WP2 – Nitrogen Removal

D2.4: Feasibility of mainstream nitrogen removal and biomass production with duckweed bioreactor

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Deliverable 2.4	*Feasibility of mainstream nitrogen removal and biomass production with duckweed bioreactor*
Related Work Package:	WP2
Deliverable lead:	VWT
Author(s):	David Kahlert Christian Loderer
Contact for queries	Christian.loderer@kompetenz-wasser.de
Grant Agreement Number:	n° 641661
Instrument:	Horizon 2020 Framework Programme
Start date of the project:	01.07.2015
Duration of the project:	36 months
Website:	www.POWERSTEP.eu
Abstract	The goal is to demonstrate and market a new wastewater treatment concept using duckweeds heading towards energy positive wastewater treatment plants. The investigations were first carried out on a laboratory scale to identify suitable duckweed species, the optimal duckweed mat density, relative growth rate (RGR), doubling time and the ammonium removal under the given conditions at the case study. Subsequently, the results were used to test on a large scale on a sewage treatment plant.

Dissemination level of this document

X	PU	Public
	PP	Restricted to other programme participants (including the Commission Services)
	RE	Restricted to a group specified by the consortium (including the European Commission Services)
	CO	Confidential, only for members of the consortium (including the European Commission Services)

Versioning and Contribution History

Version	Date	Modified by	Modification reasons
v.01	2018-02-15	David Kahlert (KWB)	1st Draft
	2018-03-01	Rabea-Luisa Schubert (KWB)	1st review
v.02	2018-03-15	Sebastian Kirchner (KWB)	2nd Draft
	2018-04-10	Henrique Sanchez (Annox)	2nd review
v.03	2018-04-20	Sebastian Kirchner (KWB)	Final version reviewed Christian Loderer
Final	2018-05-10	Sebastian Kirchner (KWB)	Based on the Feedback from Christian Loderer
v.04	2018-06-25	Sebastian Kirchner (KWB)	3rd Draft (adding latest Data)
	2018-06-29	Christian Loderer (KWB)	Final Version

Table of Content

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

List of figures

List of tables

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Executive Summary

One aim of the EU-funded research Project POWERSTEP is to investigate the applicability of duckweed in wastewater treatment in removing nitrogen based on the principle of the APS duckweed plant system. The motivation for this investigation is the intended combination of the Hydrotech drum filter with the APS duckweed plant system at case study one of the POWERSTEP project. The goal is to demonstrate and market a new wastewater treatment concept heading towards energy positive wastewater treatment plants. The investigations were first carried out on a laboratory scale to identify suitable duckweed species, the optimal duckweed mat density, relative growth rate (RGR), doubling time and the ammonium removal under the given conditions at the case study. Subsequently, the results were used to test on a large scale on a sewage treatment plant. From the four tested duckweed species Lemna Minor, Lemna Minuta, Landoltia Punctata and Spirodela Polyrhiza, the species Lemna Minor and Landoltia Punctata adapted best to the given wastewater composition. In a mix population of Lemna Minor and Landoltia Punctata a mat density of 0.075 $g \cdot cm^{-2}$ was determined to be best in suppressing competitive submerged algae growth and enabling duckweed relative growth rates of 0.072 d^{-1} and doubling times of 9.93 days. Based on the APS duckweed plant system, mean daily ammonium removal of 0.56 $g\ N \cdot m^{-2}d^{-1}$ and a daily ammonium degradation efficiency of 72.75% to a mean ammonium effluent of 12.26 $m \cdot l^{-1}$ was shown at a lab-scale for a retention time of 24 hours. Based on the results of this research, it can be concluded that the principle of the APS duckweed plant system under the use of Lemna Minor and Landoltia Punctata can be applied to remove ammonium from wastewater achieving high reduction rates. The experiment on the wastewater treatment plant shows that the effectiveness of the purification process is heavily dependent on climatic conditions. For example, in the summer the duckweed had a total nitrogen(TN) removal rate of 40-70%, while in winter it was only 17-40%. There were also great difficulties due to the occurrence of heavy storms. The plant switched off and was destroyed in many places which led to a dying of duckweed. There were also problems with the harvest of duckweed. Due to poor flow conditions, duckweed was not easy to clear off and could not be harvested.

Glossary

APS	Aqua Plant Solution GmbH
AOB	Ammonium Oxidizing Bacteria
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DW	Dry-weight
EPS	Extracellular Polymeric Substance
HRT	Hydraulic Retention Time
N	Nitrogen
NH_4-N	Ammonium Nitrogen
NO_2-N	Nitrite Nitrogen
NO_3	Nitrate Nitrogen
PAR	Photosynthetically Active Radiation
PE	Population Equivalent
PCS	Process Control System
SBR	Sequencing Batch Reactor
SD	Standard Deviation
SPC	Set Point Concentration
TKN	Total Kjehldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
WP	Work Package
WW	Wastewater
WWTP	Wastewater Treatment Plant

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

1. Introduction

Within the European project POWERSTEP, Work package 1 (WP 1) is dedicated to enhanced carbon extraction in preliminary clarification done via micro-screen filtration of municipal raw wastewater after the grid followed by a treatment through the use of an innovative duckweed reactor. The duckweed is especially examined for their applicability to lowering the input and increasing the usage of the energy potential of raw sewage.

Duckweeds are known for thriving well on water bodies rich in nitrogen and organic carbon compounds, partly relying on oxygen. Most of the carbon that is needed for their metabolic process is obtained via gaseous CO_2 from the air, so only a small amount of the carbon remains in the wastewater (Landolt, 1987). Duckweeds grow with a doubling time of 29.8 hours under ideal conditions, which makes them the fastest growing angiosperms in the Kingdom plantae (Appenroth, 2015). Their fast growth rate, high production of biomass and nitrogen uptake rates of up to 1.67 $g \cdot m^{-2} \cdot d^{-1}$ (Hasan, 2009) are promising features for remediating wastewaters low in carbon and rich in nitrogen.

Within the frame of POWERSTEP, the ammonium degradation and biomass growth is investigated in a half-automatic operated duckweed plant – unique in execution and operation. In preliminary studies the behavior of duckweeds under the given environmental conditions and operational performance of the duckweed plant are investigated prior to its commissioning.

The aim of this study is to carry out preliminary investigations on duckweed growth under the expected environmental conditions in the Westewitz wastewater treatment plant. The specific objectives of the study are to:

- Choose the optimal duckweed species for the remediation of the municipal wastewater at WWTP Westewitz;
- Determine the optimal mat density of selected duckweeds based on growth rate and suppression of submerged algae;
- Design and build a laboratory pilot based on the Aqua Plant Solution GmbH (APS) duckweed plant;
- Carry out test runs in the laboratory pilot plant to determine ammonium removal and identify operational issues of the APS duckweed plant concept;
- Carry out test runs in the pilot plant to determine the removal of ammonium, total nitrogen and total phosphorus and the enrichment of nitrate;
- Determine the optimal proportion of Duckweed for the methane recovery in BMP tests.

2. Theoretical Background

2.1. Duckweed

2.1.1. Overview

Duckweeds are the smallest and fastest growing flowering plants found in the plant kingdom (Wang, 2014b)_ENREF_4. They are aquatic plants floating on or below the surface of still and nutrient-rich fresh and brackish waters forming dense homogeneous or heterogeneous clonal populations (Armstrong, 2011),(Skillicorn, 1993). The duckweed family comprises of 37 different species divided into 5 genera (Appenroth, 2013) (Figure 1).

Figure 1 Left: Duck family on pond covered with duckweed (Bauribob, 2011); Right: The five genera of the duckweed family displayed on human hand (Lemnapedia, 2014)

Duckweeds can be found worldwide, though some species of duckweed are more prevalent in and better adapted to certain climatic zones. Duckweeds thriving in water bodies convert nutrients and minerals dissolved in the water column into plant biomass (Skillicorn, 1993). Duckweeds have been observed to thrive well on eutrophicated water bodies rich in nutrients. They reproduce vegetatively by forming daughter fronds budding within pockets of a mother frond (Sree, 2015). Depending on species, age and environmental conditions, frond size varies between 0.4 to 15 mm (Goopy, 2003). Under ideal growth conditions the fastest of the duckweed species can double its biomass within 29.8 hours which complies with a relative growth rate of 0.559 d^{-1} (Sree, 2015). Ideal growth conditions are given between 25°C-28°C (Landolt, 1987), a lighting duration of 24 hours at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Appenroth, 2016a) and a pH value between 5-8 (Landolt, 1987).

