Original Research

Pathways of Nitrogen Removal in Hybrid Treatment Wetlands

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Abstract

Hybrid treatment wetlands (HTWs) are composed of two or more filters with different modes of flow, allowing the benefits of both types of bed to be combined, resulting in better effluent quality (nitrogen and organic compound removal). Such a heterogeneous environment creates possibilities for different mechanisms of nitrogen removal. The objective of the present study was to investigate the removal of nitrogen versus a range of parameters such as hydraulic and organic loading, redox potential, pH, alkalinity, availability of easily decomposable organic compounds, and theoretical nitrogenous biological oxygen demand, in order to evaluate nitrogen conversion processes. The studies were carried out in a full-scale HTW designed for 200pe, over a two-year monitoring period. The HTW achieved stable and effective removal of all analyzed pollutants: BOD_5 was 97.9% for average load of 3.4 $gm^{-2}\cdot d^{-1}$ and 95.1% COD removal for average load of 6.9 $gm^{-2}\cdot d^{-1}$ (for the entire HTW). Unexpectedly efficient N removal was observed in the first stage of the treatment plant. The removal efficiency of TKN was over 46% in this horizontal sub-surface flow bed and was coupled with 80.5% BOD removal, suggesting heterotrophic competition for oxygen, the supply of which was strongly limited in this stage of treatment.

Keywords: horizontal and vertical sub-surface flow, wastewater, organics, nitrogen compound depletion, pollutant load rate

Introduction

Treatment wetlands (TWs) are designed to take advantage of many of the same processes that occur in natural wetlands, but in a more controlled environment. Wastewater treatment in TWs is a result of the interaction between plants, soil and microorganisms. Vertical flow beds (VF) are characterized by different oxygen and hydraulic conditions from horizontal flow beds (HF), thus they can favor another mechanism of pollutant removal. Therefore, using both types of TW within a treatment system provides a heterogeneous environment that creates possibilities for different mechanisms of nitrogen removal, including volatilization, ammonification, nitrification-denitrification, plant uptake, and matrix adsorption.

The sequence of a biological aerobic, autotrophic oxidation of ammonium to nitrite and then to nitrate (nitrification), followed by obligate anoxic, heterotrophic reduction of nitrate (denitrification) is generally considered to be the main process of nitrogen elimination [1, 2]. The physical and chemical variability in TWs enables simultaneous nitrification-denitrification [3-7].

During the last twenty years, alternative microbial pathways for nitrogen depletion have been reported, including: heterotrophic nitrification, anaerobic ammoni-

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Table 1. The characteristics of Wiklino HTW.

WWTP	Flow	Configuration	Effective grain size	HRT	Area	Depth	Hydraulic load	Unit area
	[m^3d^{-1}] (pe*)		[mm]	[d]	[m^2]	[m]	[$mm \cdot d^{-1}$]	[$m^2 \cdot pe^{-1}$]
Wiklino	20.5 (220)	HF I	2-6	12.3	1050	0.6	19.5	4.7
		VF	2-6	-	312	0.4	65.7	1.4
		HF II	2-6	6.3	540	0.6	38.0	2.4
Total					1902			8.5

*pe – person equivalent

um oxidation (ANAMMOX), and oxygen-limited autotrophic nitrification-denitrification [8, 9]. Recently, many alternative processes of nitrogen removal have been found in nature, e.g. in marine sediments or anoxic water columns [10, 11]. Ammonium oxidizers have a more versatile metabolism than was previously assumed – the aerobic nitrifier and ANAMMOX bacteria may be natural partners in many oxygen-limited environments. In 2007, Dang and Sun confirmed CANON (completely autotrophic nitrogen-removal over nitrite) deammonification, responsible for nitrogen loss in pilot studies with landfill leachate. The results of their studies proved that TWs can achieve treatment of high ammonia and low organic content wastewater [12].

According to Dong and Sun [12], who carried out experiments in a full-scale TW treating domestic wastewater in France, ANAMMOX bacteria can coexist with heterotrophic bacteria. In appropriate conditions, heterotrophic bacteria can consume oxygen and produce a low-oxygen environment for ANAMMOX, as well as reduce nitrate to nitrite to promote the ANAMMOX process. Furthermore, the authors observed that the ANAMMOX process was still working even when the wastewater temperature decreased below 20ºC.

Thus, TWs provide a variety of environments in which nitrogen removal is influenced by multiple factors such as design parameters, hydraulic and organics loads, dissolved oxygen, and pH. However, it is still not clear precisely which factors are responsible for which pathway of nitrogen removal in TWs.

The hypothesis of this study was that Hybrid Treatment Wetlands (HTWs) provide a variety of conditions, thus enhancing different mechanisms of nitrogen removal such as short-circuiting the classical nitrification-denitrification pathway. The study was undertaken on a full scale HTW designated for domestic wastewater treatment.

The objective of this study was to examine the removal of nitrogen with variations in accompanying parameters, such as hydraulic and organic loading, dissolved oxygen concentration, redox potential, pH, alkalinity, availability of easily degradable organic compounds and theoretical nitrogenous biological oxygen demand, in order to evaluate the nitrogen conversion processes. Simplified mass balance analyses were used to identify the processes responsible for nitrogen depletion in each stage of treatment.

