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AB 6
(January 2015)

ADVOCATE bulletin

CL:AIRE's ADVOCATE bulletins describe practical aspects of research which have direct application to the characterisation, monitoring or remediation of contaminated soil or groundwater. This bulletin describes pilot-scale studies to evaluate microbial nitrogen transformation in horizontal subsurface-flow constructed wetlands.

Nitrogen biotransformation in horizontal subsurface-flow constructed wetlands treating contaminated groundwater

1. Introduction

Groundwater is the main source for potable water and domestic use in numerous countries around the world. However, water quality can be affected by pollution, which influences the natural environment and human health. One of the most widespread pollutants in water is ammonium (NH_4^+), which is poisonous to animals. It is one of the major toxic compounds as well as a critical long-term pollutant in marine environments (Ip *et al.*, 2001), surface water (Mangimbulude *et al.*, 2012; Havens et al., 2001; Harrington and McInnes, 2009), and groundwater (Siljeg *et al.*, 2010). Ammonium, due to its toxicity to fish and because it causes eutrophication of lakes and wetlands, is a serious environmental problem. The Council of the European Union set a recommended level of 0.05 mg/L and a maximum level of 0.5 mg/L of NH_4^+ (EEC, 1998).

Constructed wetlands (CWs) are widely used in wastewater and groundwater treatment due to their low energy requirements and easy operation (Garcia *et al.*, 2010). Wetlands, both constructed and natural, are promising *in situ* water treatment methods thanks to enhanced microbial growth within the plants' rhizospheres, which creates an effective contaminant degradation zone (Kadlec and Wallace, 2008). While microorganisms play the primary role in pollutant elimination, plants improve the microbial activity to remove pollutants (Stottmeister *et al.*, 2003). Due to the mosaic of aerobic and anaerobic zones within the root zone of the plants, contaminants can be removed by a variety of processes, aerobic as well as anaerobic.

Among a variety of nitrogen compounds, the most important forms in wetlands are organic nitrogen, NH_4^+, nitrite (NO_2^-) and nitrate (NO_3^-) (Vymazal, 2007). These compounds can be affected by different processes such as microbial removal, nitrogen fixation, plant and microbial uptake, ammonia volatilisation, mineralisation, and NO_3^- reduction to NH_4^+. However, the quantification of these processes can be difficult due to the complexity of the wetland systems (Martin and Reddy, 1997; Kadlec, 2000). The removal of nitrogen from wastewater in biological treatment systems can be impacted by a number of physical, chemical and biological processes. These processes are potential mechanisms for biological wastewater treatment, such as total nitrification with further denitrification, and partial nitrification coupled with anaerobic ammonium oxidation (ANAMMOX) (Paredes *et al.*, 2007).

2. Site Description

This work was done within the framework of the SAFIRA II Project "Compartment Transfer - CoTra". The pilot scale plant was located in Leuna, Germany, and operated from 2007 through 2013 (Fig. 1). As Leuna has been a location of chemical industry since the beginning of the last century, a range of contaminants has migrated into groundwater as a consequence of accidental spillage, improper handling, and damage resulting from heavy bombing during World War II. Consequently, the contamination is complex, and the main pollutants are petroleum hydrocarbons (BTEX), methyl tert-butyl ether (MTBE), and NH_4^+ (Martienssen *et al.*, 2006).