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

2.1.2. **Morphology**

Duckweeds are monocotyledons belonging to the botanical family. Figure 2 displays all known species and their relations based upon the chloroplast gene rbcL (Les, 2002).

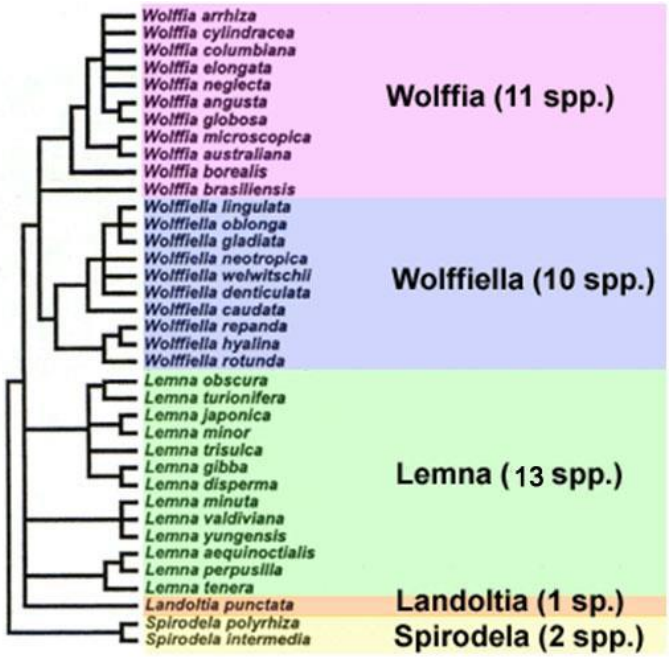


Figure 2: Cladogram displaying the relations among Lemnaceae species based on the chloroplast gene rbcL. Modified figure (Armstrong, 2011)

Duckweeds belong to the macrophytes (aquatic plants) and are classified as higher plants. The appearance of duckweeds varies between genera and species, from small and leaf-like to spherical plant bodies (Landolt, 1992). The body is organized as frond which is not differentiated into a stem or leaf (Armstrong, 2011; Wang, 2014b). Species of the genera Wolffia have the smallest frond size (0.4 mm) and the species Spirodela Polyrhiza with the biggest frond sizes (15 mm) (Goopy, 2003; Landolt, 1987). The fronds are either rootless (Wolffia or Wolffiella) or contain one or several roots (Lemna, Landoltia and Spirodela) (Armstrong, 2011). Species belonging to Lemna form one root, Landoltia two to five and Spirodela three to five. Root characteristics can be used alongside other morphological features (frond size, flower pattern, etc.) to differentiate between different duckweed species (Verma, 2015b).

Unlike most plants, duckweeds lack almost complete fibrous material as they do not need structural tissue to support their fronds (Skillicorn, 1993). Their tissue consists prevailingly of parenchyma tissue containing chlorophyll which are separated by large air-filled intercellular spaces which provide buoyancy (Goopy, 2003).

Flowers are rare in many species and number one to two per frond (Landolt, 1992). Flowering and fruiting are rarely observed in most Lemnaceae species (Armstrong, 2011; Hastie, 1992). Under unfavorable environmental conditions such as low temperatures or desiccation, some duckweed species have the ability to form modified fronds called turions. In cases of low temperatures, these turions sink to the bottom of

the water body resurfacing under more favorable climatic conditions to start a new generation of duckweeds (Ansari, 2011).

2.1.3. **Growth Conditions**

The growth of duckweed is dependent on a variety of environmental requirements – eutrophicated and nutrient rich waters supply an adequate amount of nutrients for metabolism, sunlight provides energy for photosynthesis, temperature affects metabolism rate, pH value influences nutrient absorption and a dense duckweed coverage rate helps fight against competitors like algae. Knowing the environmental requirements and effects of various environmental conditions on duckweed helps in optimizing duckweed-based processes and applications. For cultivation and maintenance of duckweed applications, natural environmental conditions have to be simulated (Skillicorn, 1993). Depending on intended performance or product achieved via the duckweed application, the ideal growth conditions varies particularly for high growth rates or starch accumulation (Cui, 2015). Duckweeds can be grown on a variety of media – in pond water, waste water from different sources (domestic or livestock farming) or specific artificial nutrient media matching their requirements. Duckweeds grown in axenic (sterile) cultures on a lab-scale for scientific studies are especially grown in artificial nutrient media. Sterile growth conditions have the advantage of determining the effect of an investigated substance etc. on the duckweed without impacts introduced by microbial metabolism (Cross, 2006). Common nutrients media for breeding duckweed (pure and modified) are for instance Hoagland (Frédéric, 2006;Vidaković-Cifrek, 2013) and Pirson & Seidel Media (Vidaković-Cifrek, 2013).

Nutrient Requirements

In nature decaying organic matter supplies them with nutrients and trace elements for growth and metabolism (Willet, 2005). Most plants absorb carbon and oxygen over the air and obtain mineral nutrients from the soil (Mengel, 2001). The free floating duckweeds remove the required nutrients, either directly from the water, or by the means of microorganisms living on their lower frond and root surface. Required macronutrients such as nitrogen, phosphorus and potassium are gained from water meanwhile CO2 and O2 are received from the atmosphere. Duckweeds prefer gaseous CO2 as C source but are able to use carbonate and bicarbonate from the water (Landolt, 1987).

Ammonium

The preference of duckweeds for ammonium over nitrate (NO_3) has been examined in several individual investigations (Fang, 2007; Lüond, 1980; Porath, 1982) and is stated throughout the literature (Hasan, 2009; ORON, 1988; Wang, 2016). Lüond (1980) for instance indicates that the growth rate of different duckweed species is higher in ammonium containing nutrient media than in nitrate containing nutrient media. Huang et al. (2013) state that for aquatic macrophytes ammonium assimilation requires less energy than NO_3, therefore it is chosen as their main inorganic nitrogen source.

Caicedo et al. (2000) investigated the influence of ammonium at concentrations of 3.5, 20, 50, 100 $mg \cdot l^{-1}$ onto the growth rate of Spirodela Polyrhiza coming to the conclusion that at low ammonium concentrations of 3.5 – 20 $mg \cdot l^{-1}$ the growth rate is higher than at

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

the higher ammonium concentrations. Wang, et al. (2014) tested Lemna Minor at different ammonium concentrations (2, 7, 28, 84, 280 and 840 mg·l^{-1}) and show with an optimal growth at 28 mg·l^{-1} a comparable result. Growth at 840 mg·l^{-1} was still visible though fronds were comparably smaller, lighter and paler and relative growth rate (RGR) at these high concentrations were significantly reduced. Furthermore, Wang et al. (2014) describe NH_4^+ induced toxicity symptoms at the highest concentration inhibiting photosynthesis pigments.

Phosphorous and potassium

Phosphorus is taken up mostly as phosphate with an optimal concentration differing greatly between species; Spirodela Polyrhiza 3 – 30 mg P l^{-1} (max. 54 mg P l^{-1}); Lemna Minor 0.43 – 10 mg P l^{-1} (max. 54 mg P l^{-1}); Lemna gibba 0.08 – 54 mg P l^{-1} (max. 271 mg P l^{-1}) (Landolt, 1987). Phosphorus is essential for rapid growth and is next to nitrogen the major limiting nutrient (Hasan, 2009). According to Hasan and Chakrabarti (2009) highest growth rates are already achieved at 4 to 8 mg P·l^{-1}.

