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Original Research

Effects of Supplemental Aeration on Total Nitrogen Removal in a Floating Helophytes Filter (FHF) for Wastewater Treatment

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Abstract

Although different systems of Constructed Wetlands (CW) for wastewater treatment have been in operation for more than 30 years, some aspects could still be much improved, such as the nutrients removal percentage and the avoidance of substrate clogging. This paper reports on an experiment conducted to assess the performance of a floating helophytes filter (FHF) to remove total nitrogen (TN) and the effect of supplemental aeration on nitrification and denitrification. The supply of external aeration to the FHF system reduced significantly ($p<0.05$) the TN final concentration in spring, summer, and autumn experiments, whereas in the winter experiment a strong N-NO_3^- accumulation was recorded in the FHF-aerated treatment that resulted in a poorer TN removal percentage ($p>0.05$). Low organic matter concentration is the most likely cause of the denitrification slowdown in the winter experiment.

Keywords: constructed wetlands, floating helophytes filter, nitrogen removal, supplemental aeration

Introduction

Constructed wetlands (CW) have become a feasible and reliable technology for secondary and tertiary wastewater treatment of wastewater from different origins, e.g., municipal sewage, industrial wastewater, urban runoff and landfill leachates. CW are particularly suitable for rural areas due to their removal efficiency, cost-effectiveness, simple technical maintenance requirements, and environmentally friendly designs. Different systems of CW have been designed that reproduce the biochemical natural processes responsible for the removal of pollutants and meet the quality standards set down by the regulator. However, there are still some common limitations that should be improved and that require further research, like substrate clogging after 10-15 years of operation in subsurface flow systems (SSFS) and effective nutrient removal at different temperatures [1-4].

Clogging avoidance was researched by Fernández during the 1990's, and resulted in the granting of a patent for a wastewater treatment (WWT) system based on the formation of an emergent macrophytes floating mantle [5]. Since then, several WWT plants have been constructed using the floating helophytes filter (FHF) design, and currently there are about 40 installations in operation at different Spanish locations [6]. Recently other authors have proposed the term "Floating Treatment Wetland" (FTW) for this type of CW based on helophytes species growing hydroponically on a consolidated floating mat [7].

The removal of nitrogen (N), a process that is dependent on oxygen levels, is another key issue in CW performance. Dissolved oxygen (DO) in wetlands comes from the photosynthetic activity below the water surface and oxygen transfer from air across the water surface or convective transport through helophytes aerenchyma down to the roots, and ultimately leaking into the rhizosphere [8, 9]. The oxygen available for microorganisms in this media is used

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first by organic matter decomposition bacteria, and later by the nitrifying bacteria population to carry out their own metabolic processes. Since the heterotrophic organic matter removal bacteria compete with the autotrophic nitrification bacteria for oxygen and natural aeration in CW does not often meet the oxygen demand required for both types of microorganisms, undesirable levels of nitrogen in CW effluents are frequently produced [10, 11]. Hence different proposals have been made to enhance aerobic areas in CW systems in order to improve N removal, namely: the use of passive pumps [12, 13], tidal or water level fluctuation [14, 15], intermittent loading [16], the design of combined or hybrid systems [17], and engineered wetlands with supplementary mechanical pumping of air [18-20].

This work deals with the performance of a closed pilot-study scale FHF system for wastewater treatment using aquatic macrophyte *Typha domingensis* Pers. Total nitrogen (TN) removal efficiency and the effect of supplemental aeration are studied for each of the four seasons of the year, in an experimental WWT plant with an FHF design.

Materials and Methods

Experimental Design

The experimental work was carried out in the period between June 2008 and September 2009 in the Experimental Fields of the Agricultural College of the Technical University of Madrid (UPM), in Madrid, Spain (latitude 40º26'36"N, longitude 3º44'18"W). In accordance with the objectives of the work, the prime factors in the experimental design were: the seasons of the year and supplemental aeration. Four experiments – summer, autumn, winter, and spring – were conducted in order to determine the capability of an FHF system to remove TN from wastewater in all seasons, over a wide range of temperatures, and at different plant growth stages, as well as to evaluate the effects of supplemental aeration on TN removal efficiency. Four treatments were conducted per seasonal experiment:

- Treatment "p+ar": floating macrophytes filter (FHF) with supplemental aeration
- Treatment "p": FHF without supplemental aeration;
- Treatment "ar": control treatment with supplemental aeration; wastewater covered with a styrofoam lid and with supplemental aeration
- Treatment "bk": blank or control treatment without supplemental aeration; wastewater covered with a styrofoam lid and no extra aeration.

Three experimental units (three replicates) were set up per treatment and season. In Fig. 1 the layout of two of the experimental units (EU) representing one replicate of treatment "p+ar" (FHF with supplemental aeration) and one replicate of treatment "ar" (aerated control) is shown. Experiments were concluded when any of the experimental units (EU) reached persistent background TN concentration values, which happened at different times for each seasonal experiment.

Water tanks of 35.8 cm inner diameter, 66.5 cm height, and 60 L volume were used as EU containers; a tape was stuck to the internal side to measure wastewater level variations. Tanks were placed outdoors under a transparent polyethylene film cover laid as a thin roof to prevent rainfall entry and to allow light to reach the plant leaves at the same time.