Description of Wetland and Method

The studies were carried out on a full-scale hybrid treatment wetland (HTW) situated in the village of Wiklino near Słupsk in northern Poland. After treatment in a three-chamber settlement tank (with two days hydraulic retention time) the wastewater was pumped into the wetland responsible for biological treatment. This system consisted of two HF beds (HFI and HFII), one before and the other following a VF. The basic characteristics of the applied effluent are presented in Table 1.

The HTW was built in 1995 and, since 2000, two parallel beds of the VF stage have been operated with intermittent loading. The facility was designed for 200pe, but from 2006 served 220pe. At the time of the study, the HTW was 95% covered by *Phragmites australis* with a plant density of 100-150 plants per m^2. The remaining surface was planted with *Typha latifolia* and sedges (*Carex* spp.).

The granulometric and permeability coefficient analyses of the filtration bed media were performed in July 2007. Two types of soil sample were collected: disturbed-structure samples, for grain size analyses; and undisturbed-structure samples, for the measurement of permeability coefficients. The measurements were carried out according to Polish Norm PN-88/B0481.

The matrix medium was coarse sand with porosity, ε_g=0.4 and permeability coefficient k=110 md^{-1} (according to the Hazen eq.). The coefficient of particle uniformity, $U=d_{60}/d_{10}$, was equal to 0.35.

In spring 2007 piezometers were installed approximately half-way along each of the horizontal flow beds in order to enable the taking of intermediate samples. Daily wastewater discharge volume was calculated based on pump capacity and pump operating period. Samples were collected every two or three weeks during the period June 2007 to July 2009 (n=27, where "n" is the number of sampling events). Composite samples (6 h mixed) were taken at influent and final effluent, after each stage of treatment and at intermediate points located within the beds, following the hydraulic retention time in each stage of treatment (influent $\Rightarrow$ after 6 days ½ HF I $\Rightarrow$ after next 12 days effluents from HF I and VF $\Rightarrow$ after 15 days ½ HF II $\Rightarrow$ and after 18 days HF II effluent, according to Table 1).

The following parameters were analyzed: organic matter expressed as BOD_5 and COD, TSS, NH_4^+-N, NO_3-N, NO_2-N, and total Kjeldahl nitrogen (TKN). COD and TKN were also analyzed after filtration through a 0.45 μm membrane (millipore nitrocellulose filter) in the aqueous phase. In addition, the volatile suspended solids content of the total suspended solids was determined, by loss on ignition. Alkalinity and pH were also measured as nitrogen transformation indicators. The procedure used was that of Hach Chemical Company (Hach, Loveland, Co) and Dr Lange GmbH (Germany). All analyses were carried out according to the Polish Standard Methods as well as U.S standards [13, 14]. The wastewater and air temperature, pH as well as dissolved oxygen and redox potential, were measured at the sampling points onsite, using a measuring probe (WTW Multi 340i/SET).

Data Analysis and Process Modeling

Water budgets were constructed for each stage of the HTW. Evapotranspiration was estimated from loss of flow between each step.

Total nitrogen (TN) was calculated from the sum of TKN and NO_x-N. Organic nitrogen (Org-N) was calculated from TKN minus NH_4^+-N. Theoretical NBOD (nitrogenous biological oxygen demand) was taken to be 4.57 times TKN, according to Kadlec and Knight, 1996 [15].

Removal efficiency (η) for each treatment stage was calculated from:

$$\eta = (L_{inf}-L_{eff})/L_{inf}$$

...where L_{inf} and L_{eff} are the influent and effluent pollutant loads, respectively.

Mass removal rate (MRR) was calculated on the basis of the following equation:

$$MRR = [(C_{inf}Q_{inf}) - (C_{eff}Q_{eff})]/A \quad [gm^{-2}\cdot d^{-1}]$$

...where:

A – area of CW [m^2]

Q_{inf} and Q_{eff} – average influent and effluent flow rates, respectively [$m^3\cdot d^{-1}$]

C_{inf} and C_{eff} – average influent and effluent pollutant concentrations, respectively [mgL^{-1}]

In order to estimate removal and transformation rates, the decay rates of organic matter (k_{pBOD}), total nitrogen removal (k_{pTN}), and organic nitrogen mineralization (k_{pMIN}) were calculated. It was assumed that the decay rates can be described by a first-order reaction constant [15-17]. Based on the retention time and concentrations of organic matter and nitrogen species, the constant decay rates for the sewage treated in the HF beds were calculated and the corresponding modified decay rates for sewage treated in the VF were calculated [18]. The values of the temperature-related decay rates ($k_{p(T)}$) were calculated using the relationship $k_{p(T)}=k_{p(20)}(1.1)^{T-20}$, using the average monthly temperature of wastewater.

The results were evaluated using StatSoft STATISTICA 8.0.

Table 2. The characteristics of wastewater discharged to HTW in Wiklino.

