STØTTET AF

Promilleafgiftsfonden for landbrug

Internship report

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Increase of constructed wetlands efficiency by adding bacteria

(Picture by Charlotte Kjærgaard)

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1. Introduction

1.1 Importance of nutrients and effect on aquatic environment

Nitrogen constitutes a major element of several key compounds such as proteins, nucleic acids, enzymes and chlorophyll involved in biological processes. Furthermore, it is one of the main chemical elements required for plant growth and reproduction as a component of chlorophyll, which is essential for photosynthesis *(Brady and Weil., 2010*).

The biggest N source is the atmosphere itself, however, molecular nitrogen (N_2) is not readily available for most of the plants. Another important source of N is soil organic matter. The amounts of plant-available N released during the decomposition of soil organic matter depends on many factors and therefore the rate of available N needs detailed consideration. It must be notified that N can be the most limiting factor for plant growth. Therefore, the use of N fertilizers is one of the most common agricultural practices to ensure a high crop yield *(Haygarth and Jarvis., 2001).*

Fertilizer provides two easily plant-available N forms, namely ammonium (NH_4^+) and/or nitrate (NO_3^-). While ammonium is positively charged and binds to negatively charged soil particles, depending on soil pH, nitrate is negatively charged and therefore highly mobile in soil solutions *(Hatch, 2002).* According to this, nitrate can be easily migrating below the plant root zone. It is documented that around 38 – 50 % of field applied fertilizer can be potentially lost from the production system (*Gardner and Drinkwater., 2009)*, which results in economic loss for the farmer. At the other hand this loss is causing detrimental ecological problems as deterioration of water quality. As a water pollutant, surplus of N can cause algal blooms, also known as eutrophication and as a result, water becomes nutrient rich (eutrophic) and submerged water plants might die. In particular cyanobacteria and unicellular blue-green algae are toxic to animals and humans. The overgrowth of algae blocks sunlight for underwater plants and subsequent to die of algae and oxygen in the water can decline during decomposition, which may even cause fish kills *(Heathwaite, 1993).*

Similarly, as with N, around 40% of phosphorus (P) reaching the sea comes from agricultural P losses associated with use of fertilizers *(Heckrath et al., 2008).* As P strongly binds in soil, loss of P is mainly associated with mobilization of soil particles and partly P is released from sorbent to solution, transportation e.g., though the soil matrix, macropores, drains, surface runoff *(Heckrath* *et al., 2008).* Increasing concentration of P in the freshwaters is one of the main environmental concern associated with P, which significantly reduce water quality. When P enters a watercourse, it may as N, also cause water eutrophication depending on prevalent limiting conditions. Water eutrophication results in an increased growth and development of aquatic plants such as algae, which lead to the same consequences as it was described above. The main difference between N and P eutrophication is that N eutrophication mainly occur in the N-limited coastal areas, where P eutrophication is more typical for P-limited lakes and rivers *(Brady and Weil., 2010).*

In Denmark, the awareness towards the state of the aquatic environment can be described with the DPSIR model and started in 1986. A group of fishermen sailed into a port with their catch – dead Norway lobsters. Two years earlier, in 1984, the plan included restrictions for the farmers on their handling of manure, and restrictions towards point sources to reduce nutrient leaching was presented. From year 2003, the Water Environment Plans was implemented to fulfil the EU Water Framework Directive (WFD), that aims to protect all terrestrial surface waters and sets environmental goals for all water bodies to achieve good ecological status *(Brozek, 2019).* According to WFD all waters including surface and ground waters should achieve good quality by year 2027. However, it can be challenging as these targets may potentially impact agriculture, industries, and the household sector, since compliance with the WFD can result in costly investments. Therefore, an in-depth knowledge of the current ecological and chemical status is needed to ensure targeted and cost-effective measures.

1.2 Constructed wetlands as a mitigation measure

Constructed wetlands (CW) are manmade engineered water treatment systems that can perform multiple treatment principles such as biological, chemical and physical processes, likewise occurring in natural wetlands (*Zhao et al., 2018).* It was widely demonstrated that CW are a very perspective mitigation measure and can significantly reduce the amount of nutrients in the effluent waters. Furthermore, they can also play a role in reducing the frequency and intensity of floods by acting as natural buffers, soaking up and storing a significant amount of floodwater.

1.2.1 Wetland types and characteristics

Nowadays, constructed wetlands have two main types of engineering: surface flow systems and subsurface flow systems. The surface flow constructed wetlands can be described as a vegetated systems that also include open water surfaces that are close to natural wetlands and according to a water flow regime can be classified into free water surface flow systems and integrated constructed wetlands (*Haberl et al., 2003).*

Free water surface flow constructed wetlands

Typically consists of open water flowing horizontally through floating vegetation and emergent plant attached to parallel basins, canals or ditches. The flow regime can be regulated with the use of dikes and levees *(Kadlec and Wallace, 2009).* Typical configuration of the free water surface wetland is presented on the Figure 1.1.

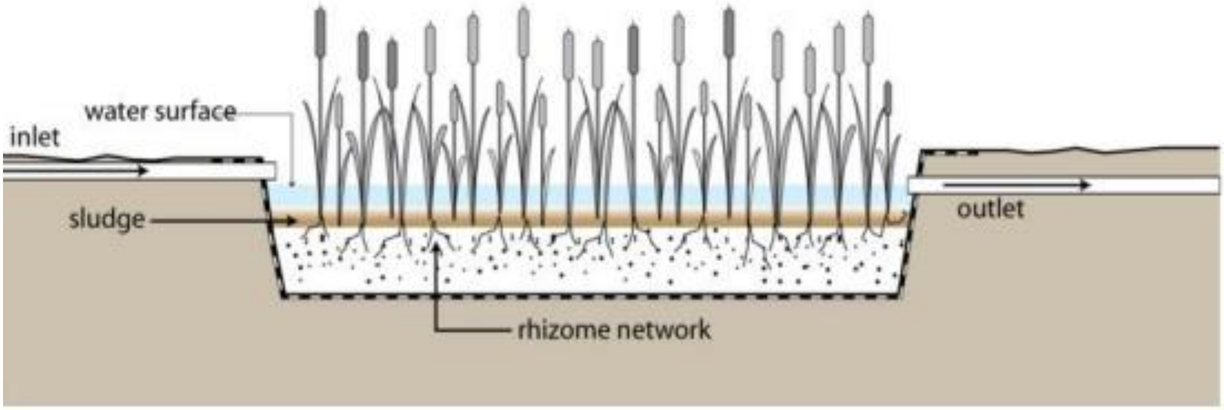

Figure 1.1: configuration of the free water surface wetland *(Sandec and Eawag, 2009a)*.

The influent water with particulate and dissolved pollutants, diffuse over the surface and percolates through emerging or submerged vegetation. Therefore, such a type of wetlands is particularly effective in removing total suspended solids (TSS) through sedimentation and filtration processes and considerably effective in N removal by nitrification and denitrification *(Kadlec & Knight, 1996).* The efficiency of such type wetlands depends on the climatic conditions. For example, during winter period, low temperature can significantly reduce the removal efficiency of all oxygen-dependent processes and inhibit the hydraulic operation. There is also a potential risk of recontamination from wildlife with pathogenic microorganisms, originating from open construction type *(Kadlec & Knight, 1996).*

Subsurface flow (SSF) constructed wetlands compared to surface flow wetlands have no free water level which is visible. The contact area of water with bacteria and substrate in such wetlands is much larger then in surface flow wetlands, and the treatment mainly occurs in the lower layers of the wetland where anoxic (no oxygen) conditions are present *(Haberl et al., 2003).* SSF constructed wetlands can be divided into two types according to a water flow direction through the porous media (sand or gravel): Horizontal subsurface flow (HSSF) and Vertical subsurface flow constructed wetland (VSSF).

Horizontal subsurface flow (HSSF) constructed wetland

Can be considered one of the most widespread subsurface wetlands and mainly consist of wetland vegetation attached on a bed which can be gravel or sand. Typical configuration of the Horizontal subsurface flow constructed wetlands is presented on the Figure 1.2.

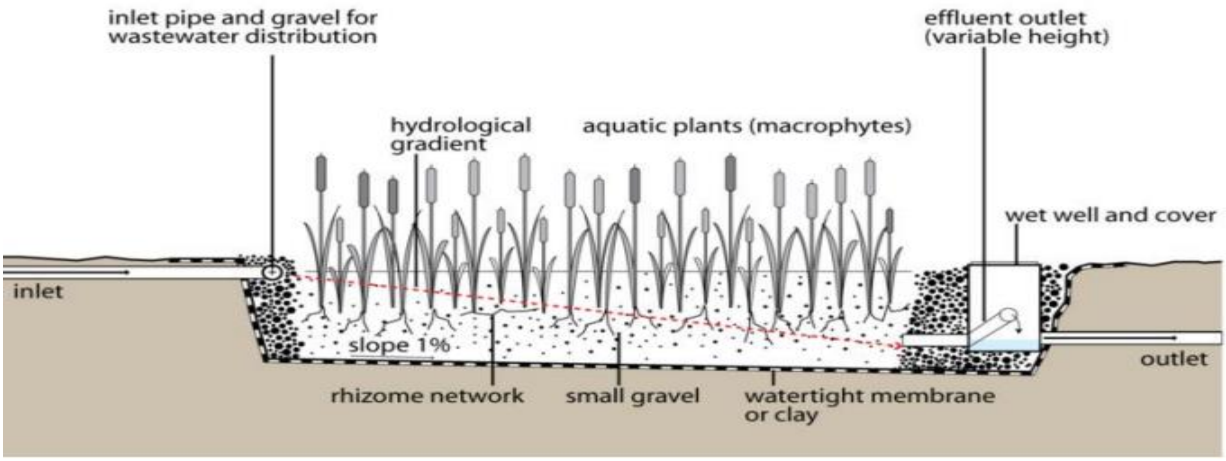

Figure 1.2: Typical configuration of the Horizontal subsurface flow constructed wetlands *(Sandec and Eawag, 2009b)*.

