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ENHANCING EFFICIENCY OF NITROGEN REMOVAL FROM WASTEWATER IN CONSTRUCTED WETLANDS

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Досліджено процеси трансформації азоту у біоінженерних ставках з горизонтальним підповерхневим потоком. Запропоновано технологічне рішення для інтенсифікації процесу очищення стічних вод у даних системах. Зокрема вивчено роль анамокс процесу у біоінженерних ставках, а також досліджено вплив внесення додаткової біомаси анамокс бактерій на ефективність видалення азоту зі стічних вод

Ключові слова: очищення стічних вод, біоінженерні ставки, видалення азоту, інтенсифікація процесу, анамокс

Исследованы процессы трансформации азота в биоинженерных прудах с горизонтальным подповерхностным потоком. Предложено технологическое решение для интенсификации процесса очистки сточных вод в данных системах. В частности изучена роль анамокс процесса в биоинженерных прудах, а также исследовано влияние внесения дополнительной биомассы анамокс бактерий на эффективность удаления азота из сточных вод

Ключевые слова: очистка сточных вод, биоинженерные пруды, удаление азота, интенсификация процесса, анаммокс

1. Introduction

Scientific research on the use of wetland plants for wastewater treatment started in the early 1950s in Germany. Already in the late 1960s the first full scale constructed wetlands (CWs) were built. Since then these systems have widely spread all over the world as a low cost, energy efficient and easy to operate engineered systems for sustainable wastewater treatment.

Horizontal subsurface flow (HSSF) CWs are one of the most popular, reliable and efficient near-natural wastewater treatment systems applied around the world

[1, 2]. HSSF wetlands are mainly designed to treat primary settled domestic and municipal wastewaters of medium and small communities, nevertheless, at present these systems are also used to treat many other types of wastewaters including industrial and agricultural effluents, landfill leachate, urban and highway runoff, etc. [3–5].

HSSF CWs are able to efficiently remove polluting organic materials, total suspended solids and pathogens from sewage, but nitrogen removal is usually of relatively low efficiency and has been reported in long term studies between 20 % and 70 % [6–8]. Moreover, researchers of CWs usually get a “missing nitrogen problem” when calculating a nitrogen mass balance, because large fraction of influent ammonia follows unknown pathways [9]. Thus it is important to investigate internal nitrogen transformation mechanisms in constructed wetlands in order to provide better management of these treatment systems and water quality improvement.

2. Analysis of published data and problem statement

Nitrogen removal, retention and transformation mechanisms in CWs are very diverse and include ammonia volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake (assimilation), mineralization, enzyme hydrolysis, anaerobic ammonia oxidation (anammox), sorption, desorption, etc. [10–12]. However, currently, science has a limited understanding of the importance of every specific process listed above.

It’s known that nitrogen removal by assimilation in aboveground wetland vegetation equals commonly to less than 10 % of the inflow load [13]. Also physical ammonia volatilization is not common for the considered wastewater types, as it is possible only at the elevated pH (higher than 8.0). But it has been discovered that the plants contribute to nitrogen removal indirectly via influence on microbial processes [14]. Firstly, wetland macrophytes release oxygen into the rhizosphere and thereby promote nitrification process [15]. Also wetland plants release root exudates and thereby supply organic carbon for denitrification [2].

Nitrification-denitrification process was believed to be the main mechanism for nitrogen removal in wetlands [2]. But environmental conditions in horizontal subsurface wetlands are not favorable for the “classical” nitrification-denitrification process, as commonly there is not enough oxygen and organic material in such systems. Moreover, nitrogen removal mechanisms in CWs are seemed to be more abundant and newly discovered microbial processes of anaerobic ammonia oxidation (anammox), heterotrophic nitrification, aerobic denitrification and methanotrophic denitrification could also play fundamental role in CWs nitrogen cycle. But importance of these processes in CWs is still unknown [9].