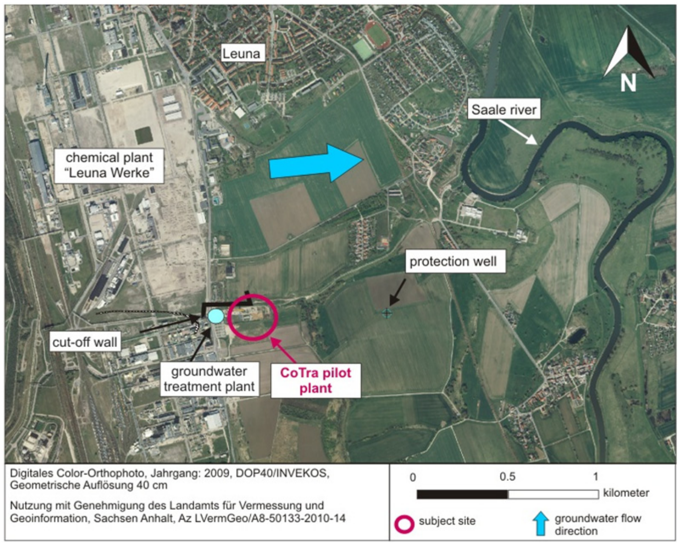

Figure 1: Megasite in Leuna

Up to now, the contaminated plume has reached an area of about 900,000 m² and become several hundred metres long. The dissolved pollutants are transported with the groundwater flow, which is directed generally from northwest to southeast. Downgradient from the oil spill, there are different receptors (e.g. river, well), which are potentially impacted by groundwater pollutants. The main aquifer thickness was estimated to be 2-4 m and the groundwater table is located about 3-4 m below ground surface. The groundwater flow velocity was estimated on the basis of water level data, pumping and tracer tests. It varies between 0.3 m/day and 1.0 m/day (Martienssen *et al.*, 2006).

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The main goal of the project was to develop a near-natural groundwater remediation technique for megasites by transferring of contaminated groundwater from wholly anaerobic environments to mixed aerobic/anaerobic environments, which enhances biodegradation/transformation processes. This was explored using constructed wetlands (CWs).

According to hydraulic water flow characteristics, CWs are classified into surface flow (SF) and subsurface flow (SSF) systems. SSF CWs are subdivided into horizontal and vertical flow systems (Lee *et al.*, 2009). Whilst all these systems were present in Leuna (Fig. 2), the focus of this research was on horizontal subsurface flow systems (HSSF CW), as they show the best performance for the removal of total nitrogen from contaminated waters.

Figure 2: The pilot scale plant in Leuna (courtesy of M. Kaestner)

3. Nitrogen cycle in constructed wetlands

The removal of NH_4^+ by CWs is well documented (Garcia *et al.*, 2010; Vymazal, 2007; Paredes *et al.*, 2007; Sikora *et al.*, 1995; Drizo *et al.*, 1997; Del Bubba *et al.*, 2000). The nitrogen cycle in CWs is characterised by complexity and the simultaneous functioning of many processes (Fig. 3).

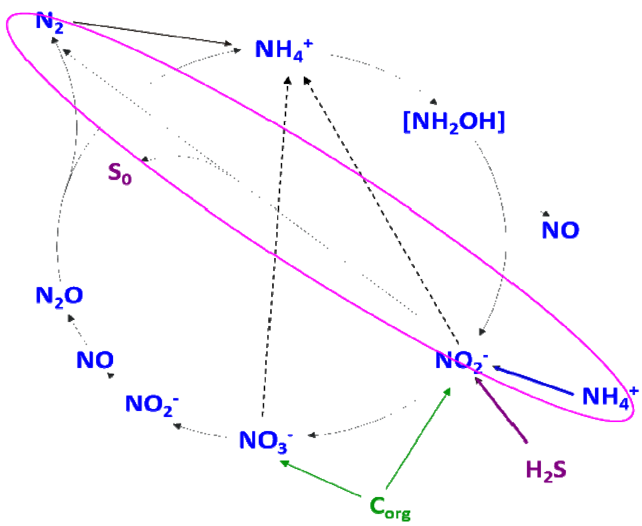

Figure 3: The pilot scale plant in Leuna (courtesy of M. Kaestner)

The most important processes are:

1. Ammonification. This is the process of biological conversion of organic nitrogen into NH_4^+. A microgradient system of aerobic and anaerobic zones in CWs is highly suitable for this process, although ammonification occurs mostly in oxygen saturated areas (Lee *et al.*, 2009). The product is NH_4^+, which is mainly removed by nitrification-denitrification processes in CWs.
2. Nitrification. Ammonium is oxidised to NO_3^- by chemoautotrophic bacteria under oxic conditions. This is a two-step process, performed by two different groups of microorganisms (Schmidt *et al.*, 2003).