The effluent water flows horizontally beneath the surface of the media and percolates slowly through the rooted zone of the vegetation until it reaches the outlet, and on its way can be involved in a series of aerobic, anoxic and anaerobic zones that most of the time take place in the rhizosphere *(Cooper et al., 1996).* However, the anoxic and anaerobic biodegradation are dominated and plays an important role in the purification of water in subsurface horizontal wetlands *(Vymazal & Kröpfelová, 2006).*

The advantages of such constructed wetlands are the fact that the water, according to its flow pattern is not exposed to the environment and therefore, there is minimal risk of recontamination and exposure to pathogens. Moreover, it is less temperature dependent due to insulation of the surface. However, logging of the media substrate is one of the main disadvantages of these systems and require more expensive maintenance *(Wallace & Knight, 2006).*

Vertical subsurface flow constructed wetland (VSSF)

Vertical flow constructed wetlands mainly differs from HSSF wetlands by the flow regime, which is vertical, meaning that the inlet water penetrating through the soil layers of a basin. Typical configuration of the Vertical subsurface flow constructed wetlands is presented on the Figure 1.3.

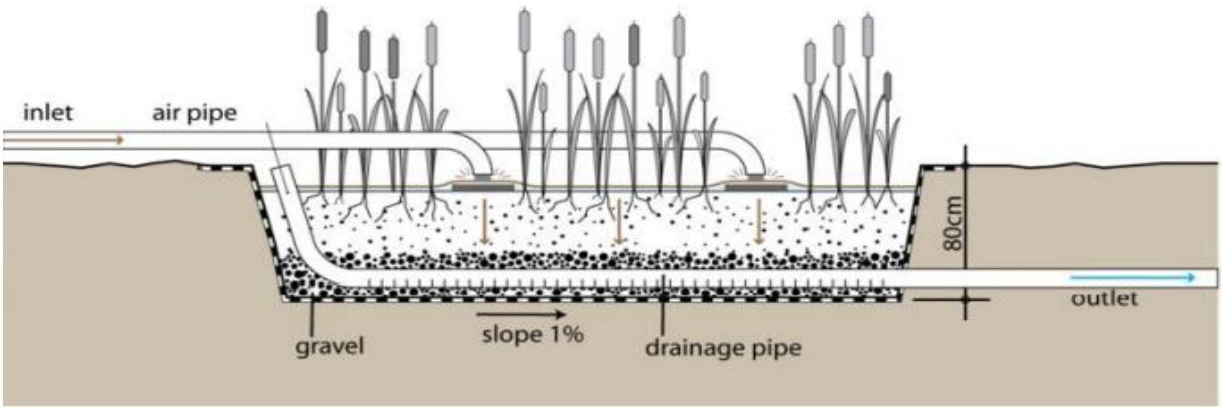

Figure 1.3: Typical configuration of the Vertical subsurface flow constructed wetlands *(Sandec and Eawag, 2009c)*.

The water enters the system through perforated pipes which are distributed through the surface of the wetland in the form of a network. The ducts carry (air pipes) a specific-sized hole that through them, a constant flow penetrates the surface *(Kadlec and Knight, 1996).* There are "upflow'' and "downflow" systems of VSSF which are related to the direction of the flow of the water. The "upflow'' is preferred in situations where the oxygen transport has to be reduced *(Kassenga et al., 2004),* and the "downflow" where the oxygen transfer is imperative, to produce a nitrified effluent. The important disadvantage of such systems is clogging of the media filter *(Winter & Goetz, 2003),* therefore, the selection of the most suitable media filter is one of the most important things to consider.

1.2.2 Nitrogen (N) and Phosphorus (P) removing process in the wetland

Nitrogen removal rates in horizontal subsurface flow constructed wetlands differs and ranging from high removals of over 90% to removals as low as 11%. In Denmark removal efficiency for nitrogen is around 22% and for phosphorus 45%. The main N removing processes in the wetland

includes nitrification/denitrification, sedimentation, microbial and plant uptake, volatilization *(Vymazal, 2002).* However, denitrification is the main mechanism of N removal. As lower parts of the constructed wetland cells do not receive enough oxygen to maintain aerobic conditions and become mostly anaerobic, this zone is suitable for a nitrate's removal by denitrification.
During the denitrification NO_3^- breaks down into nitrogen and nitrous oxide gas. These gases are then released into the atmosphere through a process called volatilization. Adsorption and plant uptake play much less important role in nitrogen removal in horizontal subsurface flow constructed wetlands *(Vymazal, 2005),* however, during a growing season a considerable amount of nutrients can be bound in plant biomass *(Vymazal, 2005).*

Phosphorus removal in the constructed wetlands can occur by the adsorption of phosphorus to the gravel media, precipitation of insoluble phosphates with ferric iron and aluminum found in media, absorption by the wetland vegetation and can be stored in constructed wetland sediments *(Sa'at, 2006).* Phosphorus is removed primarily by ligand exchange reactions, where phosphate displaces water or hydroxyls from the surface of Fe and Al hydrous oxides and therefore, if constructed wetlands does not contain great quantities of Fe, Al the removal of phosphorus is generally low.

The main controlling parameters for Nitrogen removal is water residence time (WRT) which determine the hydrology (intensity of water flow) and normally are bigger in the summertime. The bigger the WRT is, the higher removal is expected, it is due to longer water residence time in the wetland and correspondingly more time for microorganisms to remove pollutants. Other important factor is the temperature, microorganisms are normally more active with the higher temperature and therefore the removal efficiency is always higher in the summer compared to winter.

The aim of this report is to see if the microorganism mixtures, which showed a positive effect on the water quality in the wastewater treatment plants as well as in natural lakes, can have the same positive effect on N removal in constructed wetland. The project includes brief description of the N removal process in the wetlands, followed by description of the project. Finally, evaluation of the data for the internship period will be presented and discussed.

2. Project description

2.1 General information about SEGES

The company SEGES is a part of Danish Agriculture and Food Council which offers solutions for the agriculture as well as for a food sector. As such, the main objective of the company is to identify the commercial potential in agriculture and to provide the Danish farmer with the best tools for their business as well as considering the environmental aspects and animal welfare. SEGES works with all aspects of farming, which includes crop and livestock production, the environment, organic farming including finance, tax legislation, IT and much more. In this connection, SEGES constantly collaborate with research institutions, public authorities, and private companies not only in Denmark but internationally.

More than 1000 field trials are performed by SEGES annually, moreover, the company also runs the laboratory for a pig disease and health control. Aside from it, SEGES is a close partner with a pig production and a part of DanAvl business area, where the main focus is to ensure max breeding and use of the most current and best technology. Professional database of the company includes more than 120,000 articles including results and analyses as well as clarification of regulations, with around 50,000 visits per month. SEGES also provides accounting program for a farmer to overview their financial position as well as other IT tools for cattle, pig or crop production management. The organization of the company consists of five agricultural sectors where each of them has own sector board. The schematical overview of the company's organization is presented on the Figure 2.1.

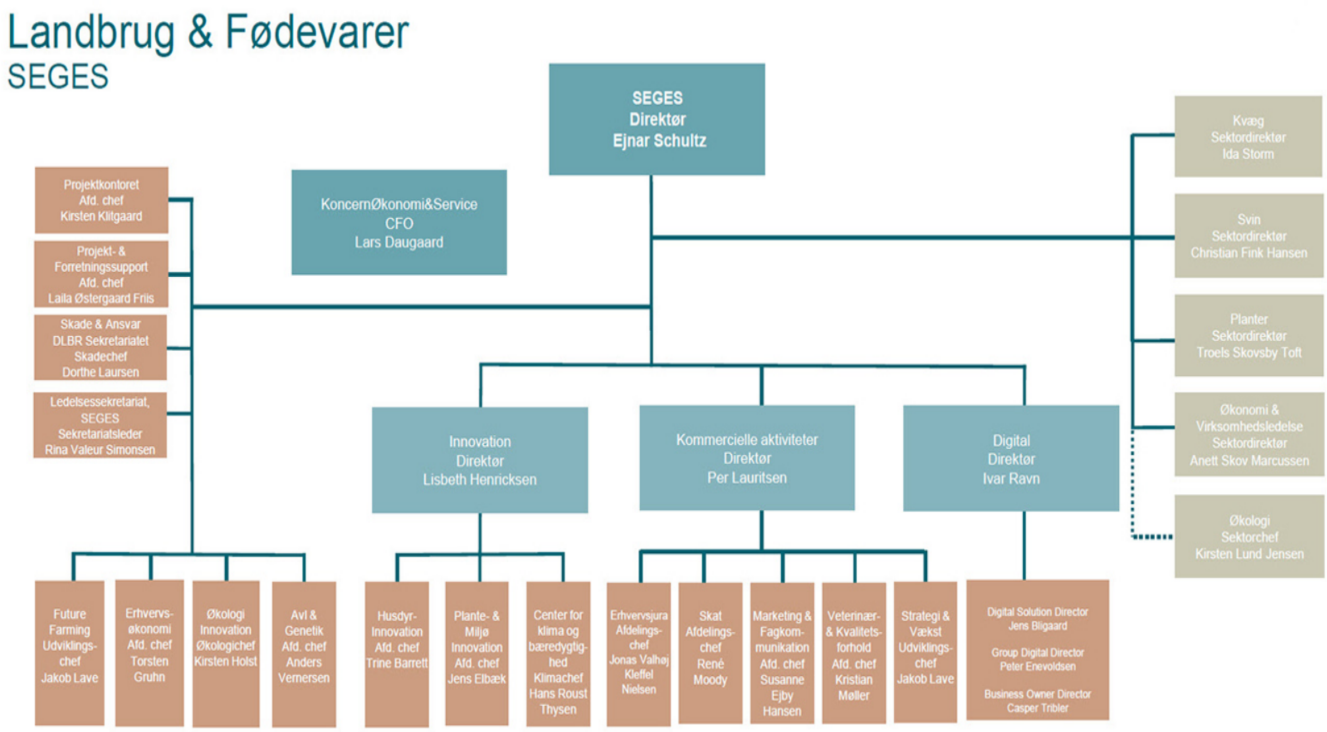

Figure 2.1: Organization of the company (SEGES official website).