Parameter	Unit	HF I Influent			
		mean	SD	median	min-max
Flow	$m^3\cdot d^{-1}$	20.5	0.7	20.3	19.9-22.1
pH	-	6.9	0.06	7.0	6.8-7.1
TSS	mgL^{-1}	392.9	79.8	348.4	162.1-922.4
VSS	mgL^{-1}	269.5	88.7	195.5	125.6-644.3
TN	mgL^{-1}	130.5	8.1	129.1	119.9-146.7
NH_4^+-N	mgL^{-1}	86.2	17.5	95.1	57.1-105.4
Org-N	mgL^{-1}	43.5	15.87	49.9	23.3-66.2
NO_3-N	mgL^{-1}	0.9	0.5	0.7	0.1-1.8
COD	mgL^{-1}	660.3	212.6	604.8	382.1-965.3
COD_f	mgL^{-1}	283.6	69.1	280.4	184.4-600.6
BOD	mgL^{-1}	323.8	126.2	280.6	194.8-500.7
Alkalinity	$mvalL^{-1}$	13.6	0.7	13.6	12.3-14.0
Ts	ºC	12.7	5.7	12.5	8.1-17.5
O_2	mgL^{-1}	0.5	0.2	0.4	0.0-0.9
Redox	mV	-242.7	21.8	-240	-301.5 – -140.5

Results and Discussion

Characteristic of Wastewater Influent

The characteristics of influent wastewater, means with standard deviation and medians, as well as the range with regard to studied parameters are presented in Table 2.

The inlet concentrations of determinants were much higher in comparison to the values given in the literature for domestic wastewater discharged to TWs [5, 23, 24].

Vymazal [5] reported influent BOD_5 concentrations almost four times lower and influent TSS over six times lower than those recorded here. Only in France, where raw sewage is treated in two-stage VF beds, have influent concentrations of BOD and TSS been observed similar to those of this study [12].

The concentration of TN in the influent was two to three times higher than that previously reported [5, 12, 23, 24]. Possible reasons for the observed higher pollutant concentrations are: lower water consumption per population equivalent (about 100 $L\cdot d^{-1}$), lack of rainwater infiltration to the sewer system, and the observed improper operation of the septic tank (causing high TSS discharge to the HCW). The nitrogen in the influent was present mainly in the form of NH_4-N (about 65%) and Org-N (over 34%), which confirmed that the septic tank provided a good environment for ammonification.

The hydraulic and pollutant loads discharged to the first stage of treatment were appropriate to protect the TW

Table 3. The characteristic of wastewater and efficiency of pollutant removal in the first-stage of HTW.

Parameter	Unit	½ HF I				HF I Effluent			
		Mean	SD	Median	min-max	mean	SD	median	min-max
Flow	$m^3 \cdot d^{-1}$	-	-	-	-	19.3	0.8	19.5	18.7-20.8
pH	-	7.0	0.1	7.0	6.8-7.1	7.05	0.2	7.1	6.9-7.4
TSS	mgL^{-1} (%)	139.4 (64.5)	16.5	138.2	116.5-166.4	80.0 (42.6)	48.6	84.6	24.6-132.6
VSS	mgL^{-1} (%)	40.1 (85.1)	20.6	42.0	10.4-66.6	43.3	33.8	28.5	10.2-96.1
TN	mgL^{-1} (%)	75.7 (41.9)	19.7	71.6	51.3-110.5	72.1 (4.7)	18.1	68.3	44.2-92.5
NH_4^+-N	mgL^{-1} (%)	47.6 (44.8)	13.3	47.3	27.2-63.8	50.8 (-)*	16.9	54.7	26.1-70.3
Org-N	mgL^{-1} (%)	22.2 (48.9)	14.7	18.9	7.1-49.3	19.1 (13.9)	10.3	18.9	6.6-33.7
NO_3-N	mgL^{-1} (%)	5.5	4.1	5.9	1.2-9.7	1.1 (80)	0.6	1.3	0.2-2.2
COD	mgL^{-1} (%)	404.3 (38.8)	113.3	417.9	196.0-530.0	234.1 (42.1)	126.6	167.2	110.4-425.7
COD_f	mgL^{-1} (%)	135.7 (52.2)	61.9	124.2	79.6-256.0	56.4 (68.4)	19.7	62.2	22.3-78.6
BOD	mgL^{-1} (%)	63.1 (80.5)	38.5	59.0	10.2-72.9	33.5 (46.9)	24.6	30.3	10.5-73.5
Alkalinity	$mvalL^{-1}$	8.0	0.5	7.9	7.0 -8.6	9.4	2.6	10.8	5.2-12.3
Ts	ºC	13.1	6.1	13.5	7.1-18.5	11.7	5,6	12.0	8.0- 16.2
O_2	mgL^{-1}	0.9	0.7	1.0	0.2- 1.9	1.8	1	1.5	0.6 - 2.1
Redox	mV	-223.5	28.8	-220	-268.5 – - 110.5	-87.3	14.2	-85	-146.5 – - 25.5

( ) – efficiency removal
*increase of pollutant concentration

against clogging, and were consistent with those reported [5, 6, 12, 23, 24].

The COD/BOD and BOD/N ratios provided information about bioavailability for microbiological transformations. Additionally, COD_f/BOD provides an indication of readily biodegradable dissolved organic matter [25]. The wastewater was characterized by a typical COD/BOD ratio of about 2.0 and a BOD/N of about 2.5. Furthermore, the COD_f/BOD ratio was less than 0.9, suggesting that organic matter was mostly present in the readily biodegradable dissolved fraction. The high content of VSS in the TSS indicated that most of the balance of the BOD was present as decomposable suspended organic matter [25].

