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Effects of hydraulic residence time in experimental constructed wetlands on wastewater treatment of a fish factory

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Abstract: The search for economically viable treatments for the effective removal of pollutants from industrial outflows has led to the study of constructed wetlands. In this work, we studied the outflows of a fish processing plant using subsurface flow wetlands scale models. The purifying capacity of two systems with hydraulic residence times of 10 and 38 hours was evaluated. A significant removal of contaminants in the outflow was observed in both systems, taking into account total suspended solids and organic matter load estimated as biological oxygen demand, chemical oxygen demand and suspended organic matter. The system with higher hydraulic residence times was more efficient in removing total suspended solids and organic matter. For both systems there was a decrease in nutrient, however the concentration difference between the input and the output was not significant. Our results corroborate that subsurface flow of constructed wetlands are efficient biotechnological tools which could be additionally used for improvement of traditional wastewater treatment of industrial outflows.

Key words: *Typha*, purifying capacity, industrial outflows, efficient removal

Resumen: Efecto del tiempo de residencia hidráulica en humedales experimentales construidos para el tratamiento de efluentes de una planta de procesamiento de pescado. La búsqueda de tratamientos económicamente viables para la remoción de contaminantes en efluentes industriales ha llevado al estudio de los humedales construidos. En este trabajo se trataron los efluentes de una planta de procesamiento de pescado mediante modelos a escala de humedales de flujo sub-superficial. Se evaluó la capacidad de purificación de dos sistemas con tiempos de residencia hidráulicos de 10 y 38 horas. Se observó una remoción significativa de contaminantes en el efluente en ambos sistemas, en función de sólidos suspendidos totales y materia orgánica tomada como demanda biológica de oxígeno, demanda química de oxígeno y materia orgánica en suspensión. El sistema con tiempos de residencia hidráulico superior fue más eficiente en la remoción de los sólidos suspendidos totales y materia orgánica. Si bien se observó remoción de nutrientes en ambos sistemas, esta no fue significativa. Nuestros resultados corroboran que los humedales construidos de flujo sub-superficial son herramientas biotecnológicas eficientes para el tratamiento de efluentes industriales.

Palabras clave: *Typha*, capacidad purificadora, efluentes industriales, remoción eficiente

Introduction

The explosive growth of human population in recent decades has impacted cities, resulting in several problems affecting human quality of life. One of the most important problems is the increased production of waste which pollutes the environment, being the most relevant the discharge of urban and industrial outflows into water courses. The solutions applied to management schemes based on the mechanical functioning of freshwater ecosystems have been considered insufficient to achieve the sustainability of the resources (Zalewski, 2002). This fact has promoted the search for more efficient and economically practical alternatives.

The use of wetlands is one of these alternatives, probably being the best example of the application of ecological engineering systems (Kangas 2005). Outflows treated with these systems reduce energy consumption and improve the ability to remove pollutants. Wetlands are areas where water is the main factor controlling the environment. They determine hypoxia or anoxia characteristics in soil, promoting biogeochemical processes as they maximize the water- sediment interface. These systems behave as accumulators of dead organic matter and are characterized by a highly primary productivity (Mitsch & Gosselink, 1993).

Wetlands are capable of removing decantable and dissolved contaminants from water (Ramsar, 1971). Obstructions (macrophyte, roots and substrate), reduce the water speed, leading to the settlement of suspended solids (consisting mainly of organic compounds). These are kept in the systems, improving degradation. Macrophytes play a important rol in relation to treatment of wastewater (Roldan, 2002), according to Brix (1997) they stabilize the surface of the beds, provide good conditions for physical filtration, prevent vertical flow systems from clogging, and provide a huge surface area for attached microbial growth. The most important nutrient removal processes taking place in these environments are: adsorption (coprecipitation of inorganic phosphorus and metals), microbial nitrification (in aerobic conditions) followed by microbial denitrification (in anaerobic conditions), ammonium (NH_4^+) anoxic oxidation (anammox) and assimilation (in plant biomass) (Vymazal, 2007).