   In the first step, *Nitrosomonas* and other groups oxidise NH_4^+ to NO_2^-:

   $$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O \qquad (1)$$

   In the next step, nitrifying bacteria, such as *Nitrobacter* and others, oxidise NO_2^- to NO_3^-:

   $$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (2)$$

   This process is considered to be a major pathway for NH_4^+ removal in both SF and SSF CWs (Lee *et al.*, 2009).
3. Denitrification. This process is defined as the biochemical reduction of NO_3^- and NO_2^- to nitric oxide, nitrous oxide, and nitrogen gas (Garcia *et al.*, 2010; Vymazal, 2007). Denitrification is carried out by a wide range of heterotrophic facultative anaerobic bacteria that are able to utilise NO_3^- (and NO_2^-) as an electron acceptor under anoxic conditions:

   $$2\ NO_3^- \rightarrow 2\ NO_2^- \rightarrow 2\ NO \rightarrow N_2O \rightarrow N_2 \quad (3)$$

   Organic carbon, which is required as an energy source for denitrifying microorganisms, can be available in CWs either from the organic pollutants contained in the contaminated water or from cell materials of microorganisms and rhizodeposition products (Lee *et al.*, 2009). It is believed that CWs have microzones that are saturated with oxygen next to the rhizosphere, and relatively anaerobic regions further from the roots. In this way, nitrification and denitrification can occur in sequence in close proximity to each other.
4. Also, a recently discovered microbial transformation pathway in the global nitrogen cycle is ANAMMOX, an anaerobic oxidation of NH_4^+ combined with NO_2^- reduction with nitrogen gas as the end product (Strous and Jetten, 2004):

   $$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (4)$$

   However, while ANAMMOX organisms have been found in many different natural environments, there is still a lack of knowledge about the role of these reactions in CWs. Further investigations are needed to learn about competition between anaerobic NH_4^+ oxidisers and other groups of NH_4^+ oxidising bacteria in the ecology of various wetland systems (Hunt *et al.*, 2005).

4. Experimental design

For the investigations, the following types of CWs were chosen: (i) planted HSSF-CW, (ii) unplanted HSSF CW, and (iii) floating plant root mat (FPRM). The HSSF CW consisted of stainless steel basins (5 m × 1.1 m × 0.6 m) planted with common reed (*Phragmites australis*). They were filled with gravel (grain size 2-3.2 mm) up to a height of 50 cm and the water level was set to 40 cm, resulting in a vadose soil zone of 10 cm. A FPRM was only supported by the densely woven root bed and was operated at a water depth of

30 cm. Inflow water was supplied from a nearby groundwater well. During the period of investigation, the concentration of NH_4^+-N in the contaminated groundwater was 23.4±5 mg L^{-1}. Sampling was carried out over the year June 2012 to June 2013 at bi-weekly intervals except for the winter season, when contaminant removal was negligible.

The hydraulic loading rate was fixed at 7 L h^{-1}. Water inflow and outflow volumes were determined by flow meters, allowing the contaminant loading rate to be calculated. Samples were taken from the inflow, outflow and along the flow path at three distances from the inlet (1, 2.5, and 4 m) and at three depths (20, 30, and 40 cm) for HSSF CWs or 30 cm for FPRM.

The following analyses were conducted:

- Physicochemical parameters, such as pH, temperature, redox potential, inorganic ions (NH_4^+, NO_2^-, NO_3^-), dissolved gases (N_2O, CH_4), organic compounds (MTBE, BTEX);
- Stable isotope methods for $^{15/14}N$ isotope signatures of NH_4^+; $^{15/14}N$ and $^{18/16}O$ isotope signatures of NO_3^-;
- Microbiological techniques of DNA extraction, DNA cloning, sequencing, PCR, Q-PCR, pyrosequencing, fluorescence *in situ* hybridisation (FISH), and confocal laser scanning microscopy.

5. Results and Conclusions

5.1 Ammonium removal efficiency

Throughout the sampling period, CWs have shown consistent removal efficiency of NH_4^+ (Fig. 4, 5, 6). Planted HSSF CW averaged 77%, unplanted HSSF CW 41% removal and FPRM 62%. There were no statistically significant differences in removal efficiencies between three investigated seasons (ANOVA, $F(2,19) = 1.719$, $p = .206$) and planted CWs (ANOVA, $F(1,20) = 4.194$, $p = .054$). Sampling in winter was not performed due to low efficiency of NH_4^+ removal during this season.