First-Stage Treatment

The concentration of all pollutants rapidly decreased in HFI. In the first piezometer (½HFI), the concentration of organics was half the inlet concentration (Tables 2 and 3) and showed further gradual, significant reduction in subsequent stages of treatment (Tables 3-5).

The effective removal of organic load was accompanied by a high reduction of Org-N and NH_4^+-N in the first stage of treatment. Removal of Org-N and NH_4^+-N upstream of sampling point ½HFI was 48.9% and 44.8%, respectively, while TN removal was 41.9%. NO_3-N concentration at ½HFI averaged 5.5 mgL^{-1} (Table 3). This value decreased to 1.1 mgL^{-1} before the HFI effluent, associated with a reduction of alkalinity from 13.6 to 9.4 $mvalL^{-1}$, which is typical of conventional nitrification. However, the pH rose from 6.9 to 7.05, a relatively small increase. According to Tanner et al. [6] fluctuations in alkalinity and pH in wastewater treated in TWs may depend on the buffering capacity of the wastewater as well as decomposition processes (e.g. organic acid generation from anaerobic fermentation). In addition, H^+ release from plant roots assimilating NH_4^+-N may have contributed to the observed pH drop.

The concentration of dissolved oxygen in the influent was close to zero (Table 2). Consequently, in HFI anaerobic conditions were found, with an average redox potential of about -242.7 mV. Such conditions do not favor conventional nitrification processes. The oxygen demand for nitrification of the HFI TKN (NBOD) was calculated to be 5.2 $gm^{-2} \cdot d^{-1}$. Thus total oxygen demand for this bed was 13.3 $gO_2\ m^{-2} \cdot d^{-1}$ (with mineralization of COD – Tables 3 and 6). Estimates of oxygen release rates from the subsurface parts of emergent wetland plant species range from 0.5 to 6.0 $gO_2\ m^{-2} \cdot d^{-1}$, while direct diffusion of oxygen from the air, in conditions of horizontal flow constructed wetlands, can be estimated to supply a further 0.11 $gO_2\ m^{-2} \cdot d^{-1}$ [15]. This gives a theoretical oxygen supply in the range 0.61-6.11 $gO_2\ m^{-2} \cdot d^{-1}$, against a demand of 13.3 $gO_2\ m^{-2} \cdot d^{-1}$.

The above assumptions lead to the conclusion that dissolved oxygen in HFI was very limited and that the N transformations observed must have occurred via shortened pathways with reduced overall oxygen requirements.

Table 4. The characteristics of wastewater and efficiency of pollutant removal in the second-stage of HTW.

Parameter	Unit	VF Influent				Efficiency, %
		Mean	SD	median	min-max	
Flow	$m^3 \cdot d^{-1}$	18.1	1.0	18.3	17.1-19.4	-
pH	-	7.1	0.23	7.1	6.7-7.4	-
TSS	mgL^{-1}	64.5	24.9	67.8	22.4-96.4	19.4
VSS	mgL^{-1}	23.6	7.6	25.0	14.2-36.9	45.5
TN	mgL^{-1}	49.0	18.3	47.8	14.7-73.5	23.0
NH_4^+-N	mgL^{-1}	33.7	16.6	33.5	7.6-53.5	33.7
Org-N	mgL^{-1}	7.9	3.4	8.9	4.2-14.1	58.6
NO_3-N	mgL^{-1}	9.1	5.4	9.1	4.2-19.8	-
COD	mgL^{-1}	75.4	31.3	76.8	35.6-115.5	67.8
COD_f	mgL^{-1}	47.0	23.3	46.4	10.0-78.6	16.7
BOD	mgL^{-1}	14.7	6.6	15.6	5.7-23.5	56.1
Alkalinity	$mvalL^{-1}$	8.5	0.7	8.6	7.7-9.5	-
Ts	ºC	9.9	5.6	10.0	8.5-11.7	-
O_2	mgL^{-1}	3.9	1.1	3.8	2.8 - 4.9	-
Redox	mV	210.5	24.5	212.0	105-320	-

( ) – efficiency removal

In the CANON process aerobic and anaerobic bacteria coexist due to oxygen and oxygen-free microzones [3, 4, 26]. Thus, ammonia is partially oxidized under oxygen-limited condition to nitrite. Then nitrite, together with residual ammonia, is converted to dinitrogen gas by the ANAMMOX bacteria.

The nitrogen mass balance for the HFI bed is consistent with the existence of a combination of the CANON and ANAMMOX processes, which can be expressed by the following stoichiometric equation [9, 10, 26]:

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+$$

After the alkalinity decrease in HFI and for every milligram decrease of NH_4^+, there were 0.47 mg N_2 and 0.13 mg NO_3^- released (Tables 2 and 3).

Second and Third Stage of Treatment

The VF effluent NO_3-N concentration was increased significantly compared to that of the inlet, and NO_3-N constituted up to 20% of N species (Fig. 1). The dissolved oxygen concentration averaged 3.9 mgO_2 L^{-1} and the redox potential +210.5 mV, indicating appropriate conditions for aerobic autotrophic nitrification (Table 4).