In detail, the company includes a board of directors consisting of 25 members in total, 22 of which are board directors and 5 is a corporate board directors. The main task of the primary boards is collaboration with a member association to ensure the business policy, social, cultural, and professional interest of farmers in Denmark.

Sector boards represents farmers in the individual sector, each sector has own strategies and purpose which can be find on the company's homepage. There are around 650 people currently employed at SEGES all over Denmark, the annual money turnover of the company is around 1 billion DKK.

2.2 Project description

The main purpose of the project is the addition of a microorganisms mix to promote denitrification and a general performance of the wetland which is denoted as bioremediation. Bioremediation is a process that uses microorganisms and higher life forms to restore an ecosystem altered by contaminants to its original natural condition. This process can contribute to ecosystem recovery by reducing organic matter, assuring nitrification, denitrification, and hydrogen sulfide reduction (odor abatement), and bio control of pathogens. Denitrification as the main targeted process in the project is performed primary by heterotrophic bacteria in the absence of oxygen. Heterotrophic bacteria use carbon as an energy source and response rapidly to any changes in substrate, resulting in population decline (Fig. 2.2).

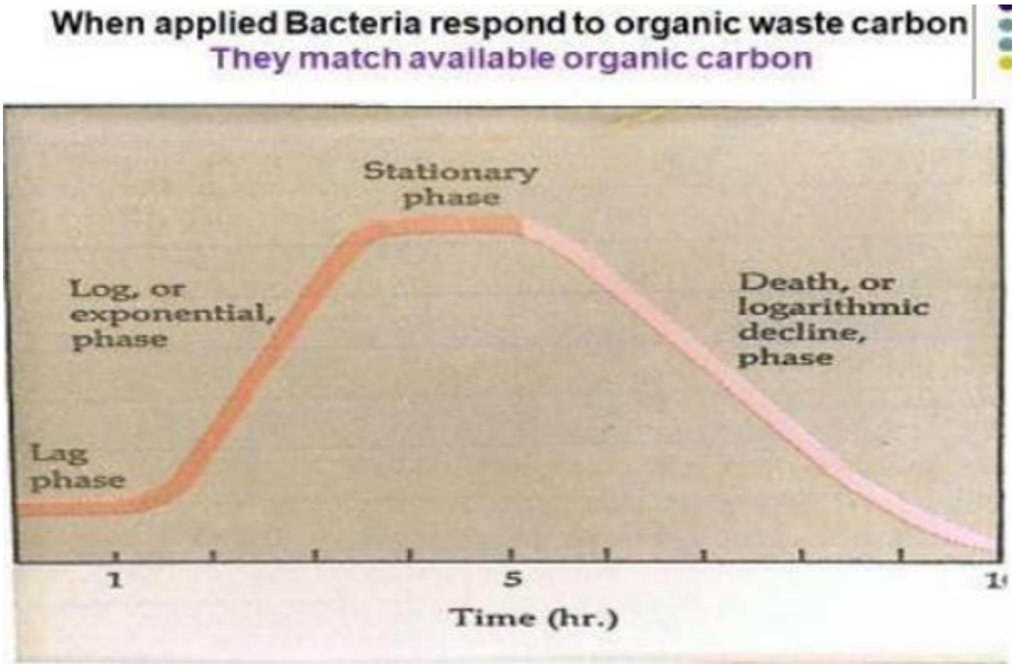

Figure 2.2: Bacteria growth curve which shows different phases of growth including their time scope (Dent.,2020)

Specifically, the bacterial growth consists of 'lag phase', (microorganisms identify the substrate and then produce the necessary enzyme reactions), 'log growth' (the microorganisms are responding to the substrate in cell growth), the 'stationary phase' (the microorganism will develop to equal the waste matter, referred as food to mass ratio). At some point, a food supply will reduce, leading to the microorganism decline – called 'death or declining phase'. It is suspected that bioremediation can be a good opportunity to enhance biomass performance by assisting the indigenous microorganism in the combined capability to breakdown and remove a greater range of pollutants at a faster rate *(Dent.,2020).*

The effectiveness of using bioremediation in water treatment can be explained by the microbe's culture consortium, which consists of a vegetative gram-positive microorganism. Gram positive microorganisms have a thick cell wall which makes them more suitable for surface applications, since their cells can withstand rigorous manipulation conditions. On addition, microorganisms are in an active state and start their process when applied, meaning that they have a very short adjustment to the environment “the lag-phase” (Figure 2.2), they will then rapidly enter “log-growth until reaching steady-state”, the stationary phase (Figure 2.2). Moreover, the culture consortium will increase in numbers as they utilize available substrate, it is assumed that there is always some continued carbon loading and recycling in wetlands and aquatic environments, stored within the wetland’s rhizosphere, within plant root zones, and in storm water basin bottom solids. The microorganisms will also attach by charge to soil particles, change their polymer structure and will function in fixed film processes (biofilms) rather than in “suspended growth” *(Dent.,2020).*

The ongoing project started in September 2021 in a collaboration between SEGES, microbes.dk and Aarhus University. An established mini wetland (2015) was selected to be inoculated with a solution containing a mixed culture of bacteria 9-times over a six months period as shown in Table 2.1. (see section 2.3 for site description).

Table2.1: Treatment plan of adding a bacteria solution to an established mini wetland (see section 2.3 for details)

Basin: SEGES-mini wetland									
Surface m^2: Around 2.450									
Expected start: September 2021									
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total:**
60 ltr	12 ltr	12 ltr	12 ltr	12 ltr	12 ltr	12 ltr	12 ltr	12 ltr	156 ltr

The total expected application of the solution with microorganism is 156 l with the biggest application of 60 l at the beginning. The targeted application interval is biweekly, however, to be suspended in case of ice covering. The treatment is performed by microbes.dk, which also provide the microorganisms mix (Figure 2.3). microbes.dk is likewise in charge to monitor the wetland state after each application. The microorganism mix was delivered in a plastic container and well mixed by shaking before filling into distribution container on an ATV (see Fig. 2.3). It is estimated that 4 liters of bacterial solution is applied per 100 m^2 water surface, which equates approximately 300 ml bacteria per 100 m^2 of wetland.

Figure 2.3: Application of the microorganisms to a constructed wetland by microbes.dk (Pictures was taken on the personal devise).

2.2.1 Product description

The bacteria mixture is developed by Ecological Laboratories in Florida, USA. It is a biotechnology company that develops and manufactures novel, proprietary, liquid microbial formulations that are suggested of solving many of the most challenging environmental problems. microbes.dk is a Danish company which acts as a distributor as well as performs bacteria spraying and monitoring.

The bacteria mixture used in the project is called MICROBE-LIFT /IND-HC for Industrial & Municipal WWT Systems (Fig. 2.4) and contains 13 naturally occurring organisms that have been enriched. The color of the product ranges from light pink to dark red, pH of the mixture is in the circum-neutral range, i.e., range between 6.9-7.5. According to the producer it is natural, nontoxic, and nonpathogenic culture which is not harmful for humans, animal, and marine life. However, Aarhus municipality also assessed the risks associated with the use of bacteria mixture in the wetland. According to assignment, the use of microorganisms is considered not at the high risk of infection to humans and animals, but it is recommended to use gloves and dust mask during spreading and no spreading is allowed under wind conditions more than 8 m/s.

Figure 2.4: MICROBE-LIFT /IND-HC for Industrial & Municipal WWT Systems used in the project

The cultural classification of the bacteria in the mixture includes:

- Heterotrophic Bacteria – Utilize organic matter as energy source
- Autotrophic Bacteria – Utilize carbon dioxide as energy source.
- Phototrophic bacteria – Utilizes light for energy. Can be autotrophic or heterotrophic in its food source. Essential to the technologies capability to restore water quality, reducing lysis in the declining phase, utilize CO_2 as an energy source.
- Chemoorganotrophic bacteria – Utilizes organic compounds for energy. Usually heterotrophic in food source.
- Chemolithotrophic bacteria – Utilizes inorganic substances for energy. The sulfide-oxidizing and nitrifying bacteria found in ecosystems are examples of lithotrophs. Can be autotrophic or heterotrophic in its food source.
- Archaea – A separate taxonomic domain from bacteria. Archaea are a major part of Earth's biodiversity and play roles in both the carbon and nitrogen cycle. They can endure high temperatures, organic solvents, and chemical toxicities that bacteria cannot.

A full list of the species presents in the mixture as well as their function are presented in the Table 2.2.

Table 2.2: List of Bacteria in the mixture used for the wetland treatment.