3. Purpose and objectives of the study

The principal aim of this study is to enhance nitrogen removal efficiency in horizontal subsurface flow constructed wetlands. The hypothesis behind the performed study was that, if anammox bacteria can become the most abundant and active microbial group in constructed wetlands (due to inoculation), higher nitrogen removal rates will be achieved, as the main limitations for “classical” nitrogen removal process (not enough oxygen and organic carbon) will become an advantage for the anammox process.

In order to achieve the aim the following research objectives have been identified:

– to investigate the process of nitrogen removal in horizontal subsurface flow constructed wetlands using laboratory mesocosms;

– to inoculate the laboratory model system with enriched biomass of anammox bacteria;

– to determine the influence of anammox bacteria inoculation on the nitrogen removal in constructed wetlands.

4. Laboratory-scale wetland mesocosms and investigation methods

4. 1. Description of experimental setup

Laboratory-scale wetland mesocosms were used to simulate horizontal subsurface flow constructed wetlands and to implement the model experiments. The experimental setup (Fig. 1) consisted of three Planted Fixed Bed Reactors (R1, R2, and R3); peristaltic pumps for feeding and recirculation; inflow tanks (IT); and gas bags (GB). Each reactor has one sampling port (SP). The effluent (EF) was collected into the outflow tanks through an overflow outlet tube. The system was operated under controlled climatic conditions (simulating a temperate climate summer) in the Phytotechnicum of Helmholtz Centre for Environmental Research – UFZ (Germany).

The experimental Planted Fixed Bed Reactor (PFR) consisted of a cylindrical plastic vessel (diameter=30 cm; height=28 cm) with a perforated stainless steel basket (diameter=23 cm; height=27.5 cm) placed inside it. In the center of the basket a perforated stainless steel pipe (diameter=4.5 cm; height=26 cm) for the suction of the process water was placed. Detailed design principles of the PFR can be found in [16]. The steel basket was completely filled with gravel (diameter=4–8 mm). The reactor was closed with a plastic lid.

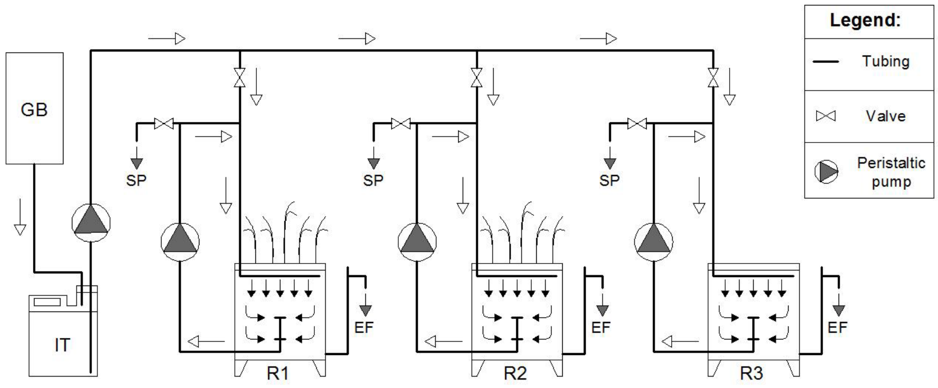

Fig. 1. Schematic diagram of the laboratory-scale experimental setup: R1, R2 — experimental reactors with plants; R3 — unplanted experimental reactor; IT — inflow tank; GB — gas bag filled with N_2; SP — sampling port; EF — effluent

Two of the PFRs (R1 and R2) were planted with a model helophyte *Juncus effusus* (around 80 shoots each). The plants were growing through the special holes in the lid (diameter=5 cm). One of the PFRs (R3) was kept unplanted as a control. Each of three experimental PFRs has a total volume of about 19.5 L and a gravel surface area of 0.040 m^2. The pore water volume amounted to about 14 L and the nominal hydraulic retention time was adjusted to approximately 7.5 days by semi-continuous feeding of artificial wastewater with average flow rate 1.8 L d^{-1}.