As in HFI, the highest removal efficiency was observed for Org-N and the lowest for TN, suggesting that only mineralization and nitrification processes were occurring. The oxygen demand for the observed removal of both TKN and COD required 17.6 mgO_2 $m^{-2} \cdot d^{-1}$. The VF provided adequate oxygen for this removal, owing to the use of intermittent introduction of effluent to the bed and the surplus oxygen dissolved in the wastewater [5, 27].

Well aerated wastewater, rich in NO_3-N, entered the last stage of treatment, where further gradual removal of both organic matter and N species took place. A 55% decrease of NH_4-N was observed in the first part of HFII, upstream of sampling point ½HFII (Tables 4 and 5).

NH_4^+-N removal decreased to 31% in the distant half of HFII, this percentage removal being similar to that of TN, Org-N and NO_3-N. Both BOD and COD_f were present in each stage of treatment, indicating the availability of organic carbon, allowing denitrification.

In HFII, both pH and alkalinity decreased and the redox potential was such that nitrification was supported.

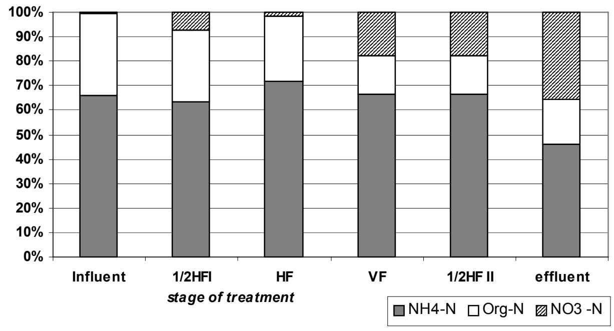

Fig. 1. Composition changes of N species through the HTW.

Table 5. The characteristic of wastewater and efficiency of pollutant removal in the third-stage of HTW.

Parameter	Unit	½ HF II				HF II Effluent			
		Mean	SD	median	min-max	mean	SD	median	min-max
Flow	$m^3 \cdot d^{-1}$	-	-	-	-	16.9	1.1	17.3	15.9-18.1
pH	-	6.9	0.3	7.0	6.5-7.3	6.9	0.3	6.9	6.6-7.2
TSS	mgL^{-1} (%)	42.1 (34.7)	20.8	43.5	18.5-70.3	25.1 (93.6)	11.1	28.3	6.5-40.1
VSS	mgL^{-1} (%)	16.5 (30.1)	12.3	12.0	1.2-39.1	9.7 (96.4)	6.7	6.6	1.2-29.7
TN	mgL^{-1} (%)	32.3 (34.1)	12.0	35.8	15.1-48.1	22.8 (82.5)	6.3	20.9	15.9-34.5
NH_4^+-N	mgL^{-1} (%)	15.3 (54.6)	9.8	12.4	1.3-31.2	10.5 (87.8)	4.9	10.1	0.7-15.3
Org-N	mgL^{-1} (%)	5.8 (26.6)	3.8	4.8	0.7-13.0	4.2 (90.3)	3.6	4.0	2.4-9.1
NO_3-N	mgL^{-1}	11.1	5.8	10.3	2.0-19.7	8.2	5.3	6.8	1.9-15.6
COD	mgL^{-1} (%)	72.3 (4.1)	18.4	62.8	57.6-98.7	59.6 (95.1)	29.8	45.7	30.4-104.3
COD_f	mgL^{-1} (%)	43.1 (8.3)	18.6	40.5	14.2-67.8	26.6 (95.6)	14.8	24.1	3.8-54.5
BOD	mgL^{-1} (%)	9.8 (10.2)	7.6	7.8	2.8-25.8	6.7 (97.9)	3.8	4.7	3.6-12.4
Alkalinity	$mvalL^{-1}$	9.1	0.8	8.9	7.9-10.0	7.4	1.0	7.8	6.7-8.3
Ts	ºC	10.7	5.8	10.0	8.4- 13.2	11.1	5.6	10.5	8.2-13.1
O_2	mgL^{-1}	2.9	1.1	3.0	1.9-4.5	2.1	1.1	2.0	1.1-3.2
Redox	mV	-223.5	28.8	-220	-268.5 – -110.5	-87.3	14.2	-85	-146.5 – - 25.5

( ) – efficiency removal

Overall Treatment Efficiency

The HTW ensured consistent and effective removal of all the contaminants analyzed.

Consequently, the concentration of pollutants in the effluent did not exceed Polish permissible values [14].

The example of the differences of TN and COD in inflow and effluent are illustrated in Fig. 2.

Considerable reduction of TN observed in the samples was general regularity confirmed by statistical tests (Fig. 2a). Because of inflow, TN and effluent TN are paired, first normality of differences distribution was checked with Shapiro-Wilk test with the significance level =0.05. Normality of the differences distribution was rejected (W Statistic =0.873, p-value = 0.003) and then differences between population distributions of inflow TN and effluent TN were tested with Wilcoxon matched pairs test with the significance level of 0.05. The hypothesis of insignificant difference between the population distributions was rejected (Z Statistic = 4.541, p-value = 0.000006). Consequently, significance of differences of distributions inflow TN and effluent TN is proved.