Typha domingensis Pers. mats previously cultivated in growing tanks were used in every FHF treatment. *Typha* age and average mat characteristics per experimental unit were the following:

i) Summer experiment: 12-month-old plants, 4.72 kg mean fresh weight, and mean number of shoots 15
ii) Autumn experiment: 15-month-old plants, 10.07 kg mean fresh weight, and mean number of shoots 31
iii) Winter experiment: 19-month-old plants, 10.39 kg mean fresh weight, and mean number of shoots 34;
iv) Spring experiment: 24-month-old plants, 10.96 kg mean fresh weight, and mean number of shoots 36

At the beginning of each experiment, *Typha* specimens were removed from the growing tanks, placed on a rack for two hours until the water was drained away from the mats, and then moved into the experimental tanks corresponding to treatments "p+ar" and "p" (FHF treatments), which had already been filled with wastewater. Plants were placed on the upper part of the EU, keeping the whole mat under the wastewater surface while the shoots remained above it, resembling a floating mantle. *Typha* mats limited free transfer of gases between the wastewater body and the air since their size fitted the upper surface area of the tanks.

The height of the wastewater surface was measured at the beginning of each experiment for evapotranspiration (ET) control. In the winter experiment, during a period of a

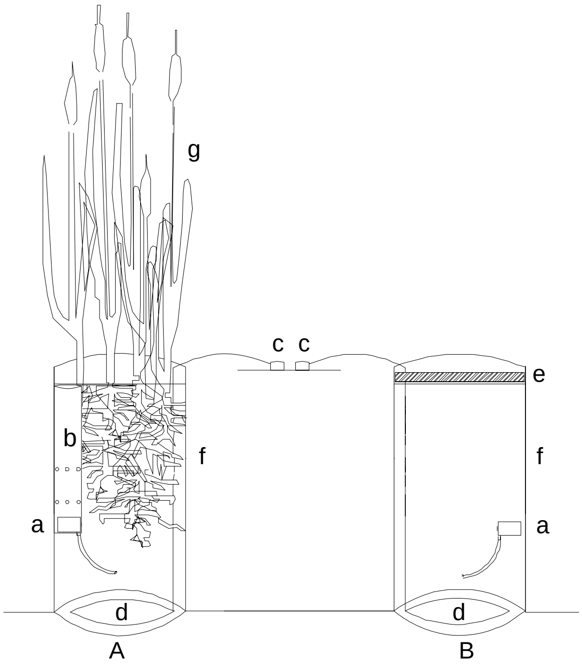


Fig. 1. Layout of experiment units. (A) treatment "p+ar": FHF with supplemental aeration. (B) treatment "ar": aerated control. Key to letters: a – water pump, b – sampling tube (perforated tube), c – air pump, d – air diffusion ring, e – styrofoam lid, f – container, g – macrophyte *Typha domingensis* Pers.

Table 1. Mean values of the parameters determined at day 0 in the influents of the experiment tanks of each seasonal experiment.

Parameter	Spring	Summer	Autumn	Winter
T_w (ºC)	23.2±0.5	24.0±0.4	18.7±0.5	6.8±0.5
pH	7.32±0.07	7.37±0.04	8.79±0.17	8.71±0.12
DO (mg/L)	0.33±0.27	1.12±0.82	2.68±2.75	0.51±0.28
BOD_5 (mg/L)	210±40	140±17	167±18	75±17
TN (mg/L)	45.0±1.8	55.1±2.5	112±4.4	76.6±1.3
N-NH_4^+ (mg/L)	33.4±1.8	37.9±3.9	3.30±0.3	60.6±3.6
N-NO_3^- (mg/L)	1.13±0.27	0.00±0.00	0.85±0.20	1.03±0.32
BOD_5:TN	4.67	2.54	1.48	0.98
N-NH_4^+:TN	0.74	0.69	0.03	0.79
N-NO_3^-:TN	0.03	0.00	0.01	0.01
Experiment duration (days)	9	8	28	28
Experiment mean T_w (ºC)	20.7±0.2	21.6±0.4	17.8±0.6	6.1±0.2

few days, small thin portions of ice formed around the lowest part of some of the shoots in the planted EU.

A small water pump (Decor 03 model, Q_{max} 300 $l \cdot h^{-1}$, 3.8 W) was placed at 40 cm depth inside the tank to simulate the water flow through a CW channel and to avoid the boundary-layer effect that might cause a reduction in the natural rates of oxygen released by the roots [21].

Air pumps (RESUN-Eolo AC 3100 model supplying an average of 2.2 $l \cdot m^{-1}$) for mechanical aeration supply were placed outside the tanks. Air distribution microtubes connected air pumps to perforated pipes that were laid as a ring (35.0 cm in diameter) at the bottom of the aerated treatment tanks so that the pumped air leaked through the pipe ring and diffused up into the tank wastewater. Air supply timing was fixed from 10 a.m. till 10 p.m. daily.

A PVC sampling tube (of 9.5 cm inner diameter and 40 cm long) was hung from the upper tank edge of the FHF treatment tanks; it had two rings of six 10 mm openings at 23 and 32 cm depth to allow the circulation of wastewater through it.

Influent was prepared in a storage tank close to the experiment tanks and a single wastewater loading was carried out at the beginning of each experiment. Forty-five litres of the prepared influent were poured into each experiment tank by means of a small portable submersible pump.

Wastewater influent was untreated pig slurry (from the Pig Welfare Laboratory of UPM) diluted in tap water. The pig slurry was previously analyzed for its content in TN and five-day biochemical oxygen demand (BOD_5) in order to calculate the dilution needed to get an influent with TN concentration and BOD_5 values within the normal range of values for municipal wastewater. That target was easily achieved for spring and summer influents, whereas for autumn and winter influents the initial TN concentration values were higher since the pig slurry had an unexpectedly low BOD_5 and an average TN concentration, which resulted in the preparation of influents with much higher TN concentration values in order to get reasonable BOD_5 values. In addition to the diluted untreated pig slurry, twelve litres of water from growing tanks were also added to contribute to the influent's population of microorganisms. In Table 1, mean values of influent parameters at the start of day 0 are given as well as their ratios and the duration of the experiments, broken down per season.