Species	Inferred function(s)	Mechanism	Specific Remarks
Bacillus amyloliquefaciens	Amine degradation; Protein degradation	Heterotrophic uptake	Not an animal pathogen
Bacillus licheniformis	Protein degradation, nitrification	Heterotrophic uptake, nitrite oxidation	Abundant In nature, opportunistic pathogen
Bacillus subtilis	Hydrocarbon degradation ; Amine degradation ; Protein degradation	Heterotrophic uptake	Not an animal pathogen
Clostridium butyricum	E. coli inhibition; Complex organics degradation	Antibiotics produced inhibit pathogens, LCFA degradation	Used probiotics in Asia, found in sour milk products
Clostridium sartagoforme	Organic Nitrogen removal; Complex organics degradation	Chitin degradation, LCFA degradation	Used as a probiotic
Desulfovibrio vulgaris	Sulfur reducing; Toxic heavy metal removal	Reducing sulfate to sulfide; Converting heavy metal to insoluble matter	Common in nature, reduces Cr (VI) to Cr (III), and tetrachlorethylene to trichlorethylene
Desulfovibrio aminophilus	Sulfate reducing; Degradation of amino acid	Amino acid oxidation, reducing sulfate to sulfide; Converting heavy metal to insoluble matter	In sewage treatment plants, anaerobic
Geobacter lovleyi	Benzene-, toluene- or xylene- (BTEX) degrading; PCE removal, Heavy metal removal	Benzene Oxidation; Reductive dechlorination, Heavy metal oxidation	Reduces chlorine compounds
Methanomethylovorans hollandica	Sulfur compound removal; Odor control	Utilization of dimethyl sulfide (DMS); Methylamine's degradation	Found in soil and groundwater
Methanosarcina barkeri	Nitrate removal; Odor control	Nitrogenase, denitrification, Methylamines degradation	Widespread in biogas plants
Pseudomonas citronellolis	Ammonia removal; Phosphorous removal, complex organics degradation	Nitrate reducing, ammonia oxidation, Accumulating poly – P, Hydrocarbon degradation	Common in forest soil

Rhodopseudomonas palustris	Phosphorous removal; Odor control; Hydrocarbon degradation	Accumulation of poly P; Degradation of LCFA, cellulose	Common in nature
Wolinella succinogenes	Sulfur reducing; Odor control; Removal of toxic organics	Oxidizes sulfide; Nitrate reduction; syntrophic degradation with sulfate reducers or Geobacter spp	Decomposes perchlorate, used for cleaning drinking water

Description of the bacteria used in the project:

- *Bacillus amyloliquefaciens* is a gram-positive soil bacterium that breaks down proteins and amino acids. The bacterium is used in agriculture to fight root pathogens, and one of the enzymes from the bacterium is used in washing powder.
- *Bacillus licheniformis* is a gram-positive soil bacterium that can break down otherwise difficult-to-degrade proteins, e.g. in bird feathers. The bacterium that can survive very high pH values, mixed in foods can cause stomach problems. A protein from the bacterium is utilized by Novozymes in washing powder.
- *Bacillus subtilis* is gram-positive soil bacterium that is also found in / on plants. It breaks down proteins, carbon compounds (eg triglycerides) and is tolerant to large fluctuations in pH and oxygen concentrations. Recent research shows that it is optional aerobic. Under anaerobic conditions, it can reduce nitrate.
- *Clostridium butyricum* is a gram-positive, obligatory anaerobic bacterium that produces butyric acid that is easily broken down by other bacteria. It surpasses the highly pathogenic bacteria Clostridium difficile.
- *Clostridium sartagoforme* is an anaerobic, gram-positive bacterium. It is found in the human intestinal system. The bacterium plays a significant role in degradation of cellulose and chitin.
- *Desulfovibrio vulgaris* is gram-negative, and is the best known of the sulfate-reducing bacteria. The bacterium occurs frequently in the wild and is a Tunisian pathogen. It is found in the intestinal systems of animals, in both fresh and salt water and in soil. It is commonly used for the treatment of soil contaminated with it carcinogenic Cr (VI).

- *Desulfovibrio aminophilus* is a gram-negative, sulfate-reducing bacterium that also effectively breaks down proteins and amino acids.
- *Geobacter lovleyi* is a gram-negative bacterium that is able to reduce metal clays and degrade chlorine compounds. It occurs naturally in sediments and used for bioremediation of contaminated soil.
- *Methanomethylovorans hollandica* is not an actual bacterium but belongs to the domain archea, which are single-celled organisms without the nuclei. It is found naturally in sediment and can decompose under difficult anaerobic conditions.
- *Methanosarcina barkeri* is an archea and is found in sediment in lakes and bogs, as well as in wastewater. The is also a normal part of the intestinal flora of ruminants, and it metabolizes efficiently carbon compounds to methane.
- *Pseudomonas citronellolis* is a gram-positive bacterium found naturally in forest soil, often near resin-producing trees. The bacterium is able to break down oil and carbon compounds that are otherwise persistent. It can also remove nitrate by denitrifcation.
- *Rhodopseudomonas palustris* is a gram-negative bacterium that can use four various forms of metabolism. It is able to break down lignin, can absorb CO_2 and N_2 from the atmosphere and can produce NH_4^+.
- *Wolinella succinogenes* is a proteobacterium found in the intestinal tract of ruminants. It is not pathogenic, although it is related to Helicobacter pylori. The bacterium can metabolize hydrogen sulfide and is thus potentially able to reduce odor nuisances.

Important functions of MICROBE-LIFT technology:

- Microorganism function aerobically, anaerobically and under anoxic oxidation reduction processes that will take place within the anaerobic zones of the wetland organic mass, where they will utilize nitrate as their electron acceptor.
- Biological oxidation reduction process will generate kinetic heat, and store this within soil layers and bottom solids to support and increase environmental temperatures as temperature decline.
- Technology contains photosynthetic culture that control cell lysis, that is ensure the integrity of microbial cells.

- Rhodopseudomonas palustris which is included in the microbial mixture can utilize light photon and CO_2 in the absence of nutrient, therefore, preventing the release of nutrient from cells.

2.3 Site description

Fensholt / Vesterskovvej is a mini wetland located in Odder, east Jutland. Being a mini wetland with a surface run-off it is established adjacent to the main drain, so that drainage water flows into open basins, where sedimentation and microbial processes occurs (Fig. 2.5). The wetland can be visually divided into seven parts with a different size and depth (for further details see Table 2.3).

Moreover, mini wetland creates a habitat for plants, insects, and other small animals and as such increase biodiversity in the area as well as act as backwater basins, which are useful for climate adaptation. The typical cost of such wetlands ranges from 100,000 to 300,000 DKK for 1 ha of wetland, however, according to the Rural Development Program, the establishment of wetland can be compensated, which make it a very attractive mitigation measure for the farmers.

Fensholt / Vesterskovvej mini wetland was established in 2015 in the catchment area of Norsminde Fjord under GUDP project. The area of the wetland is about 0,245 ha (2450 m^2) which receive drainage water from agricultural land of 33 ha (330,000 m^2) corresponding to 0.7% of the total drainage area and thus is a bit undersized in relation to current requirements for minimum 1% (Kjærgaard & Hoffmann, 2017). The drainage water flows from the field area through wetland before discharge into stream, where the main purpose of wetland is to minimize nitrogen pollution.

Before 2018 the drainage water ran via the sedimentation basin over a physical rim and into the upper part of the water column in the first deep basin. However, in November of 2018 the mini wetland was renovated resulting in design changes. According to the changes, the drainage water from sedimentation basin is collecting in two closed pipes and subsequently transported to the bottom of the first deep basin. From there, the water is distributing into the water column by 9 closed to the end perforated collectors placed in direct contact with the bottom sediments, where the water column was also raised to 1 meter (Fig. 2.6). A new design aim to ensure that the drainage water entering the wetland will be discharged into water column which results in more contact with the bottom sediments and better performance of the wetland.

Figure 2.5: Fensholt / Vesterskovvej mini wetland, general overview ((Kjærgaard.,2020).

Figure 2.6: Wetland, where yellow dot indicates the inlet, red dots are automatic sampling stations. (Zacho, (SEGES), 2021)

Table 2.3: Description of the wetland

Name	Size (m^2)	Depth (m)	Function
Sedimentation basin	153	1.2	Settlement of particulate particles
Basin 1	600	1	Water storage
Low water level basin 1	434	0.3	Water distribution, buildup of C in the system
Basin 2	227	1	Water storage
Basin 3	475	1	Water storage
Low water level basin 2	446	0.3	Water distribution, Water distribution, buildup of C in the system
Basin 4	307	1	Water storage

The drainage water enters the wetland from the drainage well into sedimentation basin showed on the Figure 2.6 as a yellow dot. The wetland is also equipped with three measurement stations, which includes precipitation meter, electromagnetic flow meter and ISCO automatic water samplers. Automatic samplings are marked on the Figure 2.6 as a red dot and are located at inlet, after first deep basin in the Low water level basin 1 showing a middle concentration, and in the outlet. The water samples are automatically taken every hour and are later on mixed to daily samples for analysis. The laboratory analyzes includes total N (TN), consisting of NO_3-N, NH_4-N, and organic N as well as total P (TP), which consists of TP filtered and unfiltered, particular P (PP), and PO_4-P.

A full monitoring of the wetland was initiated in spring of 2019 with collaboration between SEGES and the Department of Bioscience Aarhus University. From that period, sampling and analysis of data is caring out by Aarhus University and includes one grab sampling occasion every 3 weeks. Moreover, measurements also include precipitation and flow rate registration, manual spot sampling as well as emptying of ISCO automatic samplers.