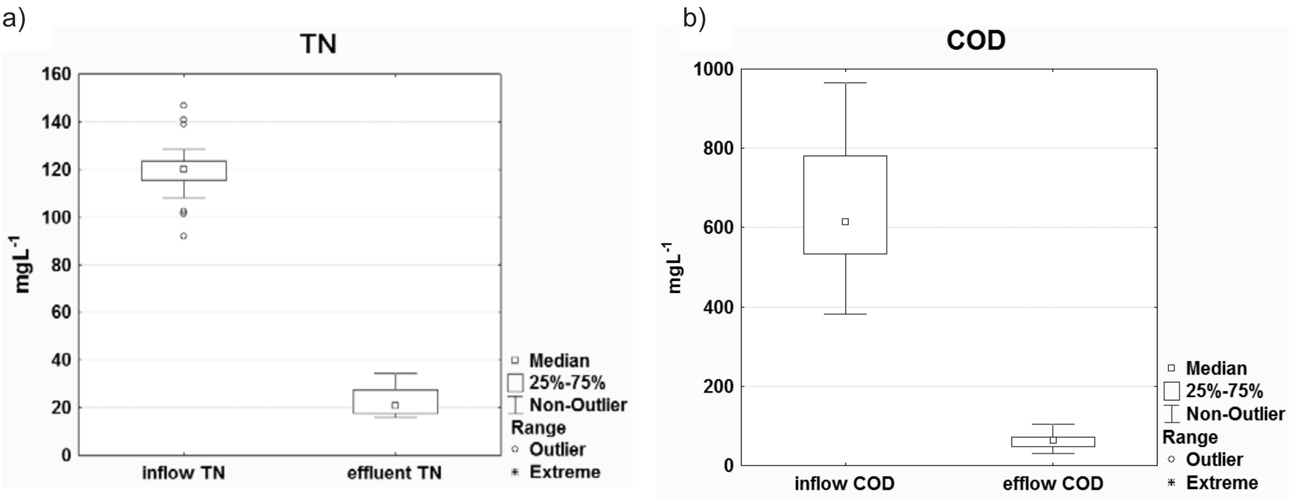

Fig. 2. Variations of TN (a) and COD (b) in inflow and effluent from analyzed HTW.

Dependence between effluent and inflow TN is given by the following simple linear regression equation:

$$\mathit{effluent\ TN} = \underset{(0.009)}{0.191} \cdot \mathit{inflow\ TN}$$

(R^2=0.174, t-value =20.915, p-value = 0, intercept is not significantly different from 0) (Fig. 3a).

Significance of differences of distributions inflow COD and effluent COD was shown in a similar way (Fig. 2b). The Shapiro-Wilk test with the significance level = 0.05 was used for differences of the paired variables: inflow COD and effluent COD. Normality of the differences distribution was not rejected (W statistic = 0.93, p-value = 0.07). T-test for dependent samples, with the significance level= 0.05, rejected the hypothesis about insignificant differences of distributions of inflow COD and effluent COD (t-value = 20.864, p-value = 0).

Dependence between inflow COD and effluent COD is given by the following simple linear regression equation:

$$\mathit{effluent\ COD} = 26.956 + \underset{(0.024)}{0.054} \cdot \mathit{inflow\ COD}$$

(R^2=0.262, t-value = 2.398, p-value=0.024 for intercept, and t-value =3.2, p-value = 0.004 for inflow COD coefficient) (Fig. 3b). In the contrast to regression equation for TN where the intercept was not significantly in the case of regression equation for COD the intercept is a significant part of the final COD concentration. These results could indicate that part of inflow organic matter concentration has already been present in non-decomposable COD [25].

The nitrogen balance carried out for Wiklino HTW indicated that the amount of nitrogen accumulated in the above-ground parts of the reed plants was 7.0% of the influent nitrogen load, while soil sorption processes accumulated about 5.9% of influent nitrogen [2]. The results obtained agree well with the investigation described in 2000 by Obarska-Pempkowiak and Ozimek [19], who found that bioaccumulation of nitrogen in above-ground reed parts can be up to 10% of the inflow nitrogen load. Further environmental conditions in the HCW were not considered to favor ammonia volatilization or dissimilatory nitrate reduction to ammonia [20, 21]. Therefore, these processes were assumed to be negligible.

Table 6. Mean values of loads, MRR of contaminants and NBOD, and first-order rate constants (k_{pBOD}, k_{pTN}, k_{pMIN}) at different stages of the HTW.