4. 2. Conditions of wetland mesocosms operation

The investigations started in the beginning of March 2013 and continued until the end of April 2014 for a total of 385 days. An establishment (pre-experimental) period for plants and microorganisms was set for three months (80 days) since the introduction of the plants into the reactors in the beginning of March 2013 until the beginning of June 2013. Since June 2013 an experimental period has started and treatment performance of the wetland mesocosms was analyzed.

In accordance with the varying operational conditions, the total experimental period was divided into three phases: Phase I (Jun '13 – Sep '13), Phase II (Oct '13 –Feb '14) and Phase III (Mar '14 – Apr '14). The defined time periods and operational conditions are provided in Table 1. Briefly, the Phase I started after 80 days of the pre-experimental period, when the model systems have reached generally stable physicochemical conditions and removal efficiencies. The Phase II started in the beginning of October 2013, when the old plants of the mesocosms R1 and R2 were removed and the new plants were introduced. The Phase III started in the beginning of March 2014, when the mesocosm R1 was inoculated with enriched culture of anammox bacteria.

Table 1

Changes in operational conditions during different experimental phases in three laboratory-scale wetland mesocosm

Parameter / conditions	Wetland meso-cosm	Experimental phase		
		I (120 days)	II (150 days)	III (60 days)
Introduction of new plants *(Juncus effusus)*	R1	-	+	-
	R2		+	
	R3		-	
Inoculation with anammox bacteria	R1	-	-	+
	R2			-
	R3			-
Nitrogen loading rate (g-N m^{-2} d^{-1})	R1	1.77		1.35
	R2			
	R3			

All systems were fed with synthetic domestic wastewater containing (in g L^{-1}): NH_4Cl (0.150 – during Phases I and II; 0.115 – during the Phase III), $KHCO_3$ (1.180), $MgCl_2\cdot 6H_2O$ (0.110), KH_2PO_4 (0.014), $FeSO_4\cdot 7H_2O$ (0.012), $CaCl_2\cdot 2H_2O$ (0.300). The resulting concentration of ammonium (as a main contaminant) was equal to 50 mg L^{-1} NH_4^+ during Phases I and II and to 39 mg L^{-1} NH_4^+ during the Phase III. Hydraulic loading rate amounted to 45 mm d^{-1}.

A trace mineral solution (TMS) was added to the artificial wastewater (1.5 mL per liter of wastewater) containing (in g L^{-1}): E.D.T.A (Titriplex III) di sodium salt (1.0), $FeSO_4\cdot 7H_2O$ (1.0), $MnCl_2\cdot 2H_2O$ (0.8), $CoCl_2\cdot 6H_2O$ (1.7), $CaCl_2\cdot 2H_2O$ (0.7), $ZnCl_2$ (1.0), $CuCl_2\cdot 2H_2O$ (1.5), $NiCl_2\cdot 6H_20$ (0.3), H_3BO_3 (0.1), $Na_2MoO_4\cdot 2H_2O$ (0.1), $Na_2SeO_3\cdot 5H_2O$ (0.02) and concentrated HCl (3 mL L^{-1}).

The air temperature was set to 22 °C from 6 am to 9 pm (simulating day time) and to 16 °C at night. An additional artificial light during day time was provided by Lamps (Master SON-PIA 400 W, Phillips, Belgium).

In order to prevent microbial degradation, artificial wastewater in storage tanks is vigorously purged with N_2 gas for approximately 20 minutes after each preparation and then the storage tanks are connected to gas bags filled with N_2 to prevent oxygen input and balance the pressure during pumping wastewater out.

The permanent recirculation of the process water was arranged in each of the reactors in order to create hydrodynamic conditions comparable to an ideal mixing – free from macro-scale gradients of concentrations. Circulatory flow was provided by a peristaltic pump, which was sucking the process water out of the gravel-free pipe in the center of the reactor and pumping it back through the distribution ring in the top of the reactor (Fig. 1).