The NH_4^+-N loads (the contaminant mass loads were calculated on the basis of water volume flow rates and contaminant concentrations) of the planted HSSF CW decreased the most significantly at 20 cm depth, which can be explained by the high root density in this zone. The loads in planted HSSF CW and FPRM decreased over distance from the inflow (Fig. 5). This indicates that NH_4^+ oxidation occurred linearly with the flow of contaminated water from inflow to outflow.

Considering that there were no depth tendencies in unplanted HSSF CW, we assume that the decrease in partial loads in this system might be due to microbial assimilation (uptake).

Planted HSSF CW could remove up to 100% of NH_4^+, which was an average of 23.4 mg NH_4^+-N L^{-1}. With an inflow rate of 7 L h^{-1}, this system could clean up the NH_4^+ contamination in the Leuna megasite in 44,000 years. This is due to a large volume of contaminated groundwater (2.7×10^9 L). Increasing the CW area would reduce the clean up time, however, CWs alone cannot be used as a single remediation strategy for contamination on such a scale.

5.2 Microbiological results

To examine the microbiological structure of the biofilms in CWs, planted HSSF CW was chosen as it had shown the best results of

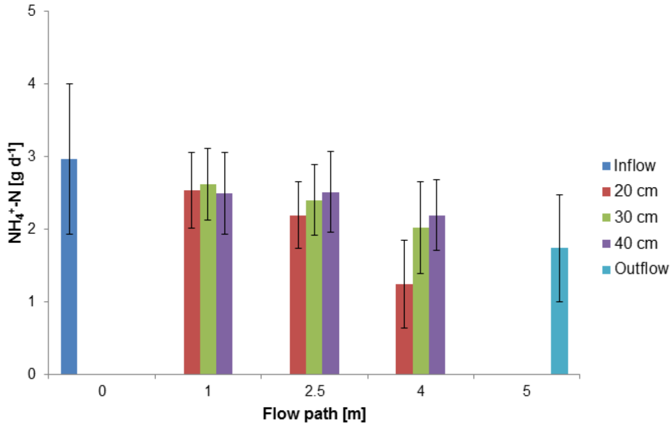

Figure 4: Average loads of NH_4^+-N in unplanted horizontal subsurface flow constructed wetland

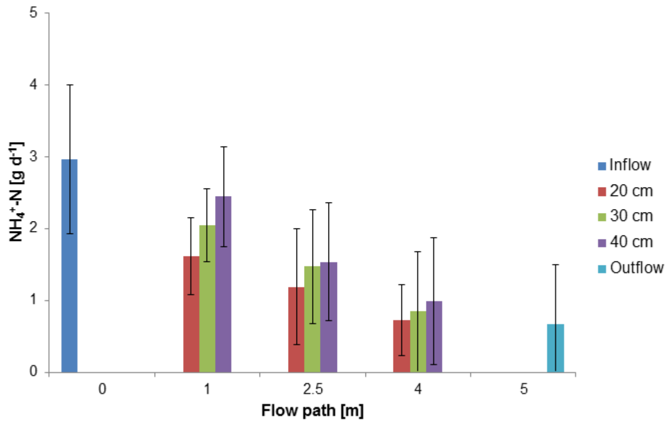

Figure 5: Average loads of NH_4^+-N in planted horizontal subsurface flow constructed wetland