During the last four decades, technology simulating processes occurring in natural wetlands has been applied to wastewater treatment using constructed wetlands (CW) (Arias & Brix, 2003; Vymazal, 2011). In CW, the subsurface flow wetlands (SSFW) (Fig.1) have a good performance removing organic load, which is the main purpose of most purification treatments (García & Corzo, 2008) as well as for nutrient removal (Brix, 1994; Vymazal, 2001). Although in general the CW have been used for the treatment of domestic sewage, currently experiences of their application to the industry are known (Vymazal, 2014). Fish processing industry generate an outflow which is particularly important due to its high content of organic matter (Mines & Robertson, 2003). On average, 30 to 40 % of fish production is consumed fresh; the remaining production is processed (Islam *et al*., 2004). The integrated outflow management is essential for production. In this regard, CW should be considered as an important component of a treatment system. Usually, CW act as advanced secondary and tertiary treatment modules (Mustafa, 2013). In most cases, space availability is the main constraint when implementing this technology. This fact, combined with the flow rate, determines the hydraulic residence times (HRT). Although HRT is a variable that defines the efficiency of contaminant´s removal, it is not the only one. Wetland geometry (Persson *et al.,* 1999) and environmental factors specific to each geographic region influencing biological processes are important as well. Some authors studying CW with a low HRT evidenced significant purification efficiency (Solano *et al.,* 2004; Sohsalam *et al.,* 2006; Guido-Zárate *et al.,* 2007; Gutiérrez *et al.,* 2010), These HRT are approximately 1-5 days.

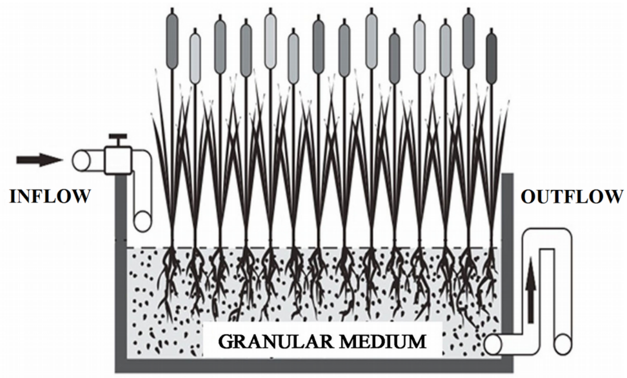

Figure 1. Scheme of subsurface flow wetland applied in this study. Water circulation is underground through a granular medium and macrophyte roots matrix.

In this work we aimed to evaluate the purifying capacity of an experimental model of SSFW in order to incorporate this technology to a wastewater treatment system in a fish processing plant. The efficiency in reducing organic compounds was particularly studied.
Moreover, we compared the purification efficiency of a SSFW with a HRT of 38 hours to a SSFW with a HRT of 10 hours, which needs a smaller construction area, thus addressing space limitations.

Materials and Methods

Study Area: The experiment was performed in a fish processing industry located in Canelones Department (Uruguay) (34°46'07, 00"S y 56°01'55, 31"W). This study was carried out in the spring of 2010.

In the processing plant 8.280 T year^{-1} of Micropogonias furnieri (white croaker) and 479 T year^{-1} of Chondrichthyes (sharks and rays) are processed. Water for production use is taken from Toledo stream, with a maximum of 200 m^3d^{-1} (Informe ambiental resumen, Novabarca, 2012), utilizing an intensive organic matter purification process after being used and then poured back into the stream. This process consists of 3 stages; a) a system where solid fats are retained; b) an oxidation channel and c) the water passes to facultative lagoons.