2.4. Water flow and nutrient removal

2.4.1 The inflow

The inflow (Q) to the mini wetland showed high seasonal changes during the whole monitoring period July 2017 to July 2020 (Fig. 2.7). In the second half of 2020 until mid-December there was no runoff from the drains, which was rather uncommon.

Furthermore, there was no significant difference in inflow before the renovation (year 2018) and after renovation (from year 2019).

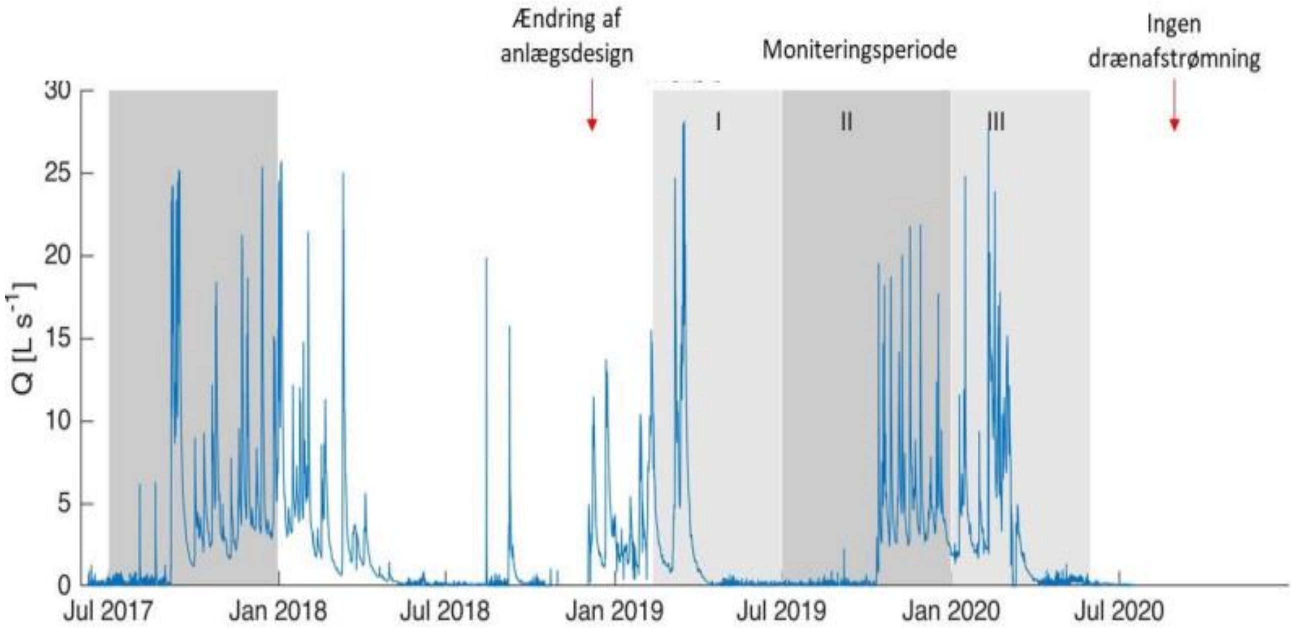

Figure 2.7: Inflow (Q) to the Fensholt mini wetland. The monitoring period for the project is marked with gray fields from February 2019 to July 2020, period from July 2017 is also included for a comparison. *(Hoffmann & Petersen, 2020).*

2.4.2 Nitrogen removal

For the measurement period of 2015-2017 before the experiment, the mini-wetland area reduces the annual average nitrogen loss in drains from 31.1 to 26.2 kg N / ha agricultural area. The average water-weighted concentration of nitrogen is reduced from 10.3 to 8.6 mg/l. Average N effect is 646 kg N / ha which is 16% of applied N and is lower than national average of 25% (Table 2.4).

Table 2.4: Total nitrogen (TN) transport and reduction from the catchment (Kjærgaard.,2020).

Monitoring period	TN inflow (kg/ha)	TN inflow (kg)	TN outflow (kg)	TN reduction (kg)	TN reduction (kg/ha)	TN reduction (%)
07/17-12/17	17.1	563	456	107	473	19
02/19-06-19	10.5	347	295	52	212	15
07/19-12/19	18.6	615	535	80	327	13
01/20-06/20	12.3	405	328	77	314	19

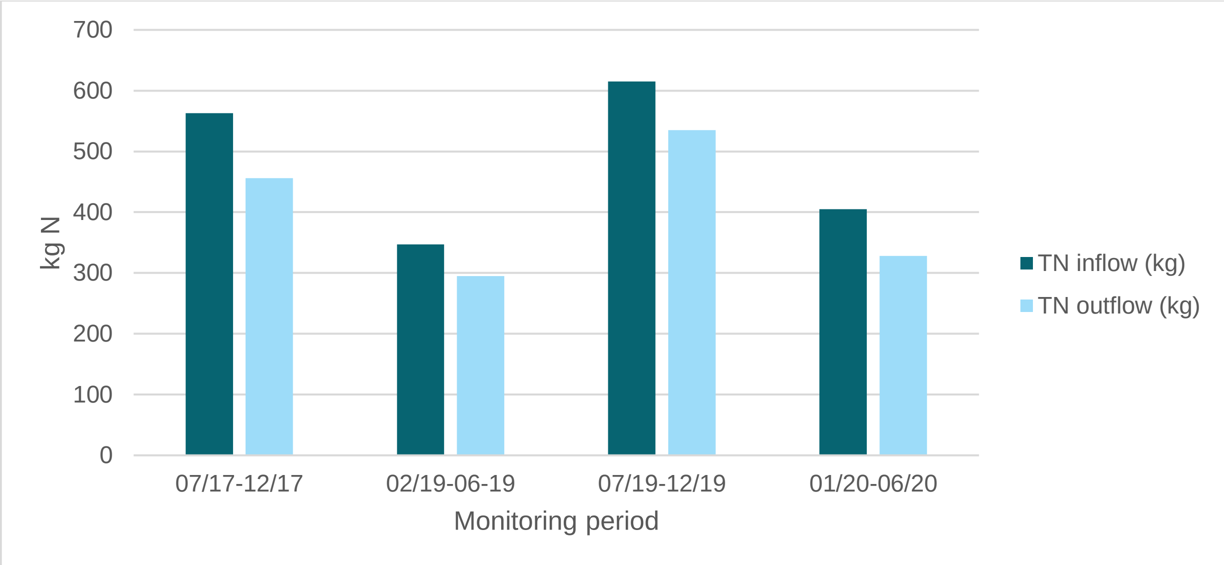

Figure 2.8: Total nitrogen (TN) transport and reduction in the Fensholt mini wetland

In the first half of 2019, nitrogen inflow was significantly lower than the rest of the year, and the overall N reduction was rather high with 19%. Overall, the N inflow was highly variable with in the monitoring period with highest amount in the second half of 2019. In this period the N reduction efficiency was the lowest recorded. In the first half of 2020 the efficiency was again 19%, the same as before the renovation, meaning that optimization of the wetland was not improving the N removal. However, due to missing data such conclusion must take with caution. The main component of the N-drainage water supply to the mini-wetland is dominated by NO_3-N and accounts to 91-92% of TN (Hoffmann & Petersen, 2020).

2.4.3 Phosphorus removal

For the same measurement period (2015-2017), the mini wetland reduced the annual average P-loss in drains from 0.883 to 0.616 kg P / ha agricultural area. The average water flow weighted TP concentration declined from 0,289 to 0,184 mg/L, which corresponds to a P removal effect of 36 kg P/ha or a P removal efficiency of 35%, respectively. As for N the P inflow was highly variable for different monitoring years (Fig. 2.9) but contrary to N the P removal efficiency was highest at highest P import (Table 2.5)

Table 2.5: Total phosphorus (TP) transport and reduction in the Fensholt mini wetland (Kjærgaard.,2020).

Monitoring period	TP inflow (kg/ha)	TP inflow (kg)	TP outflow (kg)	TP reduction (kg)	TP reduction (kg/ha)	TP reduction (%)
07/17-12/17	0.658	21.7	13.9	7.8	31.8	36
02/19-06-19	0.152	5.0	3.0	2.0	8.16	40
07/19-12/19	0.430	14.2	8.1	6.1	24.9	43
01/20-06/20	0.903	29.8	16.8	13	53.1	62

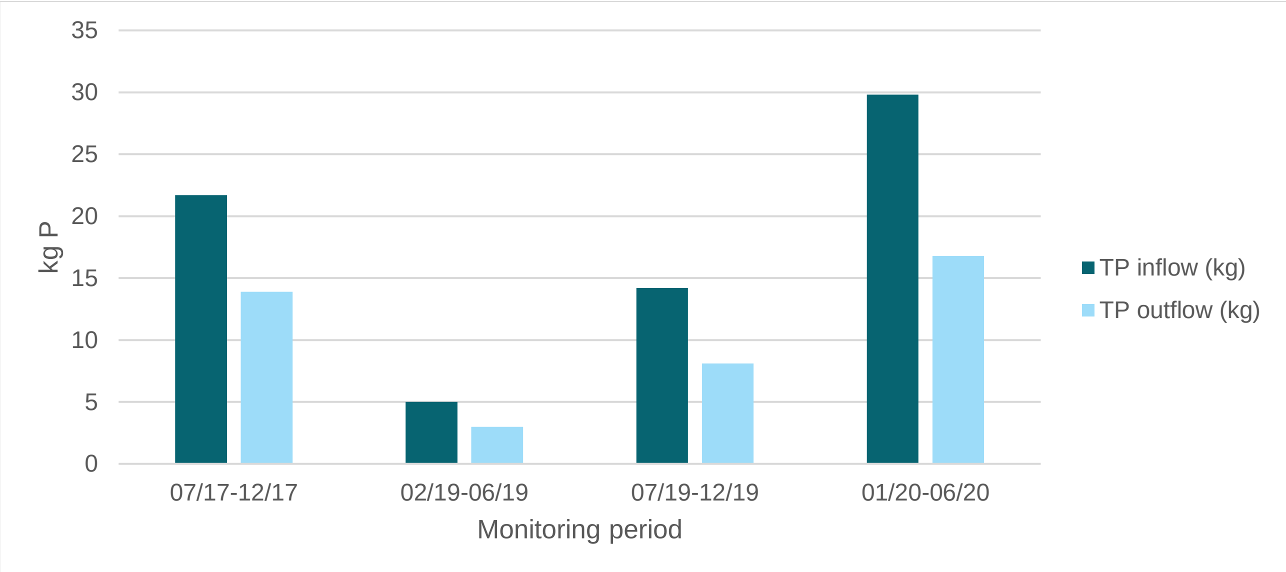

Figure 2.9: Total phosphorus (TP) transport and reduction in the Fensholt mini wetland