Winter and autumn influents had higher TN concentration mean values and lower mean wastewater temperatures (T_w) than the summer and spring influents, causing longer experiment duration (28 days versus 8-9 days). The winter TN concentration mean value was 50% higher than the spring value (76.6 mg/L and 45.0 mg/L, respectively) and the autumn TN concentration mean value was approximately twice the summer value (112 mg/L and 55.1 mg/L, respectively). Mean N-NH_4^+ concentration values ranged from 68.9% to 79.2% of the respective TN concentration mean values, except for the autumn influent, whose recorded N-NH_4^+ concentration mean value (3.30 mg/L) suggested a poorly mineralized N content.

Analyses of N-NO_3^- concentration in influents gave low-medium mean values for the four experiments; they ranged from a maximum value of 1.13 mg/L in spring to a minimum value of 0.00 mg/L in summer.

Mean BOD_5:TN ratio varied from a maximum of 4.67 in spring to a minimum of 0.98 in winter, this latter figure representing a potentially disadvantageous condition for denitrification during the winter experiment due to a probably insufficient availability of carbon (C) [22].

The highest mean value of dissolved oxygen (DO) (2.68 mg/L) was recorded for the autumn influent, which shows that the environmental conditions for ammonification and nitrification were more favourable in the autumn experiment than in the other experiments; however, all DO influent mean values were below the level ideally conducive (2 mg/L) to the conventional nitrification process [23].

The wastewater surface of unplanted units (control treatments) was covered – but not sealed – with a styrofoam lid (2.5 cm thick and 35.0 cm in diameter). It was placed to float on the medium in order to simulate a helophytes mat cover, but without the capacity to supply oxygen through aerenchyma. It also prevented algae growth that could interfere or distort real system yields. Therefore, it can be assumed that most DO in wastewater during the experiments was supplied either through aerenchyma and/or mechanical aeration.

Analytical Methods

Hach-Lange tests LCK 138, 238, and 338 were used for the analysis of TN concentration, LCK 302, 303, and 304 for N-NH_4^+ concentration, LCK 339, and 340 for N-NO_3^- concentration, and the OxiTop system for the establishment of BOD_5.

The following portable meters were used to record pH, DO, and temperature (T) in wastewater samples: CRISON 407 pH-meter, and HACH HQ40d for DO and T measurements.

Wastewater Sampling and System Operation

Wastewater characteristics were periodically monitored during the experiments. Wastewater from each experiment unit was sampled just before aeration started at 10 a.m. and promptly analyzed. Before and after each sampling, the wastewater surface level was measured to determine and compensate for possible evapotranspiration (ET) losses in the experimental units. EUs were consequently refilled with tap water as needed, keeping at least 90 minutes before sampling. As a result, the wastewater volume could be estimated throughout the whole period of the experiment as it was the initial volume of the experiment minus the sampling volume taken up to date. ET losses, pH, T, and DO values were recorded *in situ* at the sampling time. Samples were then kept in a refrigerator until BOD_5 and N analysis.

The sampling volume was the same for all tanks in the experiment. It was kept to the minimum necessary for TN, N-NH_4^+, and N-NO_3^- concentration analyses plus the volume needed for BOD_5 determination, since what was wanted as a closed system with a single wastewater load of 45L per tank at the beginning of each experiment. Following the OxiTop methodology, the sample needed for BDO_5 could represent a significant volume in some cases (it depended on the expected BDO_5 values); the highest volume required was 436 ml (determination of low BOD_5 values). Therefore the decision was taken that for the experiments of winter and autumn – which were expected to last longer – the sampling frequency for BOD_5 determination would be reduced.

External aeration was continuously supplied for the aerated treatments for 12 hours a day, from 10 a.m. until 10 p.m., during the whole experimental period in the summer and spring experiments; for treatments "p+ar" in the winter and autumn experiments, the supplemental aeration was interrupted when N-NH_4^+ concentration values <1 mg/L were achieved; this happened at day 16 in autumn and between days 21 and 25 in winter.

Data Elaboration and Statistical Analysis

TN concentration values recorded for each treatment were referred to the influent TN concentration (TN value at the starting point of the experiment) since mean influents of different seasons had different TN concentrations (Table 1). Hence TN values were expressed as a percentage (TN%) calculated for each experimental unit and sampling date as:

$$TN_i\% = \frac{TN_i}{TN_0} \times 100$$

...where: TN_i – concentration of TN (mg/L) at sampling date 'i' and TN_0 – concentration of TN (mg/L) at day '0' (beginning of the treatment).

Removal (R%) of TN, BOD_5, and N-NH_4^+ concentrations were calculated for each experimental unit and sampling date as:

$$R\% = \frac{C_0 - C_i}{C_0} \times 100$$

...where: C_0 – concentration (mg/L) of TN, BOD_5, or N-NH_4^+, accordingly, at day 0 (beginning of the treatment), and C_i – concentration (mg/L) of BOD_5, TN or N-NH_4^+, accordingly, at sampling date 'i.'

Statistical evaluation of TN removal and/or concentration (percentages) differences between treatments within each season was assessed by ANOVA followed by multiple comparison of means using Tukey's method. The arcsine square root transformation was used for data of removal and concentration percentages. SPPS version 5.0 software was used for all statistical analyses; the significance level was set at $p<0.05$, and all intra-experiment variables had homogenous variance.

Results and Discussion

The Effect of Helophytes Presence – Assembled as FHF – on TN Removal

The values of TN% reached at the end of each experiment ($TN_f\%$) were much lower for the EU with helophytes than those recorded for the unplanted ones (control treatments) in all seasons (see graphs in Fig. 2 for $TN_i\%$ per season and treatment). Differences between $TN_f\%$ of planted and unplanted treatments were always statistically significant ($p<0.05$) in the four experiments performed (four seasons).