Experimental units: Scale-built SSFW experimental units, with *Typha latifolia* monoculture inside a plastic container of 1.13 m length, 0.93 m width and 0.70 m depth were used. This container was filled with 0.30 m of gravel (0.02 m diameter and about 0.6 porosity) which is the depth that *Typha sp.* roots reach (Tanner 2001). In each of the experimental units 12 rhizomes of similar length (Fig. 2b) were sown. These plants are abundant in the study area, they show acclimation to local conditions and they are suitable to use in removing nitrogen and organic matter (Ansola, 1995; Shutes, 2001; Sohsalam *et al.,* 2006). The design was randomized with 3 replications and 2 treatments: HRT of 10 hours (treatment one) and HRT of 38 hours (treatment two) (Fig. 2a). Water from the facultative lagoon entered the SSFW drip, flushing through the gravel matrix. This water was taken from the facultative lagoon using an electric pump and then stored in a closed tank to feed the experimental units with a constant water flow.

Replicates were connected with pipes of 50 mm of diameter. Valves to regulate flow were installed, allowing working with different HRT. After filling the supplying tank, water continued its way through gravity, before discharging into the stream. In the output of each experimental unit an external siphon was placed at 0,30 m to maintain water inside the SSFW (Fig. 2c and 2d).

Sampling design and analysis: The pilot wetlands were installed in the end of winter, during August 2010. Samples were taken in the end of spring, the 12th (I), 19th (II), 26th (III) of November, at noon. No rainfall during the days before sampling was recorded.