In the year 2017 prior to the wetland optimization experiment, the P-transport was generally high, and the TP retention was 36% (Table 2.4). In the monitoring period after wetland optimization the TP effect of the mini wetland significantly increased up to 62%. However, the limited monitoring period in the fall of 2020 make it difficult to conclude on the TP effect after optimization, but the results indicate that there may be a positive effect on the TP retention. Dissolved reactive PO_4-P is the dominant P fraction in the drainage water supply accounting to 65-89% of TP, except for the first half of 2020 where PO_4-P only makes up 38% of TP in inlets. There is some evidence that the distribution of P may have an effect on the net TP retention.

3. Methods

Water samples for analysis were taken by ISCO automatic water samplers placed in the wetland. Every 30 minutes these samplers take a sample over 24 hours which is combined in one bottle to a composite sample both at the inlet, outlet and after first deep basin. From spring 2019 sampling, analysis and quality assurance of data is carried out by the Department of Ecoscience Aarhus University. Aside from automatic sampling, every 3 weeks water samples are taken manually.

The analysis of the samples is performed according to European and Danish Standards, for example, nitrate and total nitrogen determination follows the standard method called high-performance liquid chromatography (DS / EN ISO 10304 2009). The ion chromatography (illustrated in Fig. 3.1) is likewise used for determination of dissolved ions of bromide, chloride, fluoride, nitrate, nitrite, orthophosphate, and sulfate in water. The detection limit of application is 0,05 mg N/l for nitrite, and 0,1 mg N/l for nitrate. The analytical range may be expanded to lower concentrations (0,01 mg N/l) if an appropriate pretreatment of the sample is applied, and/or if an ultraviolet (UV) detector (for bromide, nitrate and nitrite) is used.

Anions of interest (nitrates and nitrites) are separated by different solubility in stationary phase (ion exchange resin) and in the mobile phase (aqueous solutions of salts of weak mono- and dibasic acids).

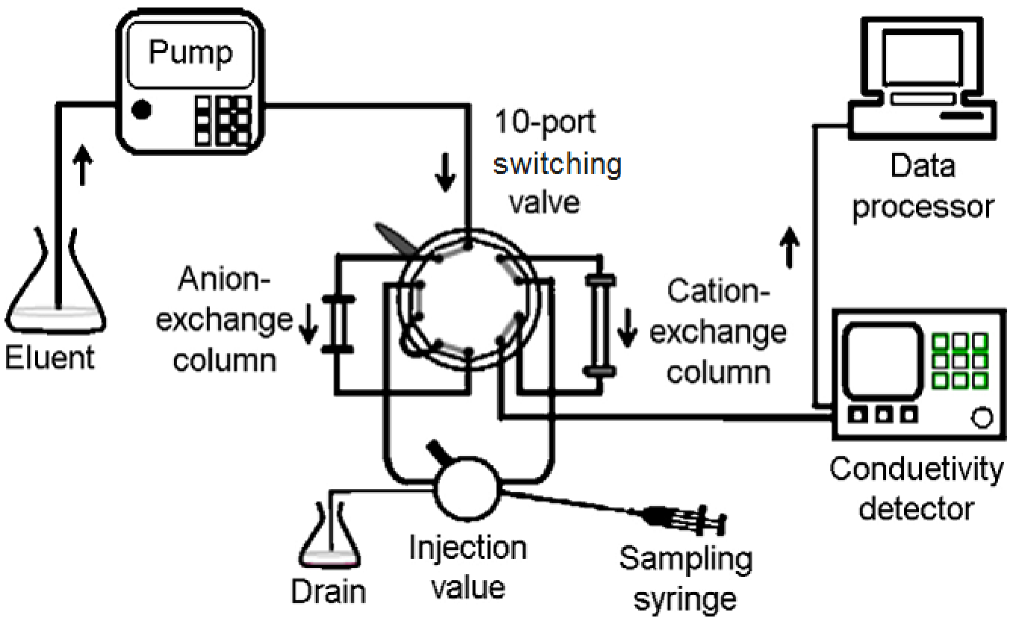

Figure 3.1: Ionic chromatographic system (Moustafa&Morsi.,2012)

The whole analytical process is divided into five steps:

Step 1: The eluent ($NaNO_3$ for nitrate and $NaNO_2$ for nitrite) loaded onto the column and displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.

Step 2: A sample containing anion A and anion B are injected onto the column

Step 3: After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample moves through the column, anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.

Step 4: As the eluent continues to be added, the anion A moves through the column in a band and ultimately is eluted first.

Step 5: The eluent displaces anion B, and anion B is eluted off the column.

After separation is done, the suppressor reduces the electrical conductivity of the eluent and increases the electrical conductivity of the analytes, so they are delivered to the detector. A computer and software are used to control the system, acquire, and process the data. The single analysis is recorded as a peak graph which is used for calculation of mass concentrations of anions in the solution given as mg/l (EN ISO 10304 2009).

4. Results and discussion

Mass balances for TP and TN were conducted for 01/01/2020 till 01/06/2020 (without microbial treatment) and 12/01/2021 till 18/10/2021 monitoring periods to proof the effect of adding a microbial mix on the nitrogen removal efficiency of a mini wetland. This approach has some limitations since no replication for the microbial treatment could be done during the short time of the project. For the interpretation of data, it is also important to consider seasonal effects like varying temperature and water flows as wells water quality changes which might overwhelm the effect of microbial treatment.

For the period from 01/01/2020 till 01/06/2020 data was extracted from an annual report (Kjærgaard.,2020) and includes TN, TP inflow (kg), TN and TP reduction in kg and in % (Table 4.1).

Table 4.1: Total phosphorus (TP) and total nitrogen (TN) transport and reduction in the Fensholt mini wetland for a period from 01/01/2020 till 01/06/2020 (without microbial treatment).

Pollutant	Inflow (kg)	Outflow (kg)	Reduction (kg)	Reduction (%)
TN	405	328	77	19
TP	29,8	16,8	13	62

For the same period a daily inflow (Q) was plotted into graph to see a seasonal variation as well as periods with high and low inflow rate (Fig. 4.1).

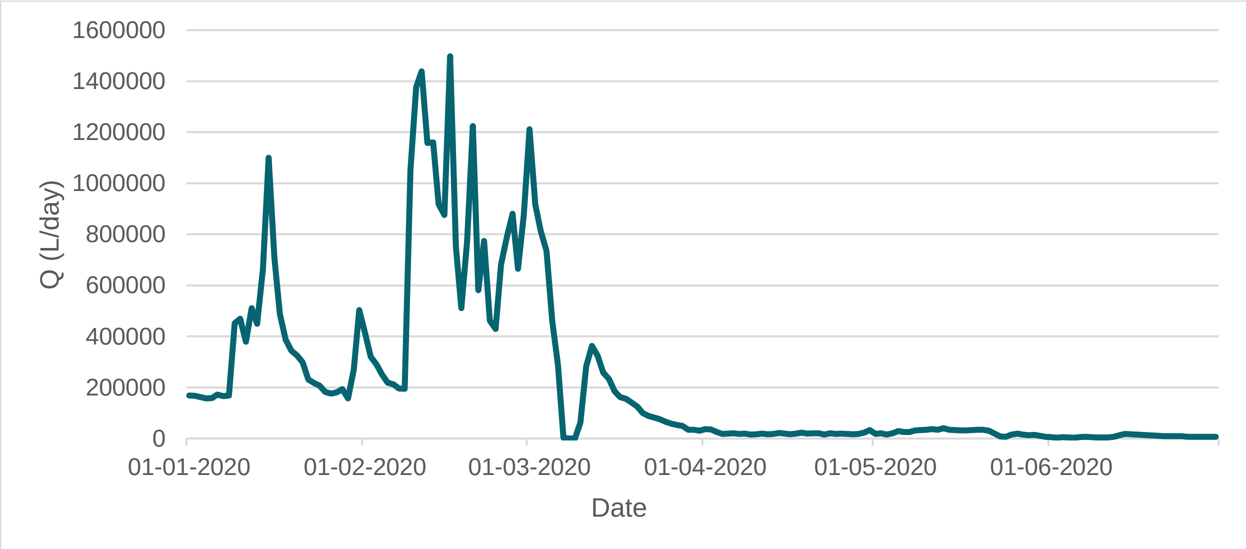

Figure 4.1: Inflow (Q) to the Fensholt mini wetland during the period from 01/01/2020 till 01/06/2020

As it can be seen from the Figure 4.1 water inflow to the wetland starts sharply in the end of January with varying peak flows higher than 600000 L/d until the end of March. In the late spring period, inflow dropped down to flow lower than 20000 L/d and almost no water flow during summer. Removal efficiency for P was 62%, which is higher than during other monitoring periods. The reason for that might be design change in 2018, forcing the drainage water to flow along the bottom of the wetland basin rather than at the surface. This hydrological change might have enhanced the contact with bottom sediments and thus increase the P removal by sorption processes.