In all conditions, the supplemental aeration *per se* did not compensate for the absence of plants. For this factor, the

Table 2. The mean results of TN removal per treatment and season.

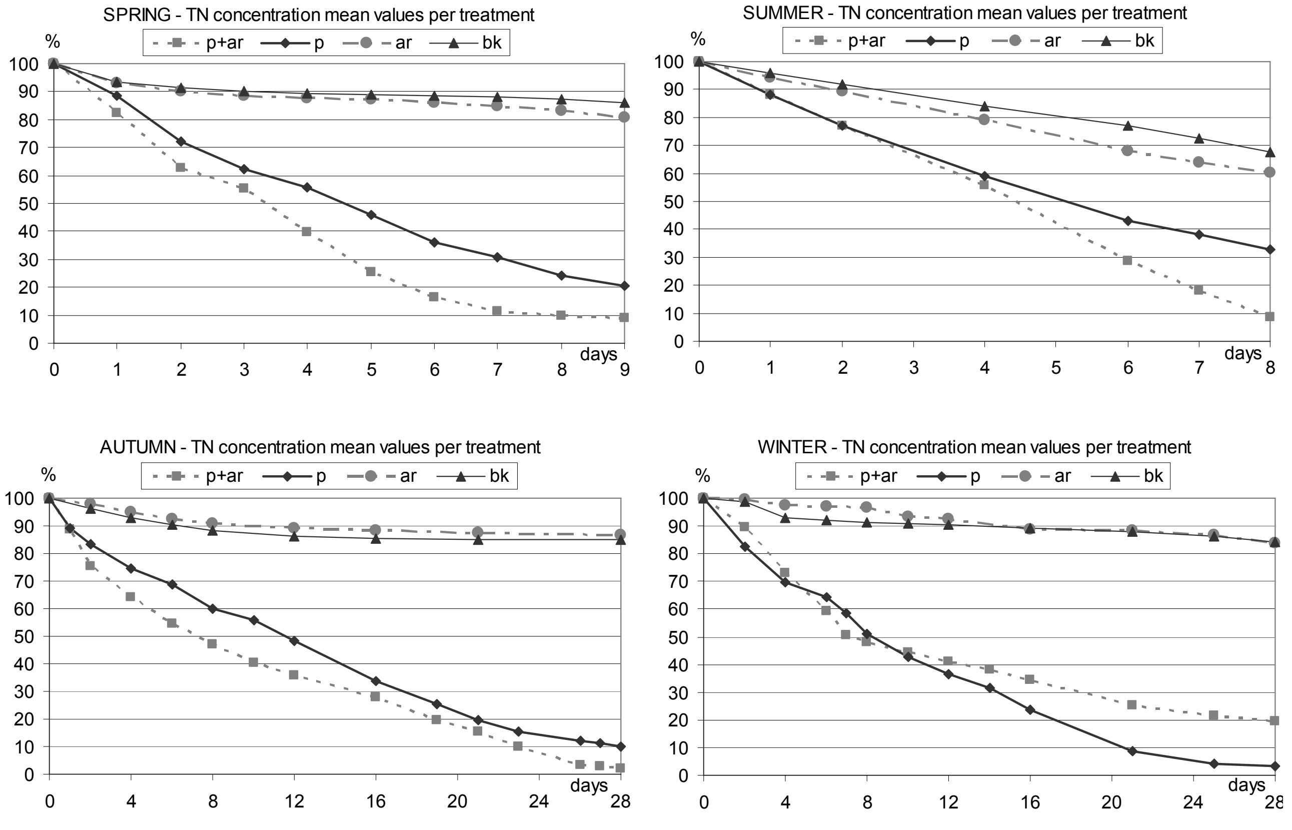
Season	Influent mean TN $(mg \cdot l^{-1})$	TN – Final mean Removal (%)				Experiment length (days)
		p+ar	p	ar	bk	
Spring	45.0	91.1±0.5	79.3±2.0	19.2±0.2	14.0±1.0	9
Summer	55.1	91.2±1.1	67.3±1.2	39.8±0.9	32.5±1.4	8
Autumn	112	97.8±0.4	90.2±0.7	13.2±0.6	15.0±0.9	28
Winter	76.6	80.6±0.3	96.7±0.3	16.3±0.5	15.9±0.7	28

differences between the $TN_f\%$ of planted and unplanted treatments were considerable; the lowest $TN_f\%$ recorded for treatment "ar" (60.2%, summer experiment) was much closer to the minimum $TN_f\%$ value of treatment "bk" (67.5% in summer) than to the highest $TN_f\%$ experiment values of treatments "p+ar" and "p" (19.4% in winter and 32.7% in summer, respectively). In the same way, "ar" $TN_f\%$ were lower than the ones recorded in "bk" EU for the spring and summer experiments, with statistically significant differences ($p<0.05$); however, for the autumn and winter experiments the differences between the two control treatments were not statistically significant ($p>0.05$).

Accordingly, the values found in this work show that EUs assembled as FHF systems have a better removal capacity of TN in comparison to unplanted EU regardless of the supplemental aeration (mean results of TN R% are detailed in Table 2), a finding that concurs with the conclusions of other experimental studies that aimed to evaluate the positive role of macrophytes in improving nutrient removal capacity of natural systems for wastewater treatment [24, 25].

Effect of Aeration on the TN Removal Efficiency of Planted EUs

In spring, summer, and autumn experiments, planted EUs with supplemental aeration (treatment "p+ar") achieved significantly lower $TN_f\%$ ($p<0.05$) in comparison to planted EUs without supplemental aeration (treatment "p"). The highest intra-season significant difference between planted treatments (treatment "p+ar" versus treatment "p") was recorded in summer, when the $TN_f\%$ of treatment "p+ar" was 23.9% lower than for treatment "p," and the lowest significant difference (7.6%) was recorded in autumn.