Parameter		Unit	HFI	VF	HFII	Total
COD	Load	$gm^{-2}\cdot d^{-1}$	12.9	14.5	2.5	6.9
	MRR	$gm^{-2}\cdot d^{-1}$	8.3	9.8	0.5	6.0
COD_f	Load	$gm^{-2}\cdot d^{-1}$	5.5	3.5	1.6	3.0
	MRR	$gm^{-2}\cdot d^{-1}$	4.4	0.6	0.7	2.7
BOD	Load	$gm^{-2}\cdot d^{-1}$	6.3	2.1	0.5	3.4
	MRR	$gm^{-2}\cdot d^{-1}$	5.7	1.2	0.3	3.3
TN	Load	$gm^{-2}\cdot d^{-1}$	2.5	4.5	1.6	1.4
	MRR	$gm^{-2}\cdot d^{-1}$	1.1	1.4	0.9	1.2
TKN	Load	$gm^{-2}\cdot d^{-1}$	2.5	4.3	1.4	1.4
	MRR	$gm^{-2}\cdot d^{-1}$	1.2	1.7	0.9	1.2
NBOD		$gm^{-2}\cdot d^{-1}$	5.2	7.9	4.1	5.5
COD		$gm^{-2}\cdot d^{-1}$	8.1	9.7	0.5	6.0
k_{pBOD}		d^{-1} (md^{-1}*)	0.152	0.043	0.291	-
k_{pTN}		d^{-1} (md^{-1}*)	0.094	0.032	0.191	-
k_{pMIN}		d^{-1} (md^{-1}*)	0.061	0.025	0.068	-

*modified first-order rate constant for VF, in md^{-1}.

Neither MRRs nor first-order reaction rate constants (Table 6) showed clear correlations with corresponding COD removal or concentrations for each stage of treatment. The highest MRRs were observed for organic matter (COD and BOD) in HFI. The COD_f MRR was slightly lower than the BOD MRR, indicating that less than 100% of the dissolved COD was easily biodegradable. N compound MRRs

a) b)

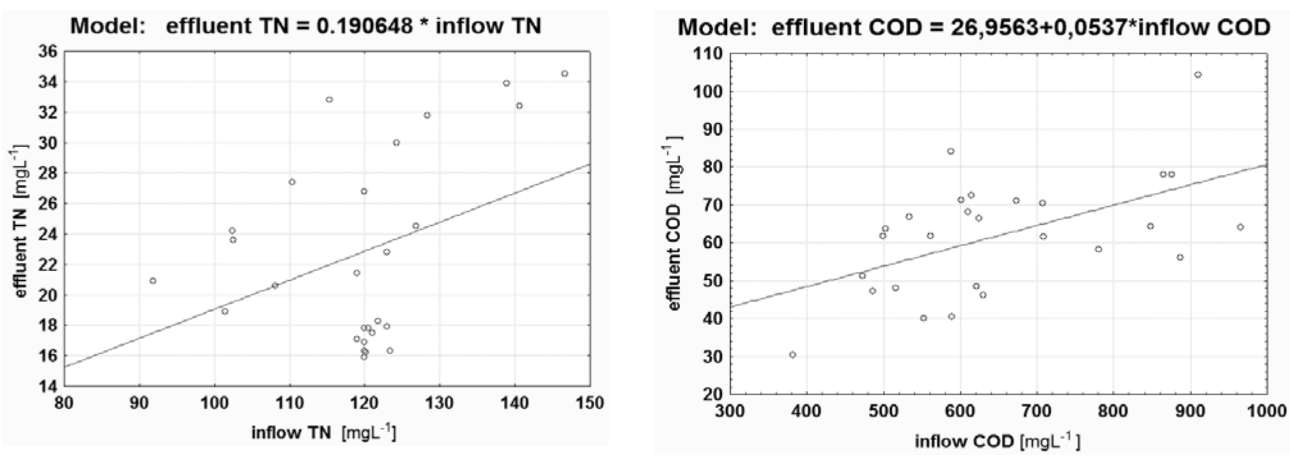

Fig. 3. The dependence between inflow and outflow TN (a) and COD (b) concentrations.

were higher in the VF than in the HF beds. These results suggest that the MRR of pollutants per m^2 changed in proportion to the loading values and were correlated with the bioavailability of organic compounds as well as the working conditions of the TWs.

The TKN and TN MRRs in this study (Table 6) were almost double the value of 0.7 $gm^{-2} \cdot d^{-1}$ reported for systems in Denmark by Brix et al. [28] and half the MRR values reported by Tanner et al. [6].

Under the working conditions of HTW in Wiklino, COD and TN treatment remains stable. Mean COD removal, shown by the slope of the regression line, was 95.1% with the optimum COD load between 5 and 7 $gm^{-2} \cdot d^{-1}$ (Fig. 4a). Total nitrogen mean removal rate is estimated at 79.11% with the optimum load from 1.3 to 1.4 g TN $m^{-2} \cdot d^{-1}$ (Fig. 4b). In the case of COD and TN, the scatter diagrams (Fig. 2a and b) show a strong and clear correlation between the received load of pollutants and the removed for the whole studied HTW. Contrary to that, the correlation between TN-treated and COD-treated has no statistical importance ($p=0.1942$), and the test of significance given by the r^2 value is equal to 0.0665 (Fig. 4c) [22]. This lack of correlation could suggest competition for oxygen among heterotrophic and nitrifying bacteria. When compared with the high efficiency of nitrogen compound removal, it could also suggest that nitrogen in the studied HTW was removed in the alternative pathway with oxygen limited demands.