Organic compounds

Heterotrophic uptake of small organic compounds is said not to be of importance for the metabolism of macrophytes, but in comparative studies of wastewater treatment ponds with and without duckweed it has been shown that the biological oxygen demand (BOD), COD and total suspended solids (TSS) removal efficiency was higher in ponds containing water hyacinths (Zimmo, 2003). An explanation could be given by the activity of microorganisms attached to the surface of duckweed in non-sterile cultures removing organic compounds from the wastewater (Szabó, 1999).

Highest growth rates are achieved in nutrient rich environments, meanwhile a high starch accumulation in duckweed fronds – interesting for biofuel production - is reached under nutrient starvation (Cui, 2011; Landolt, 1987).

Temperature

Growth rates of duckweed are greatly dependent on temperature with varying optimal requirements for different duckweed species (Landolt, 1987). They grow at water temperatures between 6 and 33 °C (Leng, 1995). Optimum growth rates for duckweed species between 25 and 31 °C are reported throughout the literature (Iqbal, 1999). Some species can tolerate temperatures near freezing; in general growth rate declines at low temperatures (Edwards, 1992). If water temperature drops below 0 °C some duckweed species sink to lower warmer levels of the water body and reemerge on the water surface under more favorable conditions (Iqbal, 1999) and others survive in starch filled bodies called turions which sink to the bottom of a water body and remain dormant until warmer temperatures trigger normal growth conditions (Edwards, 1992).

pH Value

The pH-value is a numeric scale described by the negative logarithm of the hydrogen ion concentration H+ to indicate whether a solution is acidic or alkaline (Taylor, 2000). The influence of the pH-value on nutrient absorption of water plants is complex. The pH-value is particularly of importance for the uptake of ions (Nultsch, 2012). Duckweeds grow well at pH values between 5 and 8, best around 6 (Landolt, 1987).

Reproduction

Reproduction within the duckweed family is dependent on the genus. Reproduction takes place vegetatively (asexually) which can cause explosive development of clonal duckweed population under favorable environmental conditions (Mitchell, 1974). In the genera Spirodela, Landoltia and Lemna, daughter plants are produced vegetatively in 2 lateral flattened budding pouches. In Wolffiella, daughter plants develop in triangular shaped budding pouches and in Wolffia, in the form of a funnel-shaped budding both at the basal end ((Armstrong, 2011). Figure 3 displays exemplary the duckweed species Lemna Minor including daughter fronds at different stages of development.

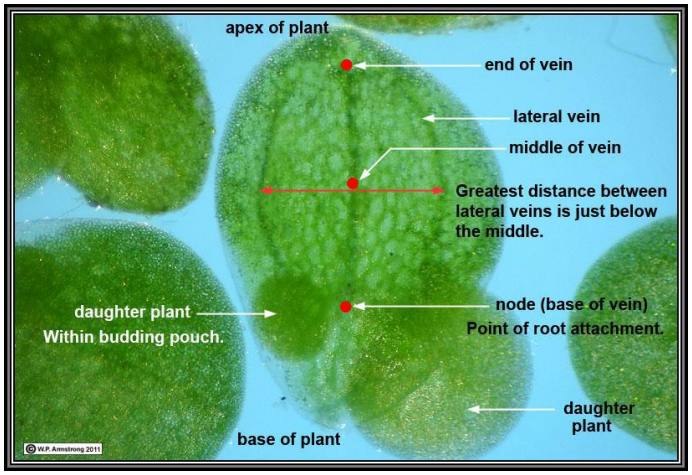


Figure 3: View of a Lemna Minor mother plant with two daughter plants in different development stages (Armstrong, 2011)

2.1.4. Relative Growth Rates, Doubling Time, Specific Yield

In science and praxis the biomass growth of duckweed under given conditions is estimated by the relative growth rate, doubling time and relative yield (Landolt, 1987; Poorter, 2013; Verma, 2015a).

Duckweeds have some of the highest bioaccumulation rates in the plant kingdom (Appenroth, 2015). The species Wolffia globosa is with a measured doubling time of 29.8 hours under optimal growth conditions, making it the fastest growing flowering plant known in the plant kingdom (Appenroth, 2015; Sree, 2015). With a doubling time of less than 30 hours it is nearly twice as fast as other fast-growing flowering plants and conventional crops (Wang, 2014a). Under ideal conditions and optimal nutrient supply, duckweeds nearly have an exponential growth (Landolt, 1987).

The growth rates and doubling time of duckweeds vary greatly between species and even between different clones (Sree, 2015). For optimal growth conditions, duckweed requires *sufficient nutrients, space, ideal temperature and available light of which temperature and light are the crucial parameters* (Hasan, 2009).

The relative growth rate indicates plant productivity relative to the size of the population. It is defined as the increase of material per unit of material present per unit of time (Mitchell, 1974).

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

2.1.5. **Applications of duckweeds**

The ability of duckweeds to thrive on nutrient rich media, their fast growth rate, their biomass composition, relative easy cultivation and harvesting methods are best prerequisites for a variety of applications. Especially, in respect of decreasing natural resources and increasing demand for sustainable and natural products based on renewable resources duckweeds have the possibility to contribute their share. Duckweeds can be applied in wastewater treatment, bioenergy production, feed and food supplement, as fertilizer, in integrated farming systems, bioassay for water toxicity testing and many more applications.

In wastewater treatment duckweeds are of interest especially because of their ability to thrive on nutrient rich media and remove nutrients from the water by binding them into their biomass. The biomass composition of duckweeds is of interest for bioenergy production as well as feed and food supplement.

Duckweeds can be used for energy production by obtaining biofuels from them, such as ethanol, butanol and biogas (Cheng, 2009). The usefulness of duckweeds for energy production depends on its starch content. The starch content of duckweed can vary between 3 –75% of its dry weight in dependence on environmental conditions (Van den Berg, 2015). Highest starch contents are achieved under nutrient starvation and at low temperatures (Landolt, 1987). Energy from duckweed can be obtained by hydrothermal processing and liquefaction, thermo-chemical conversion and bio-chemical conversion (Verma, 2015b). Xu et al. (2011) determined an overall starch conversation rate of 94.7% in ethanol production based upon duckweed with a starch content of 31% per dry matter. Verma and Suthar (2015b) state that due to certain sets of limitations duckweeds cannot be used as sole feed in anaerobic digestion tanks, wherefore a co-fermentation with other conventional sludge is recommended.

3. Study area

3.1. Wastewater treatment Westewitz

The wastewater treatment plant Westewitz (WWTP Westewitz), representing case study 1 (CS1) within POWERSTEP, is allocated in Saxony, Germany, between Dresden and Leipzig (Figure 4). It is designed for a wastewater volume equivalent to 2000 inhabitants which classifies it as a plant size of class 2 according to the German federal regulation ("Abwasserverordnung"). The plant has been operated in 2009 by OEWA Wasser und Abwasser GmbH since December. The catchment area includes three settlements and a specialist hospital which are connected to WWTP Westewitz via a separate drainage system (OEWA, 2012).

Figure 4: Top view of the WWTP Westewitz (Left); Front view of Plant (middle), View of Surrounding Area (right)

The requirements for the discharge quality of WWTP Westewitz, shown in Table 2, are stricter than the demands set by the German federal regulation. These stricter limits have been imposed by the OEWA itself.

Table 1: Loads and concentration for dimensioning WWTP Westewitz (OEWA, 2012)

Parameter	Thresholds for Discharge Quality
BOD_5 [mg/l]	< 40
COD [mg/l]	< 70
NH_4-N [mg/l]	< 10 (for °C >10°C)
TN [mg/l]	< 8
TP [mg/l]	< 8

The average inflow loads reaching WWTP Westewitz and the typical quality of domestic wastewater in Germany are shown in Table 1 which represents the basis for the design of WWTP Westewitz.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

WWTP Westewitz Operation in Brief

The WWTP Westewitz consists of a mechanical treatment (primary treatment) and a biological treatment (secondary treatment). The initial WWTP Westewitz, excluding extensions met within the scope of POWERSTEP, comprises of the following components:

- Inlet pumping station
- Primary treatment (compact system for sieve and sand classifier)
- Sump shaft to feed the SBRs
- 2 Sequencing Batch Reactors (SBR)
- Sludge tank for thickening and storage

The raw sewage is pumped over a pressure line to the primary treatment which comprises of a 6 mm sieve and a sand classifier. After the primary treatment, the wastewater flows by gravity to the sump shaft where it is pumped to the sequencing batch reactors (SBRs). Due to the process changes and extensions within the scope of POWERSTEP, the preliminary treated wastewater is pumped to the Hydrotech drum filter, where particulate carbon is extracted. Subsequently, the drum filter effluent flows into the SBRs for secondary treatment. The approach and principles of the drum filter are described in chapter 3.3. The operation mode of WWTP Westewitz including the process changes on basis of the drum filter are displayed in Figure 5.