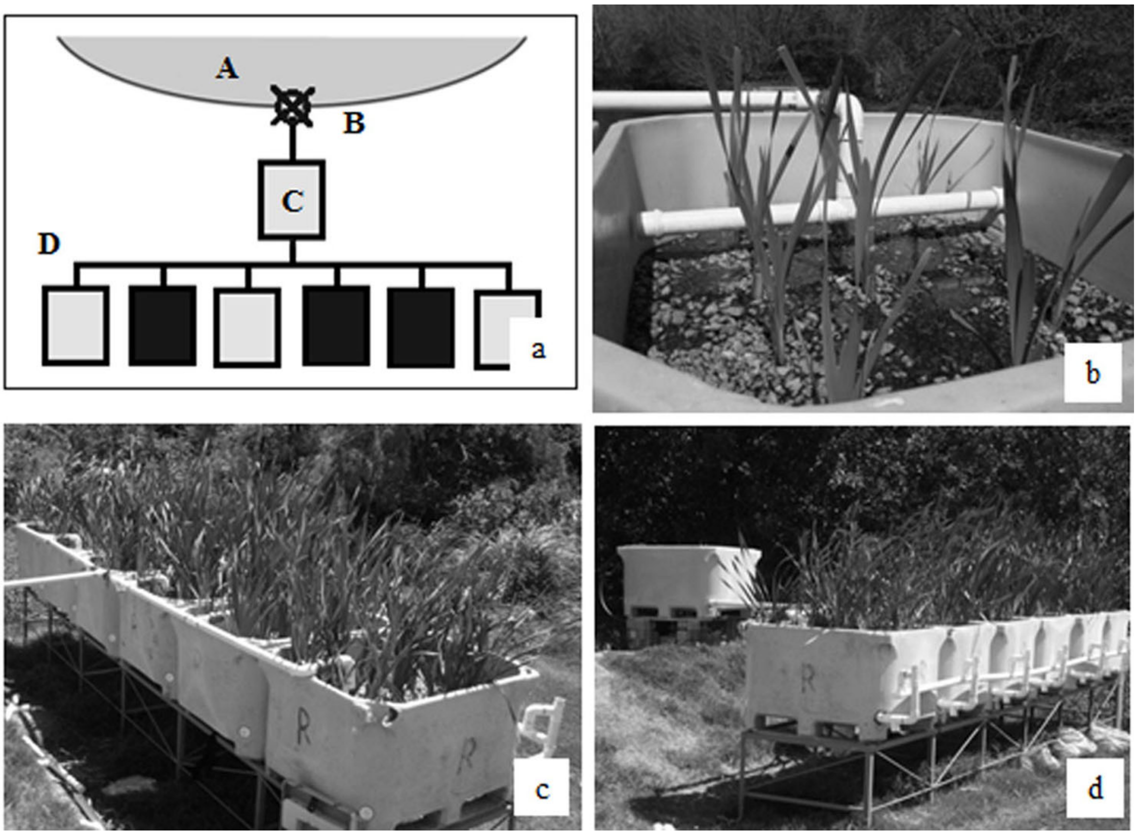

Figure 2. Scheme of the circulation flow through the experimental model. A- Facultative lagoon (end of the current treatment). B- Electric pump. C- Feed tank to D. D- Replicates where both hydraulic residence times are marked, in black the 38 hours treatment and in light grey the 10 hours treatment. b) Drip system in a wetland with growing rhizomes. c) (left) Supply pipes (detailed in b) and (right) the siphon. d) Growth of *Typha* 43 days after being planted- C- Tank and a line of the siphones of the replicates.

Measurements of temperature and dissolved oxygen were taken *in situ* with a D-25 Horiba sensor; measurements of pH were taken with a Waterproof Testr 20 sensor and conductivity was measured with a Waterproof ECTestr 11+ sensor. Organic matter concentration was assessed by three methods representing different fractions: suspended organic matter (SOM), biodegradable organic matter (BOD_5) and refractory plus biodegradable organic matter (COD) (APHA, 2005). Total suspended solids (TSS) were also determined (APHA, 2005)

Fractions of inorganic nitrogen were calculated by the following methods: NH_4^+ by indophenol blue method (Koroleff 1970); nitrate (NO_3^-) by sodium salicylate methods (Müller & Weideman, 1955); nitrite (NO_2^-) by sulfanilamide method (Strickland & Parsons, 1972) and total nitrogen (TN) by high temperature and pressure digestion of previously filtered samples (Valderrama, 1981). Phosphorus was analyzed according to molybdenum blue method (Murphy & Riley, 1962) and for soluble reactive phosphorus (SRP) and total dissolved phosphorus (TP) the same method applied to TN was used.

Organic nitrogen was calculated by subtracting from TN the sum of inorganic nitrogen fractions. For this calculation the concentrations of N_2O and NO have not been taken into account (Vymazal, 2007). Similarly, the dissolved fraction of non-reactive phosphorus was calculated subtracting SRP to TP.

Statistical Analysis: The treatment effects for BOD_5, COD, SOM and TTS variables were analyzed by ANOVA for repeated measures over time, given that variables met the assumptions of normality and homogeneity of variances. Sampling times were considered as a constant effect. The covariance matrix allowing the best fit of the model was the compound symmetry. In order to determine the HRT effects on the pollutant removal at each time, confidence intervals for each portion of substance removed were conducted from the maximum likelihood function adjusted to binomial distributions (Casella & Berger, 2001).

Significance between the average values obtained in the outflow and in the inflow were analyzed using t-tests.

In order to consider the variations in the HRTs between replicates of the same treatment, pollutant values were expressed as loads.

Equation: Load= [contaminants]*Q/A,

Where Load is expressed in (g l m^{-2} d^{-1}), contaminants expressed in (g), A is the SSFW area expressed in (m^2) and Q is the flow rate expressed in (l d^{-1}).

To assess the significant differences in removal capacity between treatments, values obtained in the tests were compared using two-way ANOVA with repetition, considering the HRTs as a fixed factor. For this analysis proportional removal values were compared with the inflow. In all the cases considered the significance values were $p < 0.05$.

Results and discussion

Physicochemical variables: The average value of conductivity in treatment 1 outflow was 2.0 ± 0.2 $mScm^{-1}$ and in treatment 2 outflow was of 1.9 ± 0.2 $mScm^{-1}$ (Table I) . There were no significant differences between mean conductivity values of outflow and inflow treatments.

The pH value was always lower in the output outflows, showing significant differences between the mean of the replicates and the inflows ($t= 4.39$ and $p= 0.0118$; $t= 8.48$ and $p= 0.0011$ for treatments 1 and 2, respectively). Average pH for treatment 1 was 7.66 ± 0.25 and for treatment 2 was 7.34 ± 0.12; while for the inflow pH average was 8.22 ± 0.17. Dissolved oxygen measures, both in the inflows and outflows, allow us to characterize these waters as hypoxic, varying between 0.2 and 2.59 mg l-1, being in all cases lower in the outflow (Table I). The pH lower values observed in the outflow (Table I) can be related to the organic matter degradation processes undertaken by microorganisms also promoting a reduction in the oxygen concentration.

