW_t0	1,68E+07	3,82E+04	0,002269
SGP_Naturalea_Outflow CW_t1	1,30E+06	9,83E+03	0,007582

Table A2. Quantitative evolution of total microbial populations (eubacteria (16S rRNA gene) and fungi (ITS1 region) in SSF system at Sala Graupera. T0 represents Feb-2015; T1 represents June-2015.

	16S rRNA gene	ITS1 rRNA	<i>nosZ</i> gene	Ratio ITS/16S
SGP_Naturalea_Inflow SSF_t0	8,83E+05	7,09E+03	1,59E+02	8,03E-03
SGP_Naturalea_Inflow SSF_t1	2,58E+05	0,00E+00	1,22E+03	0,00E+00
SGP_Naturalea_SSF_SandA_t0	2,10E+07	3,66E+06	2,66E+04	1,75E-01
SGP_Naturalea_SSF SandA_t1	1,37E+07	3,02E+05	3,58E+03	2,21E-02
SGP_Naturalea_SSF SandB_t0	1,49E+08	4,05E+07	2,26E+05	2,73E-01
SGP_Naturalea_SSF SandB_t1	3,14E+07	8,62E+05	1,39E+05	2,74E-02
SGP_Naturalea_SSF SandC_t0	1,48E+07	2,00E+06	2,22E+04	1,35E-01
SGP_Naturalea_SSF SandC_t1	3,17E+07	4,83E+05	5,92E+04	1,52E-02
SGP_Naturalea_Outflow SSF_t0	1,68E+06	8,59E+04	1,10E+03	5,12E-02
SGP_Naturalea_Outflow SSF_t1	4,66E+05	1,05E+04	1,51E+03	2,25E-02