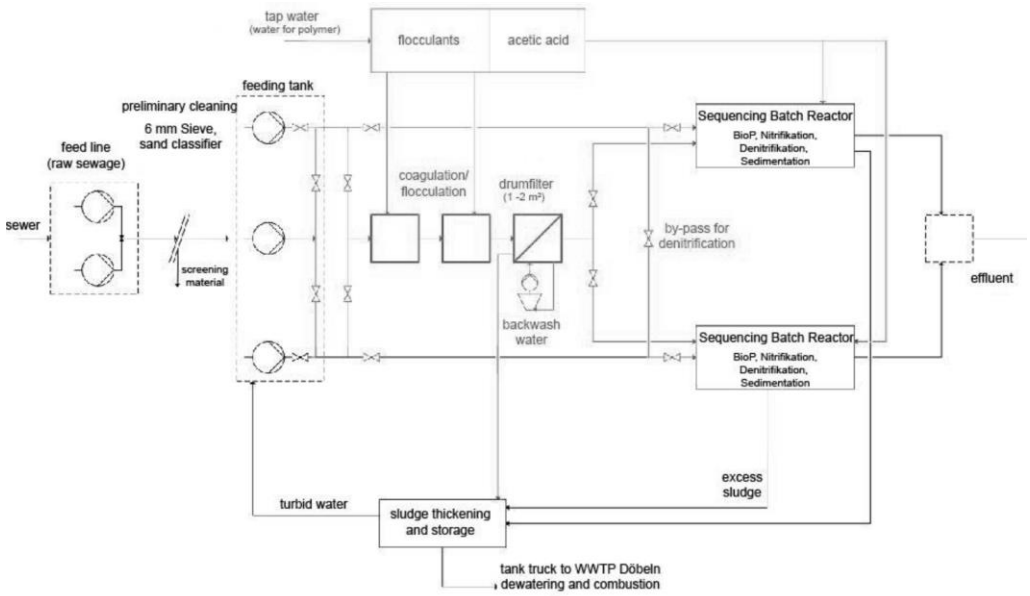


Figure 5: Schemata of Westewitz WWTP after retrofitting with drum filter

The biological treatment at WWTP Westewitz consists of two SBRs. The wastewater is treated within the SBRs by means of the activated sludge process build up on nitrification, denitrification, biological phosphorous elimination and a settlement phase. The last phase facilitates the removal of purified wastewater. The excess sludge is pumped to a sludge tank for thickening (Figure 5). The treated wastewater is discharged directly to the receiving water body "Freiberger Mulde" – a water body of

water quality category 1 according to the European Water Framework Directive 2000/60/EC (OEWA, 2012).

3.2. Full-scale Duckweed reactor

The greenhouse with the duckweed plant inside is situated at CS1 Westewitz onsite the wastewater treatment plant and form part in POWERSTEP within WP2 – nitrogen removal in main stream. The greenhouse and duckweed plant on pilot scale are designed by Aqua Plant Solutions GmbH (APS), a project partner within POWERSTEP. The approach of treating domestic wastewater over an almost automatic treatment method via a multiple layered duckweed plant hasn't been tested in this execution and scale yet. Its design resembles the execution of indoor vertical farming with the same purpose of increasing the productivity per base area. Within the scope of the APS plant that means the available tray surface area per base area.

The main purpose is to eliminate nitrogen and phosphate components from the wastewater stream. The filtered wastewater is taken from the effluent of the Hydrotech drum filter. The APS duckweed plant comprises of 11 levels consisting of two trapezoid trays per level facilitating an overall surface area of 110 m² on a base area of the greenhouse of 36 m² (Figure 6).

Figure 6 Left: Front view of Greenhouse containing the APS duckweed plant; Right: duckweed plant trays of the APS system inside the greenhouse

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

4. Material & Methods

4.1. Material

The experimental setup is divided into three main chapters, representing independent test series building up on each other. All were carried out at the WWTP Westewitz.

- Laboratory experiments
- Laboratory Plant
- Pilot Plant

4.1.1. Lab experiment

Four different duckweed species were tested for their applicability to remediate the preliminary filtered wastewater at WWTP Westewitz: Lemna Minor, Lemna Minuta, Landoltia Punctata and Spirodela Polyrhiza. All four duckweed species were provided by the KWB, obtained from the company GMBU in Halle. During the experiment the concentrations of COD, total nitrogen (TN), total phosphorus (TP), nitrite, nitrate, ortho phosphate (PO_4^{3-}), pH value and temperature within the growing media were examined. The removal efficiency for ammonia, COD and TP were determined by the concentration difference before and after the change of nutrient media. TN, nitrite, nitrate and the pH value were examined to observe potential activities of nitrifying bacteria or algae. The temperature served as a control parameter for stable environmental conditions.

4.1.2. Laboratory Plant

The construction of the laboratory plant was planned and executed following technical design and operational parameters of the APS duckweed plant. The laboratory pilot plant corresponds to 12% of the reactor size of one tray of the APS duckweed plant and permits operation during winter periods. The laboratory pilot plant aims to examine potential ammonium degradation efficiencies of the APS design concept and determine possible operational difficulties.

Laboratory Plant Design

The available space for the laboratory plant limited the duckweed reactor surface to 0.6 m² (100cmx60cm). This complies with 12% of the reactor surface area of 5 cm² of one tray for the APS duckweed plant.

The duckweed reactor is constructed out of a trapezoidal perspex sheet comprising of 15 grooves which complies with the amount of grooves as executed for the APS duckweed plant. Each trapezoidal groove has a height of 1.9 cm. The short sides amount to 2.7 cm and the long sites to 4.3 cm (Figure 7). The perspex sheet is placed horizontally. The reactor area is realized by gluing 9 cm high rigid foam pieces on the edges rectangular towards the groove and perspex plates parallel towards the first and last groove. This modification allows variable supernatant of the reactor areas up to 4 cm above the grooves.

The reactor surface area is formed by 14 of the 15 grooves with a raise in between each groove due to its trapezoidal shape (Figure 7). The 15th groove – the last groove – is connected to an automatic gate valve which regulates the removal of treated water and duckweed. The duckweed reactor is fed over an inlet in the upper right corner and drained by the gate valve in the lower left corner which is intended to enable an even distribution of filtrate.

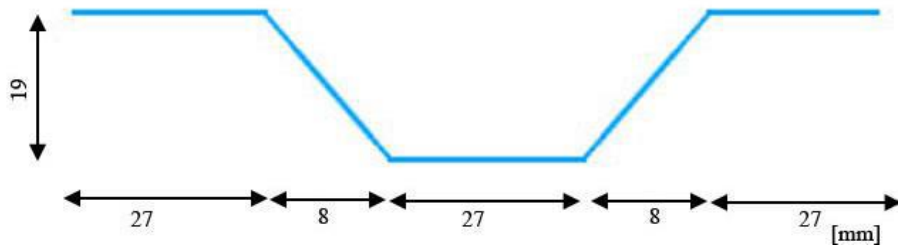


Figure 7: Trapezoidal shape of tray used for laboratory plant construction

The design of the laboratory plant is conducted based upon the reactor surface area of 0.6 m² which defines the required pump size, the size of storage containers, light requirement and the dimensions of construction and insulation material.