However, there was no such an effect detected on N removal, which was before and after the re-construction 19%. Accordingly, the design change didn't improve N removal. In the following year in 2019 the N reduction was even smaller, i.e., accounted around 13%. This drop down might be related to wetland design. Higher water flow at the bottom may increase the redox potential in the bottom sediments and even cause resuspension of the sediments impairing denitrification but this needs detailed investigations. At the same time, it can be hypothesized that the lack of denitrifying bacteria explains the low removal efficiency. The latter was proofed by the on-going microbial-lift experiment.

Unfortunately, data from 12.01.2021 to 10.12.2021 are lacking, corresponding to two microbial applications, since automatic water sample was broken and therefore the results include data only until 18.10.2021 corresponding to one microbial application. Moreover, also previous data are not complete due to suspended sampling. Interpolation was done to fill these data gaps using following aquation:

$$Bx = \frac{(Ax2 - Ax1)}{(\mathrm{ROW(Ax2)} - \mathrm{ROW(Ax1)})}$$

where B_x is the unknown data; Ax1 are known coordinates that are above unknown values; Ax2 are known coordinates that are below unknown values.

As there was no data provided on precipitation and evaporation in the area, the difference in inflow and outflow was neglected and considered to being the same. The obtained results are summarized and presented in the Table 4.2.

Table 4.2: Total phosphorus (TP) and total nitrogen (TN) transport and reduction in the Fensholt mini wetland for a period from 12/01/2021 till 18/10/2021 (with bacterial treatment from 23.09.2021)

Pollutant	Inflow (kg)	Outflow (kg)	Reduction (kg)	Reduction (%)
TN	48	25	23	48
TP	3,5	1,3	2,2	63

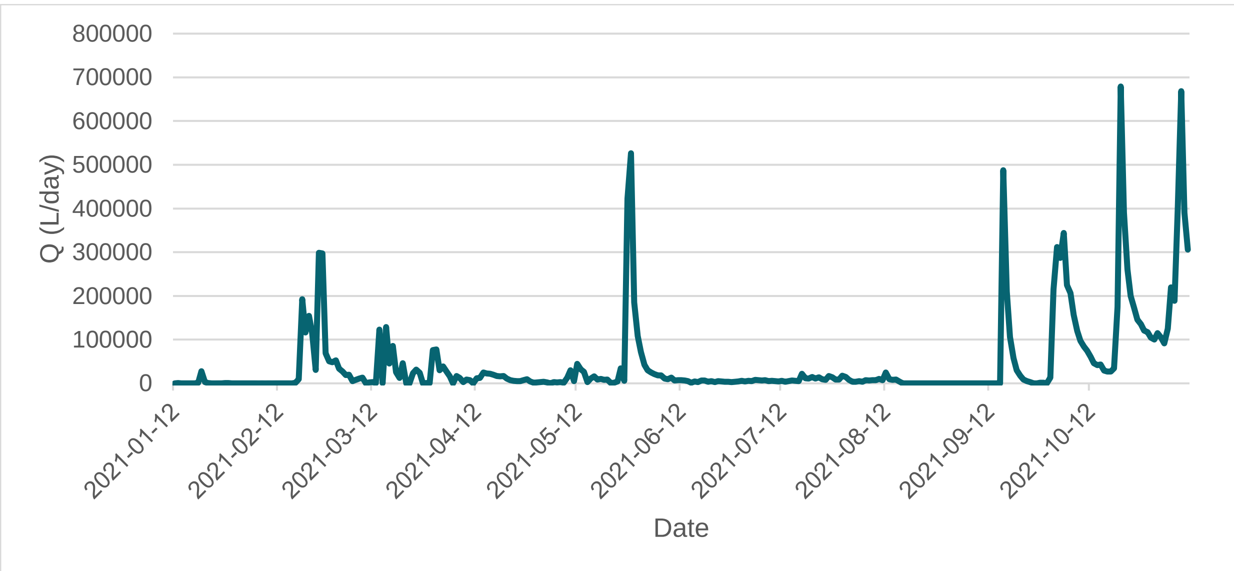

Figure 4.2: Inflow (Q) to the Fensholt mini wetland during the period from 12/01/2021 till 18/10/2021

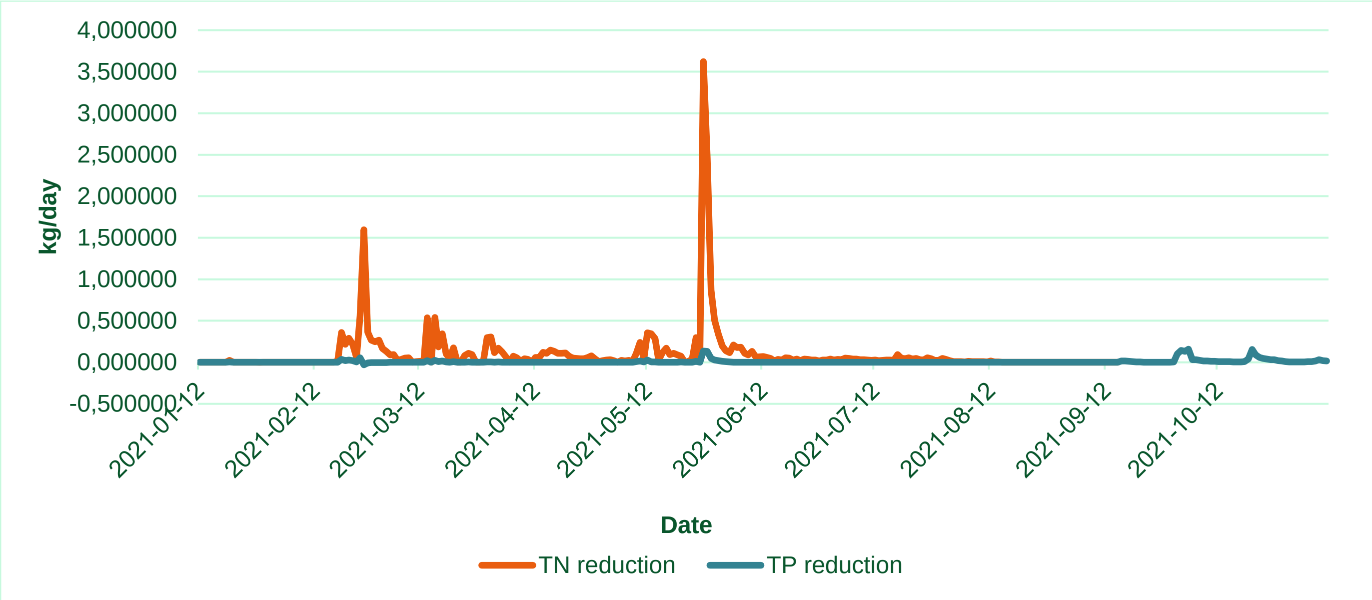

Figure 4.3: Tn and TP daily reduction for a period from 12/01/2021 till 18/10/2021

The P removal of 63% in 2021 was the same as in the previous monitoring period, so that an effect of microbial mixture on P removal can be excluded. However, any effect on P removal neither an increase nor decrease by adding the bacteria was not expected although a higher N removal by bacterial growth could be also associated with bacterial P uptake.

Nitrogen removal efficiency obtained during calculation was surprisingly high (48%) which is twice higher than any other recorded values starting from the wetland establishment. One of the reasons for a such high removal efficiency might be bacteria mixtures, but as the project is still ongoing and the calculation include only one microbial application, meaning that the highest removal efficiency should be obtained after full bacteria treatment (9 applications), the results must be still interpreted with caution. This holds also true because the lack of data, and necessity to extrapolate some of the data, which resulted in a low accuracy of the results.

Moreover, according to the Figure 4.2. the inflow to the wetland from 12/01/2021 to 18/10/2021 is very low to compare to the period from 01/01/2020 to 01/06/2020. And as it can be seen from the Figure 4.2. whole monitoring period can be described as very dry except for short inflow peak in June 2021 and in the end of October 2021. That might be the reason for such a high TN removal as lower inflow will result in longer WRT meaning that bacteria have more time to denitrify and therefore higher removal efficiency. Unfortunately, no result of N and P inflow was obtained from the end of October representing data with a high inflow are not included into calculation which might significantly influence the removal efficiency.

To sum up, further data are needed to draw sound conclusions on the effect of microbial mixture on the N removal efficiency. Since the monitoring is ongoing and will continue over a longer period, the effect of microbial mixture might be better emphasized in future. So far, primary climate conditions are proofed to be significant parameters influencing the efficiency of wetlands. Beside of complete and reliable data investigations should be replicated elsewhere to unravel the impact of bacteria adding's on the N removal efficiency of such constructed wetlands.