With regard to treatments, temperature did not vary significantly between the inflow and the mean of the samples. However, it was observed a continuous increase in the environmental temperature as the sampling took place, with significant variations between sampling dates I and II (Table I).

Organic compounds: BOD_5 removal (Fig. 3a) increased during successive samplings; this is particularly observable between samplings II and III, for both treatments. Treatment 2 is more efficient in BOD_5 removal compared with treatment 1, particularly observed in sampling I and II where the plotted intervals do not overlap. For treatment 1, BOD_5 input loads (inflows) for samplings I, II and III were 29.73; 35.01 and 16.92 gO_2 m^{-2} d^{-1} respectively, and BOD_5 outflows were 23.63; 23.58 and 6.58 gO_2m^{-2} d^{-1}, respectively.

Table I. Chemical - physical variables measured in the inflow and the outflow of each treatment. Samples were taken for 3 consecutive weeks.

Date	Sample	Variables	Inflow	Outflow treatment 1	Outflow treatment 2
12th of November	I	Conductivity (µScm^{-1})	1911	1795±12	1713±34
		pH	8.05	7.49±0.08	7.28±0.04
		Dissolved oxygen (mg ml^{-1})	1.64	0.43±0.23	1.16±1.00
		Temperature (ºC)	21.7	21.0±0.5	23.8±0.5
19th of November	II	Conductivity (µScm^{-1})	1900	2075±79	1955±26
		pH	8.25	7.74±0.05	7.43±0.03
		Dissolved oxygen (mg ml^{-1})	1.67	0.05±0.01	0.26±0.12
		Temperature (ºC)	23.2	22.7±0.5	23.4±0,3
26th of November	III	Conductivity (µScm^{-1})	2200	2133±17	1970±18
		pH	8.37	7.76±0.04	7.30±0.04
		Dissolved oxygen (mg ml^{-1})	2.59	0.74±0.47	1.60±0.55
		Temperature (ºC)	29.9	27.4±1.9	28.8±0.3

For treatment 2, inflows were 7.71; 8.94 and 17.34 $gO_2m^{-2}\ d^{-1}$ and outflow were 5.22; 4.67 and 6.26, respectively.

COD load removal (Fig. 3b) shows an irregular behavior. In treatment 1 there was a larger removal in sampling III with respect to samplings I and II, which was not observed in treatment 2 samplings. There is a smaller removal in sampling II as compared to I and III. When comparing removals between treatments, treatment 2 was significantly more efficient in all samplings. For treatment I, COD inflow were 108.11; 63.65 and 47.58 $gO_2m^{-2}\ d^{-1}$, while COD outflows were 88.74; 57.72 and 24.19 $g\ O_2\ m^{-2}\ d^{-1}$. For treatment II, COD inflows were 28.02; 16.26 and 48.77 $gO_2m^{-2}\ d^{-1}$ and COD outflows were 14.75; 12.07 and 13.76 $gO_2m^{-2}d^{-1}$.

Removal rates of organic matter were low when compared with other studies; García & Corzo (2008) presented a practical guide to constructing and exploring wetlands (including the SSFW) indicating that BOD_5 and COD removal vary between 75 % and 95 %, reaching in the outflow concentrations lower than 60 mg l^{-1} for COD and lower than 20 mg l^{-1} for BOD_5. In a 10-year revision of CW functioning in the Czech Republic, Vymazal (2001) analysed 101 operating systems, 95 of which were SSFW. As a result he observed that these systems removed on average 86.5 % of BOD_5, being their average income loads of 3.52 $gO_2\ m^{-2}d^{-1}$. Brix (1994) analysed the development of CW for two decades, focusing on surface flow wetlands (70 study cases) and SSFFs (104 study cases) built for treating urban outflows. Results indicate that the removal average of BOD_5 in SSFFs was of 85 %, with an average concentration of 41 mg l^{-1} in the inflow.