The essential plant components which allow a semi-automatic operation as intended for the APS pilot duckweed plant are a peristaltic pump, a gate valve, LED lamps for lighting and storage containers. Additionally, due to climatic conditions, a heating device and insulation are installed to withstand outdoor temperature fluctuations.

The duckweed reactor was built as a fully insulated box. The insulation is achieved by 2 mm water impermeable extruded polystyrene foam attached to all internal surfaces of the box.

The illumination within the box is achieved by two 15Watt LED lamps with a red/blue diode ratio of 7:1 and 6:1 with a light intensity of max 20 µmol·m-2·s-1 at a distance of 22 cm. The distance between the two lamps complies with the optimal distance between the LED Lamps for the APS duckweed plant suggested by Wang (2016). Within the 60 cm reactor length, the lamps are arranged 15 cm parallel to the edges, which results in a distance between both lamps of 30 cm.

Heating of the water temperature above outside temperatures is achieved by a heating cable laid directly underneath the trapezoidal perspex sheet. The heat supply is controlled with a thermostat ITC-308S from the company Inkbird with a temperature range between -50°C to 120°C.

The reactor volume of the 14 grooves including the 0.5 cm rise amounts to 9 liters. With an additional supernatant of 0.5 cm to enable duckweed to spread out to the last groove, the reactor volume amounts to 12 liters. The influent and effluent containers as displayed in Figure 22 have a holding capacity of 60 liters each, which enable an automatic operation for several days depending on the chosen HRT. The laboratory pilot plant with its main components is displayed in Figure 8.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

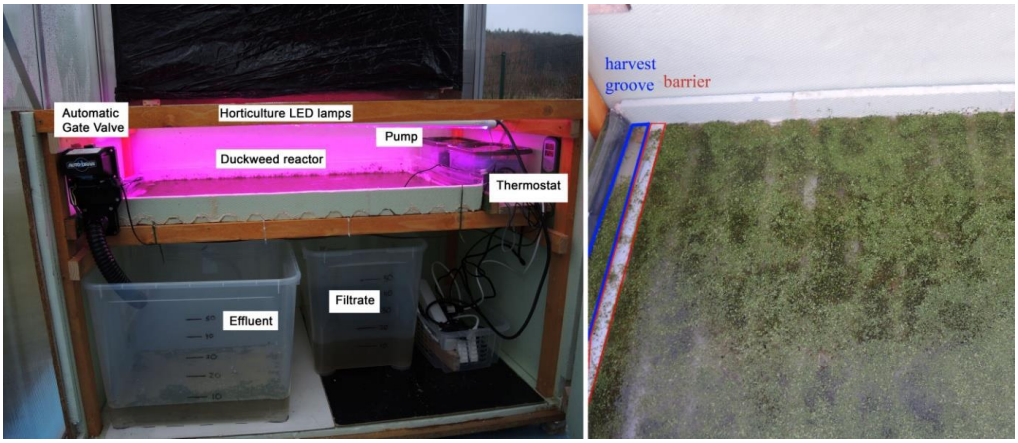


Figure 8 Left: Laboratory pilot plant; Right: duckweed reactor including barrier and harvest groove (blue and red coloured)

4.1.3. **Pilot Plant**

Pilot Plant Design

The filtered wastewater is fed to the trays where the duckweeds are floating on the surface. The shape of the trays is executed in a way that the trays - and with them the duckweeds – would not become dry (exceptions especially in warm periods due to evaporation).

The trays are made of Plexiglas with a trapezoidal shape. The dimensions are 2.7x105x500 (HxWxL) cm, with a material thickness of 2 mm. Due to the trapezoidal shape each tray consists of 15 grooves (Figure 9). Each tray has an influent and an effluent opening. The influent and effluent are situated at the opposite ends of each tray diagonal, shifted to prevent short circuit currents. The tray is perfectly horizontal over its 5-meter length but is slightly curved over its width with a maximum deflection of 15 mm in the middle. The purpose of the deflection is to achieve a supernatant of filtrate to form a closed water surface as indicated with two fine black lines in Figure 9.

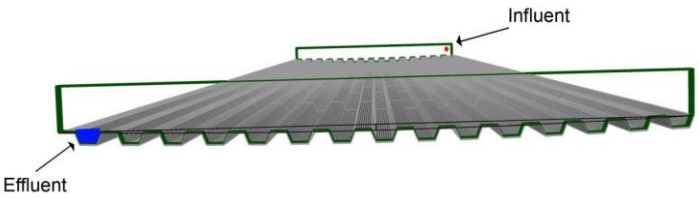


Figure 9: Design of the trapezoidal perspex sheets as used in the APS pilot duckweed plant

The idea of the APS pilot duckweed plant design is to gain a nearly automatic operation mode of feeding the plant with wastewater and removal of excess duckweed fronds and treated wastewater. A manual harvest would be substituted by automatic harvest via the harvest pits (Figure 10).

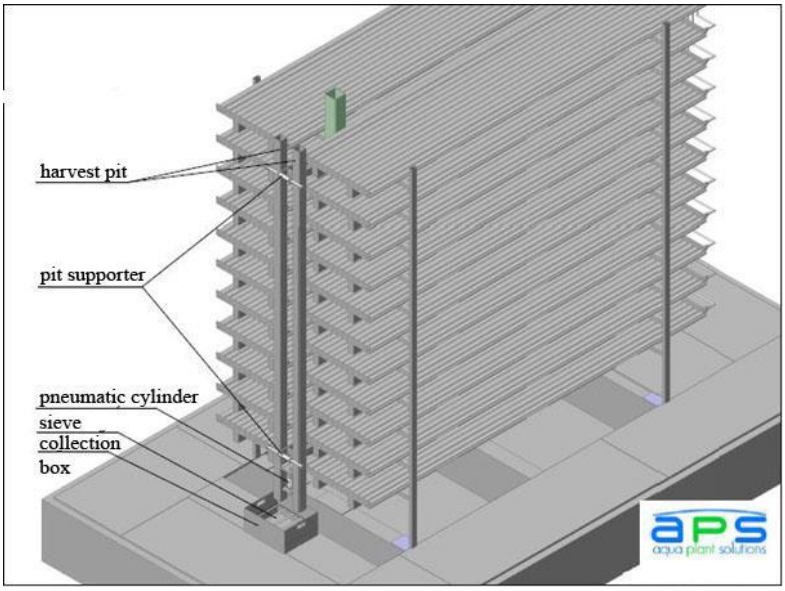


Figure 10: APS pilot duckweed plant

In the operating state, the preliminary filtered wastewater will be directed over pipes by gravity from the Hydrotech drum filter towards a one cubic intermediate bulk container (IBC) situated within the greenhouse. The IBC functions as a storage tank as well as a mixing tank for the possibility to recirculate effluent leaving the APS pilot duckweed plant. The filtered wastewater is fed from the IBC to the plant over a duct on the top of the plant. From there it is distributed to the trays. The duct is fed by a progressive cavity pump allowing varying feeding regimes. The flow rate of the progressive cavity pump amounts to 4.88 $l \cdot min^{-1}$. The inflow from the duct to each tray can be regulated via of ball valves. The hydraulic retention time of wastewater in the trays is determined by the feeding and harvest regime. The given geometry of the tray and designed harvest operation do not allow great variation in filling levels. The filling volume formed by the supernatant of the curved trapezoidal perspex sheet as shown in Figure 9 amounts to 65 liters. Every 1 mm filling level between the filled volume and the last groove results in 5 additional liters.

The last groove – indicated in Figure 9 as effluent – is drained over a hydraulic lifting device. In the closed state, the effluent opening is blocked. During the opened state, the harvest pit is lifted by a pneumatic cylinder allowing treated wastewater and duckweed to drain out of the plant. The tray is designed in a way that only the last groove and supernatant is drained. Duckweed fronds swimming on the tray's surface propagate in the nutrient rich wastewater which increase the mat density and cause a shortage in space. Consequently, excess duckweed fronds are pushed towards the sides of the tray into the last frequently drained groove.