SPRING - TN concentration mean values per treatment

SUMMER - TN concentration mean values per treatment

AUTUMN - TN concentration mean values per treatment

WINTER - TN concentration mean values per treatment

Fig. 2. Mean values of TN% per treatment and season.

As regards the winter experiment, the TN_f% recorded in "p+ar" EUs were significantly ($p<0.05$) higher than the ones recorded in "p" EUs; therefore, the TN R% values of treatment "p+ar" in winter were lower (16.1%) than those of treatment "p," contrary to what had happened in the spring, summer, and autumn experiments.

Previous studies performed in other types of CW with 24 hours supplemental aeration also reported enhancements in TN removals, e.g. from 22% to 24% [26], 6% higher in a horizontal sub-surface flow (HSSF) CW aerated during the summer-autumn period [27], and 11% and 46% increases on year average in HSSF systems planted respectively with *Typha angustifolia* and *Phragmites australis*, the removal generally being higher during the summer [28]. However, TN removal enhancements were different when aeration was supplied to another type of CW (VSSF, vertical sub-surface flow) during a shorter daily period (8 h·d^{-1}). In this study, aeration significantly improved TN removal in spring and in winter (6.9%), but no significant differences were found for the summer and autumn TN removals [29].

Nitrification and Denitrification in Planted EUs. The Effects of Supplemental Aeration

For a comprehensive understanding of TN removal in the FHF system in the different seasons, N-NH$_4^+$ and N-NO$_3^-$ concentrations were also monitored in this study, in order to gain insights into nitrification and denitrification in FHF and to know whether their removal rates could be affected by supplemental aeration and, therefore, TN_f concentrations.

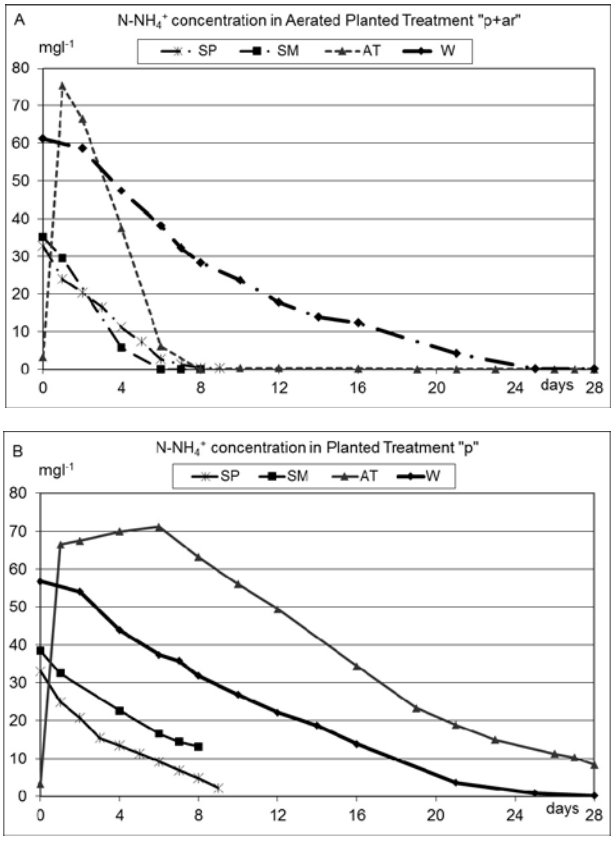


Fig. 3. Mean concentration of N-NH$_4^+$ per season: (SP) spring, (SM) summer, (AT) autumn, and (W) winter. (A): treatment "p+ar", floating helophytes filter (FHF) with supplemental aeration. (B): treatment "p," FHF without supplemental aeration.

In the spring, summer, and winter experiences, N-NH$_4^+$ mean concentration values of the influent ranged between 79.1% and 68.8% of their corresponding TN concentration values. Unlike those experiences, TN of autumn influent was poorly mineralized, N-NH$_4^+$ mean concentration value was 2.9% of TN concentration value. At day 1 it recorded a clear increase of N-NH$_4^+$ mean concentration, up to 76.8% of TN in "p+ar" treatment and 64.1% in "p" treatment, suggesting that most of the organic nitrogen of influent TN had been hydrolyzed.

Fig. 3 (A) shows that N-NH$_4^+$ concentration mean values decreased sharply in spring, summer, and autumn in treatment "p+ar", reaching < 10 mg/l mean value at day 6 and nearly 0 after day 8. In winter, 10 mg/l concentration was achieved after day 18, and about zero by day 23. These results suggest that the process of nitrification in treatment "p+ar" did not suffer any limitation in any of the seasons, although the differences in initial BOD$_5$ and TN loads and temperature variation among the four experiments seemed to have had an effect on seasonal N-NH$_4^+$ removal capacity. Regarding treatment "p," Fig. 3 (B), the mean values of N-NH$_4^+$ concentration decreased steadily throughout the four experiments. In the spring experiment, the mean concentration of <10 mg/l was achieved at day 6; however, it took more than 16 days to record values <10 mg/l in the winter experiment and more than 26 days in the autumn one, whereas for the summer experiment the final N-NH$_4^+$ mean concentration only reached 13.17 mg/l.

The mean values of final N-NH$_4^+$ R% were significantly higher for treatment "p+ar" than for treatment "p" in summer (34.4%), autumn (12.4%), and spring (5.9%). In the winter experiment, the difference between both treatments was low (0.1%) and not statistically significant ($p>0.05$).