The low removal rate of organic matter we found could be related to the pre-treatment inflow by traditional facultative lagoons, before it passes through the experimental model so remaining organic matter was mostly refractory. Abel (1999) indicated conventional treatment could remove between 75 % and 95 % of the organic matter. In our study the average DBO_5/COD ratio in the inflow was 0.36; this is in agreement with the value reported by Vymazal (2014), BOD/COD ratio >0.5 for easily biodegradable matter. The short HRTs selected and the immature stage of the wetland models planted only 5 month before starting the study, would also contribute to observed low removal rates.

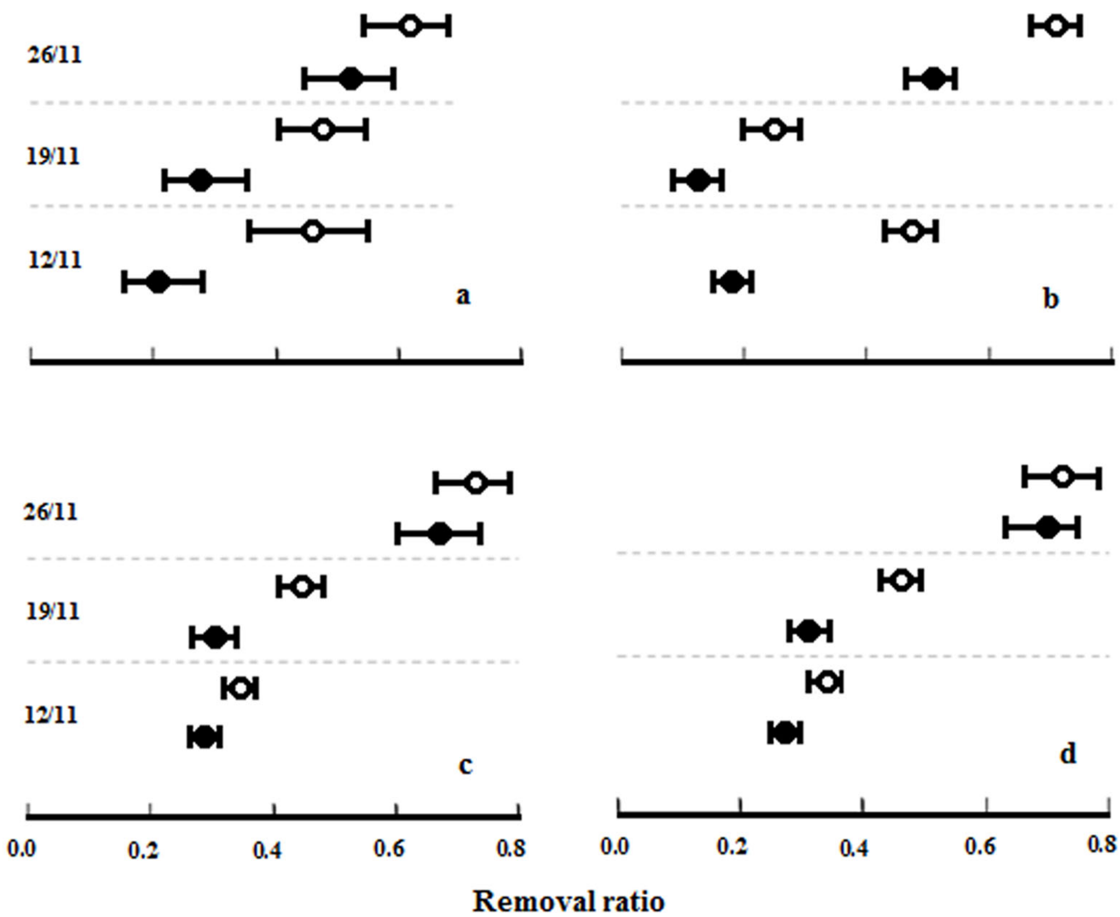

Figure 3. Proportion of contaminant removed in constructed wetlands; a- biological oxygen demand in five days, b- chemical oxygen demand, c-suspended organic matter and d- total suspended solids. Confident intervals are indicated between horizontal lines, wherein: -●- is the removal estimation for the hydraulic residence times = 10 h and -**o**- is the removal estimation for the hydraulic residence times = 38 h. Bars indicate the confidence interval (95 %). Sampling dates (12th, 19th, 26th of November) are indicated to the left of the figure.