Duckweed fronds and the treated effluent are separated via a 200 µm sieve which is situated below the harvest pit. The duckweeds accumulating on the surface of the sieve are removed frequently manually.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Operational Parameters

The operational parameters of the APS pilot duckweed plant suggested by APS are based upon outcomes of former investigations of duckweeds for wastewater remediation. The reactor volume per tray is 65 liters, with a total surface area of 6.315 m² (Figure 9). The average water depth of the tray is due to its trapezoidal shape 1.24 cm.

Table 2: APS pilot duckweed plant parameters

Parameter		Unit
Dimensions tray	500x105x2.7 (LxWxH)	cm
Design of tray	Trapezoidal	
Reactor volume per tray	65	Liters
Surface area per tray	5	m³
Effluent per harvest	7	Liters

The constant flow of the progressive cavity pump amounts to 5.5 l·min $^{-1}$. An alteration of the volume flow is technically not possible with this model, wherefore feeding of the tray has to be met in accordance of the required amount of filtrate per tray and the number of trays which tend to be fed simultaneously.

Considering a harvest of 7 liters per tray, this amount can be replaced with new wastewater, for one tray, in 76.36 seconds. The feed of two trays amounts to 153 seconds etc.

4.2. Methods

4.2.1. Lab experiment – Removal of Ammonia and COD

Experimental Operation

The four duckweed species were added into four separate beakers filled with wastewater and in tap water as a reference. A modified toolbox served as an incubator in which the beakers were placed.

The duckweeds were exposed to a white light LED 24 hour daily, positioned 22.5 cm above the duckweed surface area which represents the prospective distance of the horticulture LED lamps to the trays in the APS Duckweed Plant. At a distance of 22.5 cm the light intensity amounted to 25 µmol·m^{-2}·s $^{-1}$. 50 % (20 ml) of the filtrate was changed on a daily basis representing an HRT of 2 days. The ambient temperatures were constantly between 16 - 17°C.

The wastewater serving as nutrient media for the duckweed species was obtained from the pump shaft of the SBRs at WWTP Westewitz. To receive a wastewater comparable to the expected effluent of the drum filter, the wastewater was additionally treated in the laboratory. During the execution of this experiment the Hydrotech drum filter was not yet in operation, therefore the production of artificial drum filter effluent in the laboratory was necessary. The operational steps of the Hydrotech drum filter of polymer

dosing, flocculation and precipitation and sieving (100 µm) were conducted in a one liter beaker, following the specific recommendation for the optimal procedure set by Herrmann (2016) who determined the optimal composition and procedure of polymer, flocculation and precipitation for the wastewater in Westewitz within the scope of a master thesis on site (Figure 11).

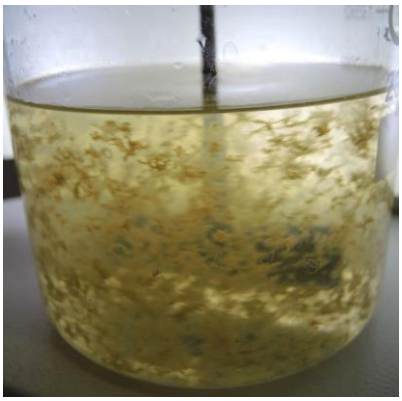


Figure 11: Filtrate after flocculation and precipitation prior to sieving (100µm)

Analytical Methods

NH_4-N, pH and the temperature were determined on a daily basis for the new filtrate and the removed (old) filtrate from the previous day (20 ml). Additionally, the COD concentration of the new filtrate was determined on a daily basis as well. TN, TP, NO_3-N, NO_2-N and ortho-phosphate were determined every other day for the old filtrate and new filtrate. The pH and temperature were determined by the pH meter type WTW Profiline pH3210 with errors of ±0.005 pH and ±0.1°C. For the determination of COD, NH_4-N, TN, TP, NO_3-N, NO_2-N and ortho-phosphate cuvette tests of the company HACH Lange GmbH were used. The error of the cuvette tests amounts ±10 % which is within the scope for permitted error deviations set by the worksheet DWA 704.

COD, TN and TP were decomposed for 15 minutes at 170°C in a high temperature thermostat HT 200 S of Hach-Lange. Ammonium, nitrite, nitrate and ortho-phosphate were filtered with 0.45 µm pore size. The photometric evaluation was carried out in the spectrophotometer DR 2800 of Hach-Lange. Table 3 shows the determination intervals of analyzed parameters.

Table 3: Determination intervals of analysed parameters

Parameter	Unit	Old filtrate	New filtrate
COD	mg/l	2day-rhythm	daily
TN	mg/l	2day-rhythm	2day-rhythm
TP	mg/l	2day-rhythm	2day-rhythm
NH_4-N	mg/l	daily	daily
NO_3-N	mg/l	2day-rhythm	2day-rhythm
NO2-N	mg/l	2day-rhythm	2day-rhythm
ortho P	mg/l	2day-rhythm	2day-rhythm

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Parameter	Unit	Old filtrate	New filtrate
pH	-	daily	daily
Temp	°C	daily	daily

4.2.2. Lab experiment - Growth and pH

During the second set of experiments the influence of mat density on the growth rate of duckweed with respect to suppression of algae bloom, pH development and self-hindering due to overcrowding was investigated also on laboratory scale.

The duckweeds used within these experiments were gathered from two ponds in Tiergarten Berlin on the 14th of November 2016. The duckweed gathered from Tiergarten seemed to comprise of a mix of duckweed species. It was estimated that they contain Lemna Minor, Lemna Minuta, Landoltia Punctata and a few Spirodela Polyrhiza.

Experimental Operation

The mixed duckweeds from Tiergarten were investigated for their growth rate at initial mat-densities of 0.05, 0.075, 0.1 and 0.15 g·cm-2. The duration per test series amounted to 7 days. In total, 8 test runs were carried out within the period 17th of January until the 23rd of March 2017. The total test run series amounted to 56 days. Three different test run settings have been applied:

- Consisting of three individual test runs with solely filtrate as nutrient media
- Consisting of three test runs with additional Blue Exit to suppress cyanobacteria growth
- Consisting of one test run with shading devices covering the beaker sides To prevent light from entering the sides of the beaker

Four separate beakers with a diameter of 78 cm² were inoculated with 3.9 g duckweed (0.05 g·cm-2), 5.85 g duckweed (0.075 g·cm-2), 7.8 g duckweed (0.1 g·cm-2) and 11.7 g duckweed (0.15 g·cm-2). In each beaker, 150 ml filtrate was added daily. 150 ml represents a filling level of 2.5 cm as indented for the APS pilot duckweed plant. A fifth beaker with only filtrate was added for comparison of pH and O2 development. On Fridays, 450 ml were added at once to bridge over the weekend. The old filtrate was not removed daily for practical reasons, but at the end of the 7-day long test period. In former tests it was observed that a 100% removal of the filtrate by the means of sieves causes stress and damage to the duckweeds fronds and roots. Additionally, the daily filtrate change caused the removal of bacteria and algae which can occur under the tested conditions. A wash off of these would sophisticate the test results as in the APS duckweed plant the duckweeds cannot be washed on a daily basis.

The latter two are unwanted in general but could occur in the APS duckweed plant and would not be possible to be washed off. For this purpose, the daily adding of new filtrate until the last day of the experiment run was chosen.

The experiments were carried out in the operation building at WWTP Westewitz. The growing media temperature was between 14 – 17°C. The air temperature within the operation building varied though out the day, rarely exceeding 21°C. The temperature

of the daily added filtrate was between 7- 10°C which caused a temporary cooling of the media temperature. The duckweeds were illuminated by a horticulture LED Lamp with 11 Watt and a red/blue diode ratio of 7:1. The light duration was 24 hours per day and the light intensity amounted to 40 µmol·m-2·s -1. The light intensity was measured by the measurement instrument PAR Meter of the company Sun System.