In order to evaluate the effect of anammox bacteria on nitrogen transformations and to enhance nitrogen removal in horizontal subsurface flow constructed wetlands, one of the planted reactors after nine months of operation was inoculated with an enrichment culture of anammox bacteria (relative abundance of 95 %). The procedure and results of anammox bacteria enrichment were published earlier [17].

4. 3. Water sampling and laboratory analysis

Due to the reactor design, the process water in the circulation flow represented actual physicochemical conditions in the whole reactor and therefore the water samples were collected from the circulation flow through the sampling ports (SP) and then different physicochemical parameters were monitored.

The inorganic nitrogen concentrations, namely ammonium, nitrite and nitrate were measured weekly by means of colorimetric method using a Spectroquant® NOVA 60A photometer (Merck KGaA, Darmstadt, Germany) and the appropriate Merck test kits (Merck No. 1.00683.0001; Merck No. 1.09713.0001; Merck No. 1.14776.0001).

The total nitrogen (TN), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were analyzed weekly using a multi N/C® 2100S TOC/TN_b analyzer (Analytik Jena, Jena, Germany) after filtration by means of a 0.45 µm syringe filter.

The methane concentration in pore water was measured at different intervals by static headspace analysis using an Agilent 7890 A gas chromatograph (Agilent Technologies, Santa Clara, CA) and an Agilent G1888 headspace sampler.

The dissolved oxygen concentration was measured at different intervals using a PreSens Fibox 3 fiber optic oxygen transmitter (Precision Sensing GmbH, Regensburg, Germany) and non-invasive oxygen-sensitive chemical optical sensor. Values were displayed and evaluated with the OxyView-PST3-V5.41 software.

The pH value was measured weekly using a WTW SenTix® 41 pH combined electrode with integrated temperature probe (WTW Gmbh, Weilheim, Germany) and a WTW MultiLine P4 Universal Pocket Meter.

The redox potential (Eh) was measured directly in the circulation flow few times a week using a WTW pMX 3000

Microprocessor pH/ION meter (WTW Gmbh, Weilheim, Germany) and a Pt4805-S7/120 Combination Redox electrode (Mettler-Toledo Gmbh, Schwerzenbach, Switzerland). In parallel the temperature was measured using WTW TFK 150 temperature sensors.

Plant transpiration was controlled by balancing the inflow and the outflow amounts of water very ten days for every wetland mesocosm. In addition, the water flow balances were used to control the inflow rate. As well the health status of the plants was monitored by calculating the plant shoots and water loss in the reactors.

5. Results of the experiments in laboratory-scale wetland mesocosms

The nitrogen species (ammonium-N, nitrite-N and nitrate-N) dynamics during the whole experimental period (Phases I, II and III) is illustrated in Fig. 2.

Within the overall experimental period in both planted systems (R1 and R2) nitrogen was present only in the form of two species – ammonium and nitrate, while in the unplanted reactor (R3) residual nitrogen was present in the form of all three inorganic nitrogen species, though the nitrite concentration were low. During the whole experiment in all three systems there was a significant decrease in ammonium nitrogen concentration, which was varying over time (over experimental phases) and among the reactors. However a considerable amount of nitrate nitrogen was formed instead and most of the time nitrate prevailed among the other nitrogen species in all three reactors.

During the Phase I there were in general stable conditions in terms of nitrogen species composition and concentrations in all three reactors. During this period both planted reactors (R1 and R2) have shown very similar performance, with total inorganic nitrogen concentration in the outflow around two times lower than in the inflow, while in the unplanted reactor (R3) the concentration was much higher. In the beginning of the Phase II there was considerable decrease in total inorganic nitrogen concentration in the reactors R1 and R2, which was increasing with time again. The performance of the reactor R3 during Phase II was similar to the one during the Phase I. During the Phase III there was observed a considerable drop of inorganic nitrogen concentration in the inoculated planted reactor R1 and a slight decrease in the reactors R2 and R3.