Improvements in ammonia and/or ammonium removal due to supplemental aeration also have been recorded in other studies performed in various types of CW systems operating with other aeration schemes and environmental characteristics; e.g. in an 8 h/d aerated VSSF CW [29] significant increases in N-NH$_4^+$ removal in the aerated units were recorded: 7.8% in summer, 9.2% in autumn, and 15.0% in spring. The supply of external aeration during 12 h/d to a HSSF system in autumn and winter also enhanced N-NH$_4^+$ R% in comparison to the removal values recorded during spring and summer without aeration; in that study removal percentages ranged from 93% to 98% with aeration and from 14% to 40% without aeration [30]. The enhancement effect of aeration on nitrification with external aeration being supplied during 24 h has also been assessed: the estimated volumetric rate constant for N-NH$_3$ removal was greater with aeration than without it (5.7 day^{-1} vs. 0.52 day^{-1}) in a pilot VSSF CW operated in a warm environment (24.6ºC) [31]. The mean N-NH$_3$ removal percentage (aerated vs. non-aerated) was 42% higher in the aerated experimental surface flow (SF) CW placed in a greenhouse [32], N-NH$_3$ mean mass reduction was significantly increased (87% vs. 78%, $p<0.001$) by artificial aeration in another piece of research carried out with a field scale SF CW during a year-round study involving a wide range of temperatures, from -8.3ºC to 22ºC, although aeration was suspended from December 24 to April 1 [33], and N-NH$_4^+$ removal monitored in winter (mean 7ºC) and summer (mean 22ºC) was significantly higher ($p<0.05$ and $p<0.01$, respectively) in the aerated tanks of an experiment performed in HSSF aerated and non-aerated mesocosms [34].

The mean values of N-NO$_3^-$ concentrations recorded per treatment and season in this study are given in the graphs in Fig. 4. The mean values of N-NO$_3^-$ concentration for treatment "p" recorded throughout the experiment duration were low and stayed within a narrow range of values in all seasons; they ranged from 0.00 ppm to 1.34 ppm. This suggests that the denitrification bacteria population in treatment "p" EUs can transform most N-NO$_3^-$ produced by nitrification in any season, even in winter, when *Typha* remains dormant and low temperatures occur.

Fig. 4 (A) shows that the mean values of N-NO$_3^-$ concentration recorded in treatment "p+ar" were not maintained within a narrow range, except in the spring experiment. In the summer, autumn, and winter experiments, low N-NO$_3^-$ concentration levels were found only in the first days of these experiments; later on, N-NO$_3^-$ concentration peaks were recorded. These higher N-NO$_3^-$ concentration values were dramatically reduced during the final stage of the autumn and summer experiments but not for the winter experiment, in which N-NO$_3^-$ accumulation was found at the end of the experiment.

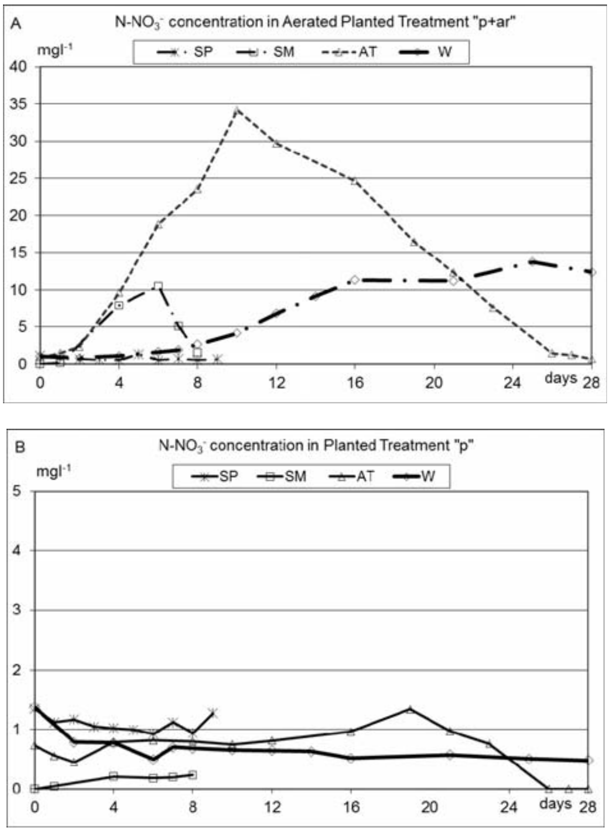


Fig. 4. Mean concentrations of N-NO$_3^-$ per season: (SP) spring, (SM) summer, (AT) autumn, and (W) winter. (A): treatment "p+ar", floating helophytes filter (FHF) with supplemental aeration. (B): treatment "p," FHF without supplemental aeration.

Effluent nitrate accumulation has been also reported in some of the above-mentioned studies, e.g., when aeration was supplied 24 h/d [27, 28, 32], as well as in a study with 12 h/d supply [30]. In this latter experiment, the effluent N-NO$_3^-$ accumulation recorded during the first year of operation with aeration was also higher in winter (75 mg/L) and lower in summer (21 mg/L), though after that year values went eventually down (5 mg/L). There are also studies reporting no significant difference between N-NO$_3^-$ contents at the outlet of aerated and non-aerated planted treatments. For instance, no N-NO$_3^-$ accumulation was recorded in any season for an experiment with a shorter aeration period (8 h/d) [29]; the same happened in the aerated (24 h/d) pilot HSSF cells of an experiment operating in winter and summer [34].

Effects of BOD$_5$:TN Ratio on N-NO$_3^-$ Removal in Planted EUs

Denitrification in wetlands is favoured by high nitrate concentrations [35]; therefore, the accumulation of N-NO$_3^-$ recorded in treatment "p+ar" samples taken during the last days of the winter experiment was probably caused by another denitrification limiter like a scarcity of organic carbon (it is considered that 5-9 mg BOD$_5$ are needed per NO$_3^-$ mg to be denitrified) or the presence of high levels of O$_2$ (oxygen solubility in water is higher when temperature is low); besides, low temperatures have an effect on nitrate removal (denitrification is optimal when temperatures are between 25ºC and 65ºC but severely declines for temperatures >65ºC or <5ºC) [36, 37].