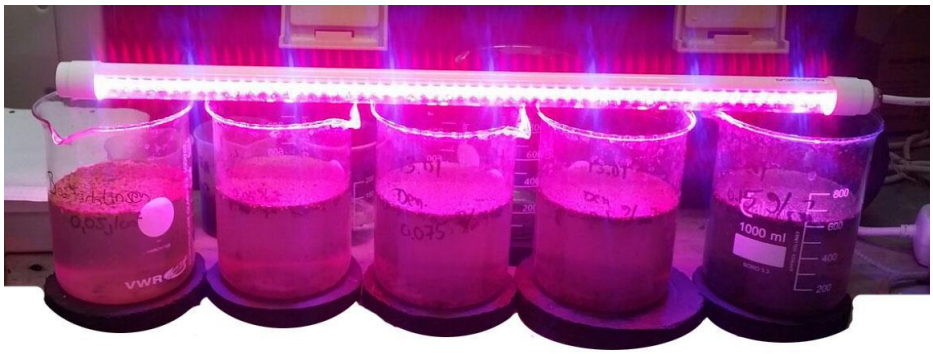


Figure 12: Experimental setup: Growth rate determination under different mat-densities

Analytical Methods

The pH value, O_2 concentration and duckweed weight were determined at least at the beginning and end of each test series. The fresh duckweed weight was determined following the procedure described by (Ziegler *et al.*, 2014). The duckweeds were poured over 300 – 500µm sieves and the dead biomass which accumulated at the bottom of the beakers was separated from the living duckweeds. Water attached to the duckweed surface was removed by paper towels for approximately 5 minutes. The determination of the weight was carried out with the scale Kern 572-32 (max. 421g, d=0.001g). The pH value was determined with pH meter WTW pH3210 as already described in chapter 4.1. The dissolved Oxygen (DO) was measured with the Multimeter 3420 of the company WTW. In the test series started on the 23.02.2017, 02.03.2017 and 09.03.2017 a 0.05% salycilate acid solution called Blue Exit from the company Easy Life was added to suppress cyanobacteria grow. The doses per beaker were 80 µl (on Friday), 100 µl (on Monday), 120 µl (Tuesday), 140 µl (Wednesday), 160 µl (Thursday) – increasing doses with increasing filling level in the beakers.

4.2.3. Laboratory plant

Seven experimental runs were carried out during the 5th of January 2017 until the 21st of April 2017 with test durations varying between 4 to 17 days. Table 4 gives a more detailed overview of the duration dates of the seven test runs carried out.

Table 4: Displaying starting and end date, test duration and measurement points per test run

Test run	Starting Date	Ending Date	Duration [days]	Measurements [days]
1st run	04.01.2017	16.01.2017	12	8
2nd run	16.01.2017	20.01.2017	4	4
3rd run	25.01.2017	10.02.2017	16	12

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

4th run	10.02.2017	22.02.2017	12	7
5th run	02.03.2017	17.03.2017	15	11
6th run	20.03.2017	31.03.2017	11	7
7th run	04.04.2017	21.04.2017	17	9

The duckweed used within this experiment originated from the same pond as used for the experiments described in chapter 4.2.2. The initial HRT was set for 24 hours, the flow rate to 10 ml·min $^{-1}$ and the pump volume flow to 50 min·h $^{-1}$. Set flow rate and pump operation time enabled a constant flow throughout the day, resulting in 12 liters per day. The thermostat was set to a target value of 25°C to allow water temperatures of 25°C inside the reactor volume. This value is based upon the lower threshold for optimal growth temperature for duckweeds cited in the literature (Iqbal, 1999; Landolt and Kandeler, 1987). The light intensity of the two 15 Watt LED lamps amounted up to 20 µmol·m^{-2}·s $^{-1}$ at a distance of 22 cm vertical underneath them, corresponding to the water surface. The light duration was 24 hours per day, as suggested by Appenroth and Lam (2016) to achieve a maximal growth rate. The microcontroller was programmed to open every 6 hours for 2 minutes which resulted in a removal of approximately 3 liters and an additional harvest of duckweed of the last groove per opening. In accordance to the HRT and flow rate the influent filtrate container was fed on a daily basis – Monday to Thursday - with approximately 15 liters – including some liters as safety measures to prevent them from drying out. On Fridays, 50 liters were fed into the influent container. The influent filtrate for the laboratory plant was obtained at the sampling point of the Hydrotech drum filter. Table 5 shows a summary of the experimental setup of the laboratory plant.

Table 5: Experimental setup of measurement series of laboratory plant

Parameter	Unit	Comment
HRT	24 hours	
Volume flow	10 ml · min $^{-1}$	
Feeding regime	50 min · h $^{-1}$	10 min break every hour
Reactor volume	12 l	
Supernatant	0.5 cm	overstand to enable surpass of duckweed
Harvest regime	2 min every 6 hours	
Volume harvested	3 l	
Illumination	2 x 15 Watt LED lamp	Red:blue dione ratio 6:1 and 5:1
Distance Lamp to duckweed	22 cm	
Light intensity	20 µmol · m^{-2} · s $^{-1}$	reached only 22 cm below lamp

Parameter	Unit	Comment
Water temperature	25 °C	target temperature
Initial mat density	750 g · FM · m^{-2}	

During the week – Monday to Friday – the pH value and temperature of a) the remaining influent filtrate inside the container of the previous day; b) filtrate of the current day; c) filtrate within the reactor volume; and d) the effluent, were measured on a daily basis. The pH and temperature determination was carried out with the pH meter WTW Profi line pH3210. Additionally, ammonium concentrations of the new filtrate and the effluent and the amount of harvested duckweed were determined on a daily basis. The ammonium nitrogen (NH4-N) concentrations were determined by

Hach Lange cuvette – tests (see chapter 4.1) and the weight detected by the same procedure as described in chapter 4.2.

The ammonium removal rate ($mg \cdot l^{-1} \cdot d^{-1}$) and degradation efficiency (%) are calculated based upon the influent and effluent ammonium concentrations. The ammonium removal rate is calculated out of the difference between the influent filtrate and the effluent two days later (e.g. difference between influent day1 to effluent day3). The actual HRT of the filtrate within the reactor volume amounts to one day. The total time until the filtrate passes entirely from the inflow container to the outflow container amounts to two days. In other words, it takes two days, until the total amount of filtrate in the influent container reaches the effluent container. Therefore, the basis for the calculation amounts to two days. The removal rate of ammonium per day ($g \cdot m^{-2} \cdot d^{-1}$) of the duckweed pilot plant is calculated based upon the degradation rate multiplied by the amount of water extracted, divided by the reactor surface area.

Removal rate per day calculation:

$$URNH4 - N = \frac{cNH_4N_{influent} - cNH_4N_{effluent}}{SA_{DW} * 1000 * HRT}[gm^2 \cdot d]$$

$URNH_4$-N describes the uptake rate of ammonium ($g \cdot m^{-2} \cdot d^{-1}$), SA_{DW} describes the surface area of the duckweed reactor (m^2) and cNH_4-N describes the ammonium nitrogen concentration ($mg \cdot l^{-1}$).

4.2.4. **Pilot plant**

The aim of the pilot experiment is to determine the actual reduction of total nitrogen, total phosphorus and ammonium and the accumulation of nitrate in the field trial at the Westewitz site using a duckweed pilot plant. The measurement is carried out with Hach Lang tests and the subsequent comparison of the results from the inlet and outlet of the duckweed plant. The samples were collected and measured over a period of several months, three times a week.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Operating principle of the duckweed reactor

The duckweed reactor is fed with the drainage of the Hydrotech drum screen. The required filtrate to the feed is temporarily stored in the greenhouse in an IBC. Here up to 1m^3 can be stored. The stored filtrate is pumped from the IBC via an eccentric screw pump into the two source bays of the system. The eccentric screw pump has a pumping capacity of 4.88 l / min. The filtrates are fed from the source shafts to the individual trays. The loading of the troughs is regulated by means of ball hoists. The settings for the feed are 'Ja' (maximum load), 'Nein' (no load) and 'wie viel' (certain flow rate). The trays each have a capacity of 100 liters (depending on the level). The filtrate is taken from the tub via the harvest shafts. At the same time, purified filtrate and duckweed are taken from the trays. The duckweeds are collected on the sieve below the shafts and the purified wastewater is collected in the sump. A submersible pump returns the treated wastewater to the inflow of the sewage treatment plant.