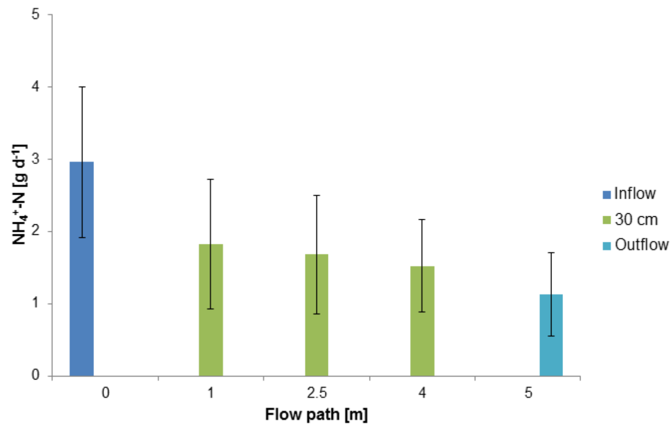

Figure 6: Average loads of NH_4^+-N in floating plant root mat

NH_4^+ removal efficiency. Samples of gravel and roots were taken at distances of 1, 2.5 and 4 m from the inlet at 20, 30 and 40 cm depth. Afterwards, DNA was extracted from the biofilms attached to roots and gravel, and Q-PCR analyses for total bacteria, ANAMMOX and ammonia oxidisers were conducted. ANAMMOX were detected in low amounts, 4-20% of total bacteria. Taking into account their extremely slow growth, i.e. metabolism rates, they could be responsible only for small parts of NH_4^+ elimination in our CWs. Thus, even though ANAMMOX communities were identified within the wetlands, coupled nitrification-denitrification and plant uptake were responsible for most of nitrogen removal.

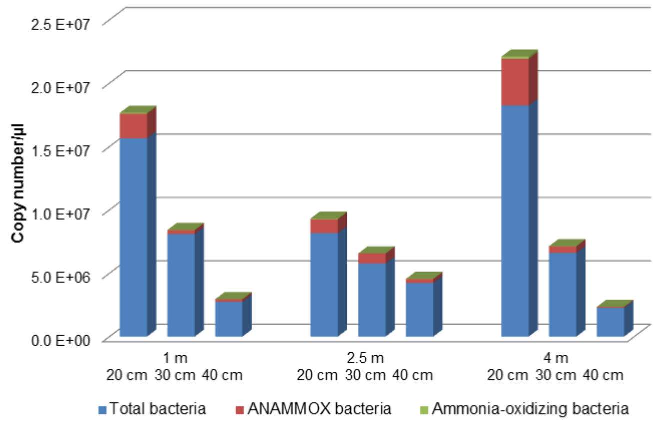

Figure 7: Copy number of different clusters of bacteria in dependence on depth and distance from inflow in planted HSSF CW

The highest number of total bacteria was found at the 20 cm depth, as well as anaerobic NH_4^+ and aerobic ammonia oxidisers (Fig. 7). These results agree well with the pattern of NH_4^+ removal, where the highest removal efficiency was achieved at 20 cm as well. The data prove that roots of plants with rhizospheres are necessary for efficient maintenance of aerobic as well as anaerobic bacterial populations and thus the occurrence of both NH_4^+ removal pathways.

6. Overall Conclusion

CWs overall show a good performance for bioremediation of NH_4^+ contaminated groundwater. Up to 100% removal of inflowing NH_4^+ was achieved in planted HSSF CW. Microbial methods helped to identify key processes responsible for NH_4^+ removal. Although ANAMMOX communities were identified within the wetlands, coupled nitrification-denitrification and plant uptake accounted for most of nitrogen removal. However, the removal efficiency is strongly seasonal (insignificant removal efficiency in winter), which has to be considered in the up-scaling of these systems for full scale contaminated groundwater treatment. Also, an understanding of the processes within the system is necessary for further technological improvement, as various factors influence the processes of the nitrogen cycle (Saeed and Sun, 2012). The present work connects removal efficiency of the CWs with the ongoing microbially promoted chemical processes of contaminant elimination and therefore demonstrates how important it is to take these into consideration when designing CW facilities.

7. Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013 under grant agreement n°265063), SAFIRA project and the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