The results of N-NO$_3^-$:BOD$_5$, N-NO$_3^-$:TN, BOD$_5$:TN ratios, BOD$_5$ and TN R% and wastewater DO, temperature and pH are detailed in Table 3.

In the spring and summer experiments, final BOD$_5$ R% was higher in treatment "p+ar" than in treatment "p" (97.3% vs. 90.4% and 96.6% vs. 85.8%, respectively). In the autumn experiment, final BOD$_5$ R% in both planted treatments was similar (93.3% vs. 93.7%), but the trend observed was different: most BOD$_5$ was removed sooner in treatment "p+ar" than in treatment "p"; at day 6 treatment "p+ar" achieved 97.1% BOD$_5$ R% vs. 64.8% in treatment "p." In the winter experiment, the final BOD$_5$ R% of treatment "p+ar" and treatment "p" were similar as well (97.0% and 97.9%, respectively), and again the period needed to achieve high values of BOD$_5$ R% was shorter for treatment "p+ar" than for treatment "p"; e.g. at day 10, BOD$_5$ R% was 89.6% for treatment "p+ar" vs. 66.8% for treatment "p" (Fig. 5).

Table 3. Mean removal percentages (R%) of BOD_5 and TN, mean values of the concentration ratios $N\text{-}NO_3^-$:TN, $N\text{-}NO_3^-$:BOD_5, and BOD_5:TN, and mean values of DO, T_w and pH for treatment "p+ar" and treatment "p." Some values in the autumn experiment are underlined to highlight the fact that supplemental aeration was interrupted between days 16 and 28.

EXP	Day	Mean BOD_5 R%		Mean TN R%		Mean $N\text{-}NO_3^-$:TN		Mean $N\text{-}NO_3^-$:BOD_5		Mean BOD_5:TN		Mean DO mg·L^{-1}		Mean T_w ºC		Mean pH	
		p+ar	p	p+ar	p	p+ar	p	p+ar	p	p+ar	p	p+ar	p	p+ar	p	p+ar	p
Spring	0	0.0	0.0	0.0	0.0	0.03	0.03	0.00	0.01	5.49	5.23	0.1	0.1	23.0	22.7	7.3	7.3
	2	85.3	69.9	44.5	37.6	0.02	0.03	0.02	0.02	1.32	2.19	0.0	0.1	21.8	21.8	7.5	6.9
	6	94.4	91.8	83.5	63.9	0.08	0.06	0.04	0.05	1.98	1.17	1.5	1.2	16.5	17.0	7.1	6.9
	7	98.1	88.9	88.5	69.4	0.15	0.08	0.20	0.05	0.84	1.80	0.5	0.4	19.1	19.4	7.1	7.1
	8	98.7	96.2	90.0	75.9	0.12	0.08	0.27	0.27	0.78	0.82	0.5	0.5	22.2	22.4	7.3	7.1
	9	97.3	90.4	91.1	79.3	0.17	0.13	0.04	0.06	1.72	2.41	0.4	0.5	24.3	23.8	7.3	7.1
Summer	0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00	2.86	2.69	1.7	0.7	23.8	23.7	7.4	7.3
	2	79.9	75.8	23.1	23.0	0.19	0.00	0.25	0.01	0.75	0.85	2.9	0.1	23.2	22.1	7.1	7.2
	6	90.3	84.5	71.3	57.0	0.50	0.01	0.70	0.01	0.98	0.96	4.7	1.9	20.9	20.1	7.1	6.9
	8	96.6	85.8	91.2	67.3	0.32	0.01	0.16	0.01	1.13	1.17	3.4	0.6	23.1	21.9	6.8	7.1
Autumn	0	0.0	0.0	0.0	0.0	0.01	0.01	0.01	0.00	1.56	1.55	0.1	0.0	18.5	18.5	8.8	8.6
	2	38.5	32.6	24.5	16.8	0.03	0.00	0.02	0.00	1.28	1.26	0.6	0.1	19.0	19.0	7.7	7.4
	6	97.1	64.8	45.5	31.4	0.31	0.01	7.82	0.01	0.08	0.81	1.1	0.1	19.6	19.5	6.5	6.9
	16	99.6	88.0	72.1	66.2	0.80	0.02	3.97	0.04	0.02	0.56	0.8	0.7	16.3	16.3	7.1	7.0
	28	93.3	93.7	97.8	90.2	0.30	0.00	0.06	0.00	4.96	0.99	0.6	0.0	16.7	16.7	7.0	7.0
Winter	0	0.0	0.0	0.0	0.0	0.01	0.02	0.01	0.01	1.17	1.22	0.6	0.8	6.6	7.4	8.6	8.6
	2	51.9	26.8	10.5	17.5	0.01	0.01	0.02	0.01	0.63	1.08	3.9	0.2	8.7	8.7	8.3	7.9
	10	89.6	66.8	55.5	57.5	0.12	0.02	0.43	0.02	0.27	0.96	6.8	0.8	6.1	6.3	8.0	7.3
	16	95.9	95.0	65.6	76.2	0.44	0.03	2.71	0.12	0.14	0.26	5.9	1.3	5.7	6.3	7.4	7.0
	28	97.0	97.9	80.6	96.7	0.83	0.19	5.28	0.28	0.17	0.80	3.6	2.7	7.3	6.8	6.6	6.8

From these results it can be deduced that supplemental aeration promotes higher BOD_5 removals, concurring with previous studies that reported significant differences in BOD_5 R% due to external aeration, e.g., 8.8% enhancement in summer and autumn experiments and 13.2% in winter and spring [29], 21% improvement in summer, 14% in autumn, 7% in winter, and 16% in spring [30]. However, no significant difference ($p=0.127$) between aerated and non-aerated treatments has been reported for final BOD_5 R% in a planted surface flow experiment [33].