Reduction of BOD_5 and COD in the SSFW outflow could be used as an estimate of organic matter removal efficiency. Comparing treatments, treatment 2 was more efficient than treatment 1. Better result can be attributed to the larger HRTs, in this case the main efficiency factor in organic matter removal. The metabolism of organisms responsible for organic matter degradation is dependent on environmental temperature. The results obtained in this work were in accordance with this fact because as the water temperature increases, the SSFW removal also increases.

The TSS load removal is shown in figure 3c. In treatment 1, there is a marked difference comparing sampling III with samplings I and II. In treatment 2, the three samplings results are remarkably different. When considering all treatments we observed that treatment 2 was more efficient in removing the first two samplings ($p < 0.05$) relative to treatment 1. In treatment 1, inflows TSS were 0.027; 0.010 and 0.001 g $m^{-2}d^{-1}$; outflows were 0.038; 0.015 and 0.002 g $m^{-2}d^{-1}$. Regarding treatment 2, inflows were 0.006; 0.002 and 0.001 g $m^{-2}d^{-1}$, and outflows were 0.010; 0.004 and 0.002 in each sample for both treatments.

Figure 3d shows SOM removal; we highlight the greater increase in the removal of samplings taken in treatment 2. This treatment, as the others, resulted to be more effective for samplings I and II, relative to treatment 1. For treatment 1, SOM inflow was 0.029; 0.013 and 0.002 g $m^{-2}d^{-1}$ and outflows were 0.02; 0.009 and 0.001 g $m^{-2}d^{-1}$. For treatment 2, inflows were 0.007; 0.003 and 0.002 g $m^{-2}d^{-1}$ and outflows were 0.005; 0.002 y 0.001 g $m^{-2}d^{-1}$.

Along the samplings we observed an increase in TSS and SOM load removal. This increasing removal was explained by a physical sieving process favoured by macrophyte roots growth (Brix, 1997).

It should be highlighted that the obtained values are not the expected ones for the suspended matter removal; the larger ones could be: larger than 90% (García & Corzo, 2008), 89% (Vymazal, 2001) and 83% (Brix, 1994). While both treatments were significantly effective, in treatment 2 better results were obtained as compared to treatment 1. Here, the incidence of the different HRTs considered is reflected.

Nutrients: Regarding to the studied compounds, removal of nutrient load did not show significant differences between treatments. In treatment 1 there was neither SRP removal nor NH_4^+ removal during the different samplings. Regarding treatment 2, there was no NO_3^- remov al in sampling I nor in sampling III. Removal values were: NH_4^+ between 5% / 20%, NO_3^- between 20% / 55%, NO_2^{-2} around 99%, TN between 5% / 23%, SRP between 25% / 40% and TP between 21% / 61% (Table II).

There were different mechanisms for nutrient removal. While assimilation in plant biomass was not quantified, the period of time in which the experiment was conducted coincided with the maximum *Typha* sp. biomass production; therefore assimilation levels must be important (Bécares, 2006; Da Silva, 2006; Mesquita *et al.,* 2013). Although algae could have assimilated nutrients, this effect would not be significant because of sunlight limitation (Chang *et al.,* 2012). In aquatic systems, denitrification is the most relevant process for nitrogen removal (Vymazal, 2007).

This process is limited by oxygen availability; hence, in our study systems it would be very restricted, as they exhibited oxygen values near anoxia.

Table II. Ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total nitrogen (N), soluble reactive phosphorus (SRP) and total phosphorus (TP) in treatments 1 and 2 inflow and outflow . For each treatment, removal is the concentration in the inflow substracting the concentration in the outflow. Standard errors are indicated.