The average values of reaction rate constants for the HFI and HFII beds, and corresponding modified rate constants for VF, are given in Table 6. The rate constants indicate that organic substance decomposition was the fastest process, while TN removal was slightly slower. The slowest process was mineralization of Org-N (k_{pMIN}). The temperature influence on the ammonification process was negligible. The average values of the constant rates $k_{pBOD(20)}$ and $k_{pTN(20)}$ were two times higher for HFII than for HFI, which suggests that these rates are influenced by factors other than temperature alone.

The average value of the modified $k_{pTN(20)}$ rate constant was 0.0247 md^{-1}. Similar results were obtained by Brix and Johansen [18] for 37 VFs in Denmark, and by Tanner et al. [6] for different types of wastewater treated in pilot cascade filters. The values of $k_{pBOD(20)}$ reported from investigations carried out in Austria and the UK ranged from 0.067 to 0.1 md^{-1} and were higher than the values calculated for the Wiklino VF, possibly influenced by higher average air temperature [1, 29].

Conclusions

1. The HTW studied ensured consistent and very effective removal of all analyzed pollutants. The overall removal of BOD_5 and COD was 97.9% and 95.1%, respectively.
2. With respect to N compounds, Org-N was removed with the highest efficiency (90.3%) while NH_4-N and TN had removal rates of 87.8% and 79.1%, respectively. An unexpectedly high removal efficiency of N species

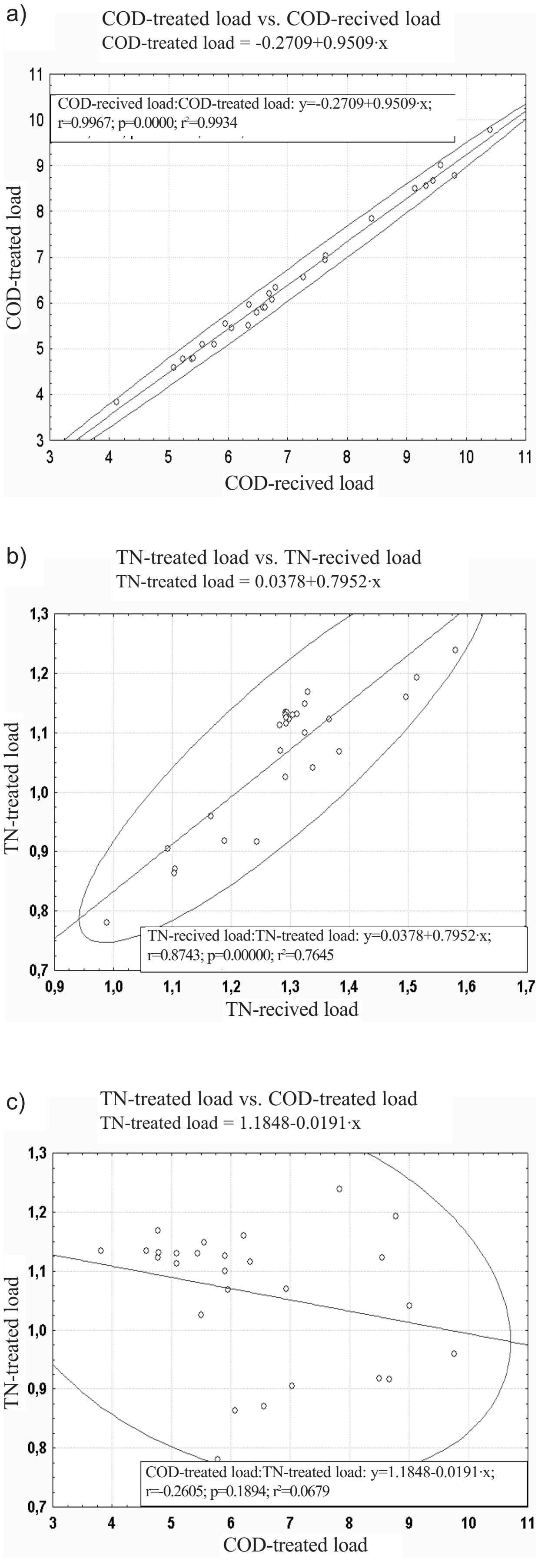

Fig. 4. The overall efficiency removal of COD (a) and TN (b) as well as of TN in dependence of COD (c), $gm^{-2} \cdot d^{-1}$, with regression lines for each example.

was observed in the first stage of treatment, HFI. The removal of TKN was over 46%, and occurred with 80.5% BOD removal, suggesting potential heterotrophic competition for oxygen, the supply of which was strongly limited in this stage of treatment.

3. The Wiklino VF beds showed the lowest efficiency of NH_4-N removal (31.1%) of the three stages of HCW, although the Org-N removal was the highest and equal to 58.6%.
4. Both BOD and COD_f were present in each stage of treatment indicating the availability of organic carbon. Thus, denitrification was not limited by this factor.
5. The observed changes of alkalinity and pH were inconsistent with the occurrence of sequential, conventional nitrification, and denitrification. Moreover, the limited oxygen supply and very effective removal of organic matter as well as the lack of significant correlation between COD and TN removal suggested the presence of alternative pathways of N compound removal with reduced oxygen demands such as ANAMMOX or oxygen-limited autotrophic nitrification and denitrification (OLAND). Simultaneous nitrification-denitrification in microzones around rhizomes is also likely to have occurred.

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