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Lab-scale study on nitrogen transformations in constructed wetlands for wastewater treatment

Constructed wetlands (CWs), also known as planted soil filters, are low cost and energy efficient systems for wastewater treatment that are able to remove polluting organic matter, nutrients and pathogens from sewage. Nonetheless present understanding of underlying microbial mechanisms, like nitrogen transformation, is only fragmentary. Thus, this study aims to investigate the role of different nitrogen transformation processes and especially the abundance of anammox bacteria and its correlation to nitrogen removal and further physicochemical parameters in the root zone of CW.

Біоінженерні ставки (БС) є низькозатратними та енергоефективними системами, що здатні видаляти органічні забруднення, біогенні елементи та патогенні мікроорганізми зі стічних вод. Однак сучасне розуміння мікробних механізмів, що лежать в основі процесу, як, наприклад, трансформація сполук азоту, є лише поверхневим. Тому метою даних досліджень є визначення ролі різних механізмів трансформації сполук азоту у БС, з детальним вивченням наявності та поширення анаммокс бактерій та їх впливу на ефективність усунення азоту та інші фізико-хімічні параметри у фільтруючій товщі БС.

Despite the fact of wide spread application of constructed wetlands (CWs) as treatment facilities all over the world for the last 30 years, science still has a lack of understanding of specific internal removal mechanisms such as specific nitrogen removal processes, microbial predatory activities, etc. [1]. This study involves investigation of nitrogen removal mechanisms in horizontal subsurface flow constructed wetlands (HSSF CWs), which comprise macrophytes planted in porous medium (gravel, sand, etc.) with a wastewater flow occurring under the surface of the bed and mainly in a horizontal direction. HSSF CWs show high removal efficiencies (80-90%) of organic matter, suspended solids and pathogens, but nitrogen removal is of relatively low efficiency and has been reported between 20% and 50 %. The main mechanism of nitrogen removal in CWs is believed to be nitrification and denitrification performed by autotrophic aerobic nitrifiers and heterotrophic anaerobic (anoxic) denitrifiers. However, nitrogen removal mechanisms in CWs seem to be more complex and include anaerobic ammonium oxidation, oxygen-limited autotrophic nitrification denitrification, heterotrophic nitrification, and aerobic denitrification. The importance of these processes in CWs is still unknown [2].

The aim of this study is to estimate the role of anaerobic ammonium oxidation (anammox) process in nitrogen removal in HSSF CWs and to attempt to enhance nitrogen removal in model experiments by inoculation of CWs with enriched culture of anammox bacteria. To reach this aim, laboratory-scale experiments are performed using Planted Fixed Bed Reactor (PFR) [3] as a tool for modeling CWs. The experimental set-up comprises three PFRs: two planted with *Juncus effusus* and one unplanted control. A synthetic wastewater containing only ammonium nitrogen, carbonate, minor amounts of potassium, phosphorus and microelements is used for the experiments. In parallel a 20 L upflow fixed bed reactor is run in order to obtain an enriched culture of anammox bacteria.

To analyze treatment performance of PFRs different parameters are being monitored, including: temperature, pH, redox potential (Eh), dissolved oxygen, pore volume, inorganic nitrogen (ammonium, nitrite and nitrate ions and nitrous oxide) concentrations, and microbial community composition and distribution. Real-time quantitative polymerase chain reaction (q-PCR) [4] with non-specific (bacterial) and group-specific PCR oligonucleotide primers are used for microbial community analysis. Group-specific primer sets targeting 16S ribosomal RNA gene sequences of

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anammox bacteria (AMX), ammonium oxidizing bacteria (CTO), and also primers targeting copper nitrite reductase gene (nirK), cytochrome *cd*$_1$-nitrite reductase gene (nirS) and nitrous oxide reductase gene (nosZ) of denitrifying bacteria are used for quantification and determination of taxonomic composition of microbial biomass in the PFRs.

After 80 days of operation, the model system has reached generally stable physicochemical conditions and removal efficiencies. In particular, the total nitrogen (TN) removal efficiencies are equal to about 50% in both planted systems and about 25% in unplanted system. Furthermore, residual nitrogen in planted systems is present only in the form of ammonium and nitrate, wherein nitrate amounts to about 80% of TN. In unplanted system residual TN is present in forms of ammonium, nitrite and nitrate with a ratio of approximately 30:10:60. But the higher rates of nitrogen removal in the pla-nted systems cannot be explained only by ammonia volatilization, plant uptake or soil adsorption, because these processes usually do not contribute more than 10% to the total nitrogen removal.

The redox potential (Eh) during daylight hours in all three PFRs amounted to 325±25 mV in planted systems and 350±25 mV in unplanted system. It should also be considered that CWs, according to R. Kickuth, are interpreted as redox multi-gradient systems, with both reducing and oxidizing conditions occurring at the same time in different zones (root surfaces, gravel bed, microbial biofilms). Simultaneously, strong oscillations are caused by day-night cycling. Those factors cause superimposed redox potential fluctuations with steep spatial differences that create favourable living conditions for both aerobic and anaerobic microorganisms. The question then arises as to which microbial processes are of the highest importance and have the biggest influence on nitrogen transformations in CWs. That is why this study intends to explore is this either autotrophic nitrification by aerobic nitrifiers, heterotrophic denitrification by nitrate and nitrite reducing bacteria or anaerobic ammonium oxidation by autotrophic anammox bacteria capable of converting ammonium and nitrite into dinitrogen gas.

In order to answer the question, molecular biology analysis was carried out. Significant differences were found between amounts of bacteria on the root and on the gavel surfaces, resulting in a larger amount of bacteria on the roots than on the gravel in planted systems. More ammonium oxidizing bacteria (AOB) were also found on the roots. Around 50% of bacterial cells detected on the gravel belong to anammox bacteria in all three PFRs, but total amount of anammox bacteria is low.

To estimate the influence of anammox bacteria on treatment efficiency of CWs, one of the PFRs will be inoculated with a biomass of anammox bacteria. Inoculation is essentially important for the study, because anammox bacteria are slow growers (cells double only once per 11–20 days). Then changes in nitrogen removal efficiencies and microbial community composition will be determined.

References

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