			Treatment 1		Treatment 2	
Compound	Sample	Inflow ($mg.l^{-1}$)	Outflow ($mg.l^{-1}$)	Removal ($mg.l^{-1}$)	Outflow ($mg.l^{-1}$)	Removal ($mg.l^{-1}$)
	I	52,18	47.99 ± 2.88	4.19 ± 2.88	44.02 ± 1.34	8.16 ± 1.34
NH_4^+	II	53,17	53.89 ± 0.29	-0.72 ± 0.29	51.74 ± 0.66	1.43 ± 0.66
	III	55,93	52.00 ± 0.73	3.93 ± 0.73	45.20 ± 0.55	10.73 ± 0.55
	I	0,32	0.15 ± 0.01	0.17 ± 0.01	0.43 ± 0.02	-0.11 ± 0.02
NO_3^{2-}	II	0,32	0.18 ± 0.04	0.14 ± 0.04	0.26 ± 0.01	0.06 ± 0.01
	III	0,32	0.28 ± 0.03	0.04 ± 0.03	0.53 ± 0.11	-0.21 ± 0.11
	I	1,23	0.01 ± 0.00	1.22 ± 0.00	0.02 ± 0.01	1.21 ± 0.01
NO_2^-	II	2,64	0.03 ± 0.01	2.61 ± 0.01	0.03 ± 0.01	2.61 ± 0.01
	III	4,49	0.01 ± 0.00	4.48 ± 0.00	0.03 ± 0.02	4.46 ± 0.02
	I	96,71	90.62 ± 11.87	6.09 ± 11.87	83.95 ± 5.13	12.76 ± 5.13
Total N	II	101,92	97.29 ± 1.26	4.63 ± 1.26	89.17 ± 8.47	12.75 ± 8.47
	III	95,84	90.91 ± 7.98	4.93 ± 7.98	92.07 ± 12.05	3.77 ± 12.05
	I	6,13	7.13 ± 0.62	-1.00 ± 0.62	4.23 ± 0.30	1.90 ± 0.30
SRP	II	5,84	6.17 ± 0.27	-0.33 ± 0.27	5.28 ± 0.56	0.56 ± 0.56
	III	5,16	5.77 ± 0.20	-0.61 ± 0.20	2.76 ± 0.15	2.40 ± 0.15
	I	8,26	8.88 ± 0.27	-0.62 ± 0.27	5.99 ± 0.48	2.27 ± 0.48
Total P	II	7,42	7.23 ± 0.02	0.19 ± 0.02	5.99 ± 0.64	1.43 ± 0.64
	III	7,53	6.74 ± 0.34	0.79 ± 0.34	3.54 ± 0.26	3.99 ± 0.26

However, removal values obtained were similar to those reported by Garcia & Corzo (2008) in CW (10-20%).

Regarding phosphorus removal in the outflow treatments, the main mechanism is mineral adsorption (iron, aluminum and calcium) with chemical precipitation (co-adsorption). We consider that the study model (rich in iron provided by the gravel) would not have had the enough maturation time, so the sediment would not be saturated (Richardson, 1985; Daza-Torres *et al.*, 2008) and therefore co-adsorption would be taking place all along the sampling period.

Conclusions

The performance of our experimental model showed a significant removal of organic matter and TSS in the fish processing plant outflow. Both HRTs improved the quality of the outflow, indicating the importance of complementary treatments. Furthermore, the wetland model operating with a 38 h HRT proved to have a better performance. Although the nutrient removal was not statistically significant, the registered values were similar to other cited studies. To improve the nutrients removal, increased oxygenation is needed, in order to favor the denitrification and phosphorus precipitation; this could be achieved by treating the outflows in combination with wetlands subsurface flow and surface flow. Our results corroborate that SSFHs are efficient biotechnological tools improving traditional wastewater treatment of organic industrial outflows. However, further research is still to be done considering longer periods of time and taking into account temporal temperature variations.

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