The dynamics of water loss (due to plant transpiration and direct evaporation) for all three wetland systems mesocosms during the whole experimental period is illustrated in Fig. 3. During the Phase I the number of plant stalks (*Juncus effusus*) in the mesocosm R2 was slightly higher than in the R1 and was decreasing with time. In the beginning of the Phase II the plants in the both reactors were replaced by new ones, moreover higher number of plants was introduced into the mesocosm R2. During Phases II and III the number of plant stalks was decreasing again.

Based on the mass balance data the nitrogen removal efficiencies (RE) were calculated. The mean values of the percent of total inorganic nitrogen (ammonium, nitrite and nitrate) removed from wastewater in three wetland mesocosms during every month of the experimental period are presented in Fig. 4.

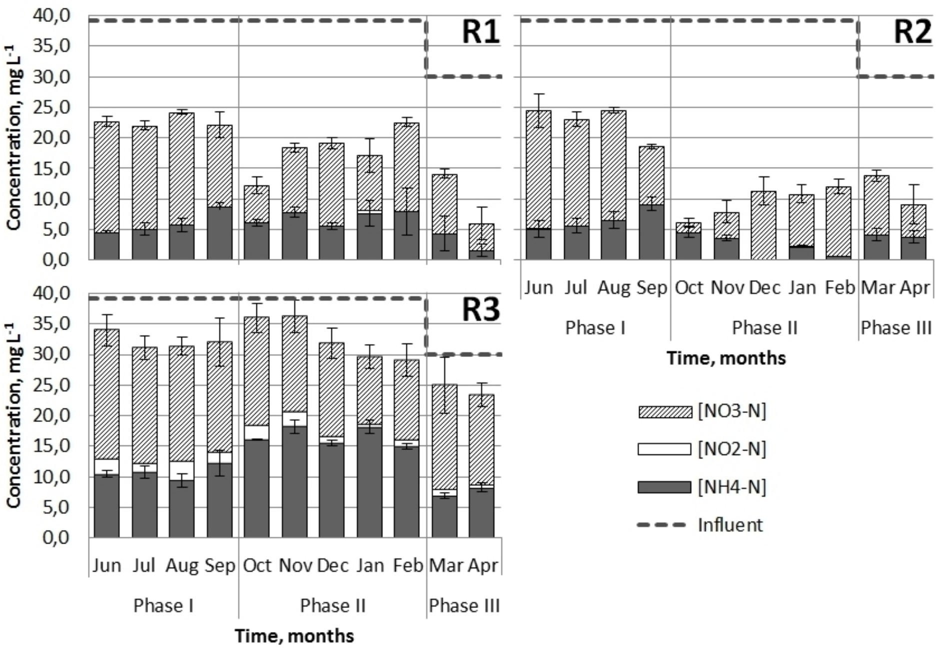

Fig. 2. Outflow concentrations of ammonium (NH4-N), nitrite (NO2-N) and nitrate (NO3-N) nitrogen in the reactors (R1, R2, R3) during the experimental period