The mean values of initial BOD_5:TN ratio in treatment "p+ar" for the spring, summer, autumn, and winter experiments were 5.49, 2.86, 1.56, and 1.17, respectively. Regarding treatment "p," the values found were very similar: 5.23, 2.69, 1.55, and 1.22. The initial BOD_5 was more than 2.5 times the TN influent concentration in the spring and summer experiments and less than 2.5 times in the winter and autumn ones.

The final values of BOD_5:TN ratio were >1 for treatment "p+ar" in the spring and summer experiments and <1 in the winter. In the autumn experiment, BOD_5:TN ratio decreased from the beginning of the experiment until day 16 (BOD_5:TN = 0.02) and then increased to 4.96 (day 28), coinciding with the interruption of the supplemental aeration on day 16, which may have caused a slow-down of BOD_5 removal; this fact, added to the decay of plant material, could have contributed to higher BOD_5 levels supporting denitrification at the end of the autumn experiment [38]. Regarding treatment "p," the final values of BOD_5:TN ratio were also >1 in the spring and summer experiments and <1 in the autumn and winter ones.

When the initial content in BOD_5 was over 2.5 times the initial TN concentration (spring and summer experiments), the high values of $N\text{-}NO_3^-$:TN ratio in treatment "p+ar" did not prevent it from achieving a better final TN R% than treatment "p" (91.1% vs 79.3% in the spring experiment and 91.2% vs. 67.3% in the summer one), unlike the winter experiment (80.6% vs. 96.7% TN R% for treatment "p+ar" and treatment "p," respectively) when the influent BOD_5 content was lower, as was the influent BOD_5:TN ratio (1.17). These results suggest that the external aeration causes a shortage of carbon available for the process of denitrification when it is supplied to an influent with similar values of BOD_5 and TN content, since aeration promotes a faster NH_4^+ transformation and BOD_5 removal, resulting in NO_3^- accumulation and less efficient TN removal. The lack of bioavailable C produced by aeration has been noted previously in other studies as one of the probable causes limiting denitrification in aerated experimental CWs [27, 28, 32].

High DO values were recorded in treatment "p+ar" when BOD_5 R% reached about 90%, concurring with N-NO_3^-:TN ratios that clearly indicated that N-NO_3^- was accumulating; DO values were particularly high (5.9 and 3.6 mg/L) during the last days of the winter experiment when N-NO_3^- concentration was high. DO values in treatment "p" were generally lower; the highest value (2.7 mg/L) was recorded at the end of the winter experiment when BOD_5 R% reached 97.9% and TN R%, 96.7%. Temperature and pH values in the winter experiment did not constitute limiting values for the denitrification process although temperatures were not as favorable as in the other three experiments.

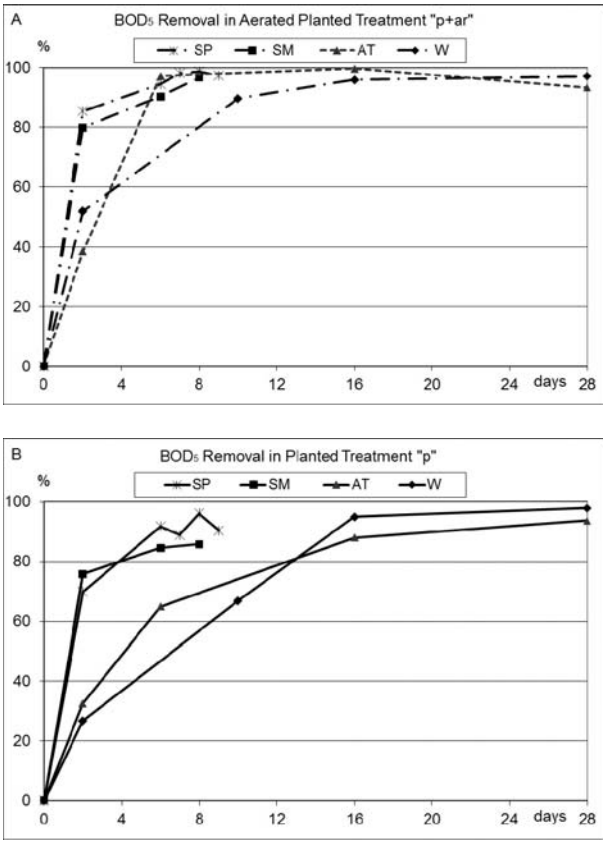


Fig. 5. Mean removal percentage (R%) of BOD_5 per season: (SP) spring, (SM) summer, (AT) autumn, and (W) winter. (A): treatment "p+ar", floating helophytes filter (FHF) with supplemental aeration. (B): treatment "p," FHF without supplemental aeration.

Conclusions

The FHF system has proved to remove TN reliably in all experiments carried out in this work – even during the helophyte dormancy period – achieving removal percentages from 67.3% in summer to 96.7% in winter for the different influent TN and residence times considered in this study. The continuous supply of external aeration during 12 hours per day significantly enhanced TN removal in spring, summer and autumn; however, in contrast, aeration seemed to be disadvantageous in winter because it led to a nitrate accumulation. Unfavorable values of BOD_5:TN ratio in winter could have limited the denitrification process in conjunction with high DO and low temperatures. Further research is needed to assess different winter aeration schemes, particularly in influents with similar BOD_5 and TN concentration values.

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