5. Conclusions

Based on the presented report following conclusions can be made:

- Phosphorus and nitrogen are essential in agriculture but can lead to eutrophication and significantly reduce water quality;
- Constructed wetland is a promising mitigation measure, where average efficiency in Denmark is 22% for N and 45% for P;
- To improve efficiency of wetlands, the project between SEGES, Aarhus university and microbes.dk has started in September 2021;
- The aim of the project is to improve N removal by adding microbial mixture, called bioremediation;
- The treatment plan includes 9 applications (156 l) and contain 13 naturally occurring organisms;
- Fensholt / Vesterskovvej mini wetland was established in 2015 and renovated in 2018, where the removal efficiency before renovation was 19% for N and 36% for P, after renovation the efficiency is 13-19% for N and 40-62% for P;
- First microbial application was conducted 23.09.2021;
- The removal efficiency after microbial application is 48% for N and 63% for P;
- The removal efficiency for P is comparable with the efficiency in 2020, meaning that no significant influence on P removal by microbial mixture were observed;
- Observed nitrogen removal was significantly higher than in 2020, which might be associated with microbial application;

- When compare inflow data from 2021 with 2020, is it clear that high removal efficiency is most likely associated with very dry conditions observed in 2021 resulted in very low inflow and high WRT rather than with microbial application;

- As project is still ongoing, the real effect of microbial mixture on removal efficiency will be clearer by the end of the project.

6. Perspectives

The wetland technology is continuously improving. A wealth of studies about wetland technology have been published in the past years, which suggest a high interest in this mitigation measure. According to Danish "Agreement on green conversion of Danish agriculture" published 4th of October 2021, to achieve good water quality by 2027 (Water Framework Directive) a total reduction of nitrogen emissions should be approximately 13,100 t. Around 1,500 t of total nitrogen reduction it is planned to achieve using collective methods, such as afforestation, natural and constructed wetlands. According to this, the increase in the number of constructed wetlands in Denmark is expected, therefore stable work and high removal efficiency in the wetland is a key parameter and high priority.

As it was mentioned in the previous sections, national average N removal efficiency by constructed wetlands is about 25%, which probably can be improve. microbes.dk provides microbial mixtures that was successfully used in wastewater treatment plants and are proven to be environmentally friendly. A success of using microorganisms in water treatment are also proven by documented examples, which includes 56 cases in total, three of which is from Denmark (examples from Denmark is provided in the Appendix A).

Moreover, MICROBIAL-LIFT products were also evaluated by Aarhus Vand, one of the largest water company in Denmark, which supply drinking water, treat wastewater, and maintain sewer systems. According to their evaluation, a positive result during the performance tests was achieved, mainly in reduction of odor and erosion processes in the pipe systems. Aarhus Vand also plans to continue using microbial product and, moreover, expend the use to other facilities.

It is expected, that in case of positive effect of microbial mixtures on Fensholt wetland performance, microbial-lift technology will be implemented in the other constructed wetlands. Currently, there are around 100 constructed wetlands established in Denmark and if the bacteria adding shows promising results there are great perspective for significant increase of national average

N effect of wetlands and achieve higher nitrogen reduction. Furthermore, use of microorganisms is not only a natural way of water treatment, but it also does not require high investments, has low energy consumption and are relatively cheap to compare to other methods used in classic water treatment. However, as MICROBIAL-LIFT products are mainly made for wastewater treatment use, one of the perspectives can be creation of microbial products which target denitrification and tolerant danish weather conditions, resulting in even further N reduction.

7. Referencies

Akratos, C. S., & Tsihrintzis, V. A. (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecological engineering, 29(2), 173- 191.

Alexandros, S. I., & Akratos, C. S. (2016). Removal of pathogenic bacteria in constructed wetlands: mechanisms and efficiency. In Phytoremediation (pp. 327-346). Springer, Cham

Barber. S.A., .1980. Soil-plant interactions in the phosphorous nutrition of plants. In: Khasawneh FE, Sample EC, Kamprath, EJ (eds) The role of phosphorus in agriculture. ASA CSSA-and SSSA, Madison, pp 591–613.

Brady. N., Weil. R. 2010. Nutrient Cycles and Soil Fertility. Chapter 12 of Elements of the nature and properties of soil, Third Edition. Pearson Education.

Brozek, M. (2019) Vandmiljø, Miljøtilstand.nu.

Cooper, P. F., Job, G. D., Green, M. B., & Shutes, R. B. E. (1996). Reed beds and constructed wetlands for wastewater treatment. European water pollution control, 6(7), 49.

Dent, D.2020. MICROBE-LIFT NOVEL TECHNOLOGY. Ecological Labarotories.

Gardner, J. B. & Drinkwater, L. E. 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of N-15 field experiments. Ecological Applications, 19, 2167-2184.

Goulding, K.W.T. and Webster, C.P. 1992. Methods for measuring nitrate leaching. Aspects of Applied Biology 30, 63–69.

Haberl, R., Grego, S., Langergraber, G., Kadlec, R.H., Cicalini, A.R., Martins Dias S (2003). Constructed wetlands for the treatment of organic pollutants. Journal of Soil & Sediments. 3 (2), 09-124.

Haygarth. P., Jarvis., S. 2001. Agriculture, Hydrology and Water Quality. USDA–ARS, Pasture Systems and Watershed Management Research Unit, Curtin Road, University Park, PA 16802- 3702, USA.

Hatch, D., Goulding, K., & Murphy, D. 2002. Nitrogen. In Agriculture, Hydrology and Water Quality CABI Publishing.

Heathwaite, A.L. (1993) Nitrogen cycling in surface waters and lakes. In: Burt, T.P., Heathwaite, A.L. and Trudgill, S.T. (eds) Nitrate. Process Patterns and Management. John Wiley & Sons, Chichester, UK, pp. 99–140.

Heckrath, G. et al. 2008. 'Review of indexing tools for identifying high risk areas of phosphorus loss in Nordic catchments', Journal of Hydrology, 349(1–2), pp. 68–87.

Kadlec, R. H. (1995). Overview: surface flow constructed wetlands. Water Science and Technology, 32(3), 1-12.

Kadlec, R. H., & Knight, R. L. (1996). Treatment wetlands. Lewis Publ., Boca Raton. Treatment wetlands. Lewis Publ., Boca Raton.

Kadlec R, Wallace S. Treatment wetlands, 2008, February, CRC press, second edition, ISBN 978-1420012514, 3.

Kadlec, R. H., & Wallace, S. (2008). Treatment wetlands. CRC press.

Kadlec, R.H., Wallace, S.D., (2009). Treatment Wetlands (2nd Ed). CRC Press Taylor Francis Group, Boca Raton, London, NewYork.

Kassenga, G., Pardue, J. H., Moe, W. M., & Bowman, K. S. (2004). Hydrogen thresholds as indicators of dehalorespiration in constructed treatment wetlands. Environmental science & technology, 38(4), 1024-1030.

Kjærgaard.2020. Innovationsplatform for Drænvirkemidler Foreløbig effektopgørelse af anlægsoptimerede minivådområder med overfladestrømning. Annual report. Plante- og Miljøinnovation, SEGES.

Moustafa. Y. and Morsi. R. 2012. Ion Exchange Chromatography - An Overview. Access from: https://www.intechopen.com/chapters/43603

Rousseau, D. P. L., Lesage, E., Story, A., Vanrolleghem, P. A., & De Pauw, N. (2008). Constructed wetlands for water reclamation. Desalination, 218(1-3), 181-189.

Sample. E.C., Soper RJ, Racz GJ .1980. Reaction of phosphate fertilizers in soils. In: Khasawneh FE, Sample EC, Kamprath EJ (eds) The role of phosphorus in agriculture. ASA-CSSA-SSSA, Madison, pp 263–304.

Sa'at S., 2006. MSc thesis entitled: Subsurface flow and free water surface flow constructed wetland with magnetic field for leachate treatment site. Faculty of Civil Engineering Universiti Teknologi Malaysia.

Sandec and Eawag (2009 a). Free water surface wetland.png. www.sandec.ch

Sandec and Eawag (2009b). Horizontal subsurface flow constructed wetland.png. www.sandec.ch.

Sandec and Eawag (2009c). Vertical subsurface flow constructed wetland.png. www.sandec.ch.

Scholz, M., Harrington, R., Carroll, P., Mustafa, A., (2007a). The Integrated Constructed Wetlands (ICW) concept. Wetlands 27, 337-354

Vymazal J., 2005. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecological Engineering 25, 478–490.

Toet, S., Van Logtestijn, R.S.P., Kampf, R., Schreijer, M., Verhoeven, J.T.A., (2005). The effect of hydraulic retention time on the removal of pollutants from sewage treatment plant effluent in a surface-flow wetland system. Wetlands, 25 (2), 375-391.

Vymazal J., 2002. The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. Ecological Engineering 18, 633–646.

Vymazal, J., & Kropfelova, L. (2006). Can we predict processes in the filtration bed of horizontal-flow constructed wetlands according to dissolved oxygen concentrations at the outflow. In Proceedings from the 10th International Conference on Wetland Systems for Water Pollution Control. Lisbon: Portugal.

Wallace, S. D., & Knight, R. L. (2006). Small-scale constructed wetland treatment systems: Feasibility, design criteria, and O&M requirements, Final Report. Water Environment Research Federation, USA, 304.

Weber, K. P., & Legge, R. L. (2008). Pathogen removal in constructed wetlands. Wetlands: Ecology, Conservation and Restoration, 176-211.

Winter, K. J., & Goetz, D. (2003). The impact of sewage composition on the soil clogging phenomena of vertical flow constructed wetlands. Water Science and Technology, 48(5), 9-14

Zhao, Y., Yang, Y., Liu, R., Morgan, D., (2018). Global development of various emerged sunstrates utilized on contructed wetlands. Bioresource Technology 262, 441-452.

Appendix A

Søer i Forstbotanisk Have ved Marselisborg Slot, Aarhus

Juni 2018 | **September 2018**

Sø i Sønderjylland ved landmand

3. juni 2017 – før

13. september 2017 - efter