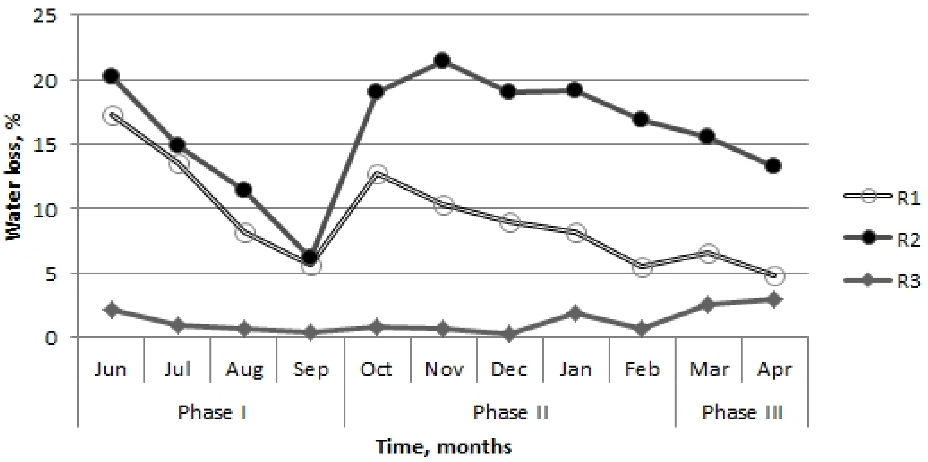

Fig. 3. Dynamics of water loss in the wetland mesocosms (R1, R2, R3)

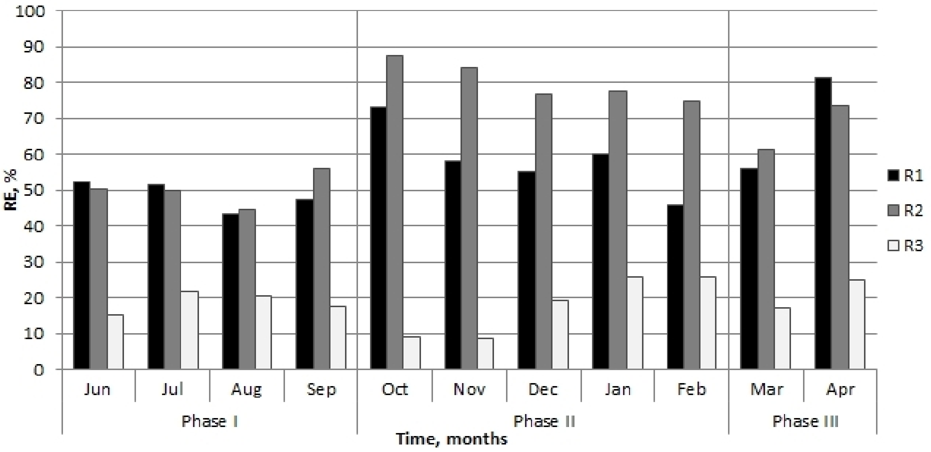

Fig. 4. Average total inorganic nitrogen removal efficiencies (RE) for three wetland mesocosm (R1, R2, R3) during the total experimental period

It is very important to consider a water budget dynamics when evaluating treatment performance of a constructed wetland and, therefore, removal efficiency is a crucial parameter. A significant difference in total inorganic nitrogen removal between planted and unplanted systems was found for all three phases. A positive correlation was found between number of plants in the reactor and total nitrogen removal efficiency. An inoculation of anammox biomass into the reactor had a positive effect on the removal efficiency, even the amount of plants in the reactor was low.

6. Conclusions

The effects of plants (Phase I), replanting of the system (Phase II) and inoculation of anammox bacteria (Phase III) on nitrogen removal in laboratory-scale wetland mesocosms were studied.

The plants have a large influence on nitrogen removal performance of experimental wetland mesocosms. Nitrogen removal efficiencies during all experimental phases in the planted reactors were few times higher in comparison to the unplanted one. Moreover the reactor with higher number of plant stalks (R2) has shown better performance than one with lower number (R1), while all the other experimental conditions were similar (Phase II).

Introduction of the new plants into the reactors resulted in rapid increase of nitrogen removal efficiency if compared to previous experimental period. However, eventually the removal efficiency was decreasing again.

After introduction of enriched biomass of anammox bacteria into one of the experimental reactors a great improvement in the performance of this reactor has been observed. Wetland mesocosm inoculated with anammox (R1) has outperformed the other planted reactor with higher number of plants and which has been previously showing higher removal efficiencies (R2).

The experimental results have shown that prompting of anammox process by inoculation of externally enriched biomass could be of tremendous importance for increasing the effectiveness of nitrogen removal in horizontal subsurface flow constructed wetlands.

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