8. References

- Del Bubba, M., Lepri, L., Griffini, O., and Tabani, F. 2000. *Nitrogen removal in a pilot-scale subsurface horizontal flow constructed wetland*. Annali Di Chimica. 90 (9-10): p. 513-524.
- Drizo, A., Frost, C.A., Smith, K.A., and Grace, J. 1997. *Phosphate and ammonium removal by constructed wetlands with horizontal subsurface flow, using shale as a substrate*. Water Science and Technology. 35(5): p. 95-102.
- EEC. 1998. Official Journal of the European Communities Council Directive Drinking water quality intended for human consumption, Brussels. p. 98/83/EC, L 330/32.
- Garcia, J., Rousseau, D.P.L., Morato, J., Lesage, E., Matamoros, V., and Bayona, J.M. 2010. *Contaminant Removal Processes in Subsurface-Flow Constructed Wetlands: A Review*. Critical Reviews in Environmental Science and Technology. 40(7): p. 561-661.
- Harrington, R. and R. McInnes. 2009. *Integrated Constructed Wetlands (ICW) for livestock wastewater management*. Bioresource Technology. 100(22): p. 5498-5505.
- Havens, K.E., Hauxwell, J., Tyler, A.C., Thomas, S., McGlathery, K.J., Cebrian J., Valiela, I., Steinman, A.D., and Hwang, Soon-Jin. 2001. *Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress*. Environmental Pollution. 113(1): p. 95-107.
- Hunt, P.G., M.E. Poach., S.K. Liehr. 2005. *Nitrogen cycling in wetland systems. Nutrient management in agricultural watersheds: a wetland solution*, ed. K.R.R. E.J. Dunne, O.T. Carton (Eds.): Wageningen Academic Publishers, The Netherlands. pp. 93–104.
- Ip, Y.K., S.F. Chew, and D.J. Randall. 2001. *Ammonia toxicity, tolerance, and excretion*, in Fish Physiology, W. Patricia and A. Paul, Editors. Academic Press. p. 109-148.
- Kadlec, R.H. 2000. *The inadequacy of first-order treatment wetland models*. Ecological Engineering. 15(1-2): p. 105-119.
- Kadlec, R.H. and S.D. Wallace. 2008. *Treatment Wetlands*, Second Edition. CRC Press.
- Lee, C.G., T.D. Fletcher, and G.Z. Sun. 2009. *Nitrogen removal in constructed wetland systems*. Engineering in Life Sciences. 9(1): p. 11-22.
- Mangimbulude, J.C., N.M. van Straalen, and W.F.M. Röling. 2012. *Microbial nitrogen transformation potential in surface run-off leachate from a tropical landfill*. Waste Management. 32(1): p. 77-87.
- Martienssen, M., Fabritius, H., Kukla, S., Balcke, G.U., Hasselwander E., and Schirmer, M. 2006. *Determination of naturally occurring MTBE biodegradation by analysing metabolites and biodegradation by-products*. Journal of Contaminant Hydrology. 87(1-2): p. 37-53.
- Martin, J.F. and K.R. Reddy. 1997. *Interaction and spatial distribution of wetland nitrogen processes*. Ecological Modelling. 105(1): p. 1-21.
- Paredes, D., Kuschk, P., Mbwette, T.S.A., Stange, F., Muller, R.A., and Koser, H. 2007. *New aspects of microbial nitrogen transformations in the context of wastewater treatment - A review*. Engineering in Life Sciences. 7(1): p. 13-25.
- Saeed, T. and G. Sun. 2012. *A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media*. Journal of Environmental Management. 112(0): p. 429-448.
- Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J. Gijs, Jetten, Mike S. M., Strous, M. 2003. *New concepts of microbial treatment processes for the nitrogen removal in wastewater*. FEMS Microbiology Reviews. 27(4): p. 481-492.
- Sikora, F.J., Tong, Z., Behrends, L.L., Steinberg S.L., and Coonrod, H.S. 1995. *Ammonium removal in constructed wetlands with recirculating subsurface flow: Removal rates and mechanisms*. Water Science and Technology,. 32(3): p. 193-202.
- Siljeg, M., L. Foglar, and M. Kukucka. 2010. *The ground water ammonium sorption onto Croatian and Serbian clinoptilolite*. Journal of Hazardous Materials. 178(1-3): p. 572-577.
- Stottmeister U., A.W.n., P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, H. Moormann. 2003. *Effects of plants and microorganisms in constructed wetlands for wastewater treatment*. Biotechnol. Adv. 22: p. pp. 93–117.
- Strous, M. and M.S.M. Jetten. 2004. *Anaerobic oxidation of methane and ammonium*. Annual Review of Microbiology. 58: p. 99-117.
- Vymazal, J. 2007. *Removal of nutrients in various types of constructed wetlands*. Science of the Total Environment. 380(1-3): p. 48-65.

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