The IBC serves as a buffer for the filtrate of the filtration plant and has a capacity of 1000 liters. Depending on the number of wells to be charged and the filtrate supplied per trays, the time to refill varies.

The filling

The filtrate is fed directly from the sampling tap on the drum screen through a hose to the IBC. It is important to ensure that the hose elements are connected to each other. It should also be noted that a dry run protection for the eccentric screw pump is set up in the IBC. This starts at a filling level of less than 150 liters, and it is fed from the duckweed reactor. For a constant operation, a sufficient filtrate quantity of at least 150 liters should be guaranteed.

Sampling

For the sampling, the eccentric screw pump was switched on and the harvest shafts opened. In Figure the setting values of the eccentric screw pump and harvesting wells for sampling are shown.

Figure 16: Setting values of the eccentric screw pump and harvesting wells for sampling

In Figure 13 the sampling of the inlet to the duckweed (left side) and the sampling of the outlet (right side) are shown.

Figure 13 Left: Sampling inflow; Right: Sampling outflow (right)

The samples were analysed in the wastewater treatment plant's laboratory, for total nitrogen, total phosphorus, ammonium and nitrate. For this purpose, appropriate Hach Lange cuvette tests were performed similarly as for the lab trials.

4.2.5. Bio-methane potential measurements (BMP Tests)

The aim of a BMP experiment is to investigate the biodegradability of a substrate and at the same time the potential to recover methane by anaerobic digestion (BIOPROCESS CONTROL, 2016). The measurement is carried out by inoculating the substrate with an anaerobic inoculum. While the substrate is then incubated at a controlled temperature, the methane production is periodically measured.

Description of the equipment

The Bioprocess Control's BMP test with the AMPTS II is based on the same measurement principle as the conventional BMP test. However, analysis and data recording are done fully automatically. The AMPTS II consists of three units (see Figure 14): the thermostatic water bath (unit A), the CO2 absorption unit (unit B) and the gas volume meter (unit C).

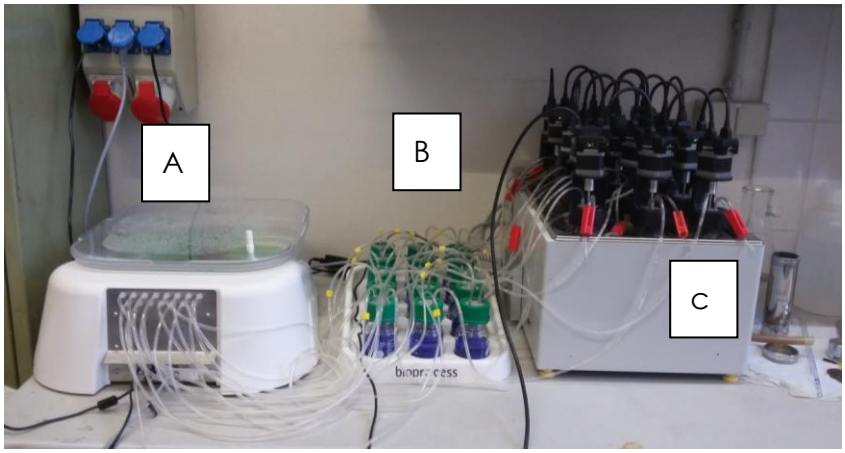


Figure 14: Bioprocess Control AMPTS II used for Bio-methane potential test

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

TR/oTR Measurement

Before starting the measurement with the AMPTS, first the dry residue (TR) and the organic dry residue (oTR) of all substances - samples, inoculum and reference substrate - must be determined. The software of AMPTS automatically calculates the composition of inoculum and substrate in each bottle with the values of oTR, indicating a desired ratio of inoculum to substrate.

Preparation of the experiment

For statistical significance, everything was measured in triplicate. Since only 15 bottles are available and due to the considerable length of the BMP test, the blanks are only measured in duplicate. The reference is measured only once, as it only shows that the inoculum works, and these values are not used for further evaluation. Through the remaining twelve bottles, four samples can be tested in each experiment. Thus, for the blank in two bottles of unit A, only inoculum is added, placed in a bottle for the reference inoculum and cellulose and for each sample to be tested in three bottles of sample and inoculum. In this case, a total amount of 400 g is always added. Depending on the oTR value of the sample, a different proportion of sample and inoculum is used, but this is also indicated by the software of the AMPTS II available from Bioprocess Control after specifying the oTR._After filling the bottles, the pH is first measured and adjusted if necessary. All filled bottles are now placed in unit A, closed and connected to the stirrer and the motor. The water bath is filled up with distilled water and the plastic cover is put on to reduce the evaporation of the water. For the absorption (Unit B), a three molar sodium hydroxide solution and a 0.4% thymolphthalein pH indicator solution was prepared. 5 ml of the indicator solution is added to one liter of the sodium hydroxide solution. From this mixture about 80 mL is added to each bottle of unit B. If the solution decolorizes again during the experiment, it must be replaced, since then the absorption capacity is exhausted. The gas volume measuring unit (unit C) is filled to the mark with distilled water. At the end, all units will be connected with Tygon hoses.
Before starting the correct measurement, all cylinders are first purged with nitrogen under a low gas flow. This creates anaerobic conditions, so that anaerobic degradation of the samples can effectively take place right from the start. Then the thermostatic water bath, the motor controller and each motor of the reactors are set. Via the software of the device, the motors can now finally be switched on and the experiment can be started for all individual bottles via the "Control" page.

5. Results

5.1. Results Lab Experiment

5.1.1. Removal of Ammonium

The focus of the first run of laboratory experiments was on the ammonium removal by duckweed. The average ammonium concentration of the used filtrate, amounted to 49 $mg \cdot l^{-1}$, with a standard deviation of 14.6 $mg \cdot l^{-1}$. Minimum concentration was 25.6 $mg \cdot l^{-1}$, maximum concentration was 66 $mg \cdot l^{-1}$. The average removal for Lemna Minor, Lemna Minuta, Spirodela Polyrhiza and Landoltia Punctata were 1.55 $mg \cdot l^{-1}$, 0.68 $mg \cdot l^{-1}$, 2.47 $mg \cdot l^{-1}$, and 4.64 $mg \cdot l^{-1}$ respectively, and their efficiency 3.78 %, 2.95 %, 7.11 % and 13.09 %, respectively. The results of the ammonium removal for each species and measurement are displayed in Table 6.

Table 6: Ammonium removal of the four tested duckweed species

Comparison of NH 4 -N Removal [mg/l]								
Measurement	L. Minor		L. Minuta		S. Polyrhiza		L. Punctata	
[days]	Removal [mg/l]	Efficiency [%]	Removal [mg/l]	Efficiency [%]	Removal [mg/l]	Efficiency [%]	Removal [mg/l]	Efficiency [%]
1	2.60	9.29	1.80	6.43	4.20	15.00	8.60	30.71
2	1.00	2.48	1.00	2.46	2.90	7.34	8.60	23.06
3	1.35	2.56	1.15	2.18	2.30	4.48	3.75	7.92
4	3.10	6.05	12.40	4.67	1.95	3.90	2.25	4.75
5	0.25	0.47	0.30	0.56	1.85	3.47	3.65	7.05
6	1.00	1.80	0.80	1.43	0.20	0.36	1.95	3.66
7					3.90	15.23	3.70	14.45
Mean	1.55	3.78	1.24	2.95	2.47	7.11	4.64	13.09
SD	0.99	2.99	0.68	2.00	1.26	5.40	2.59	9.51

The highest average ammonium removal was achieved by Landoltia Punctata, followed by Spirodela Polyrhiza, Lemna Minor and lastly by Lemna Minuta. All species showed on the first day a removal rate and efficiency higher than its average value. An explanation could be given due to the fact that the species were taken from small containers where they were floating on sterile tap water – a medium poor in nutrients. When relocated to a medium rich in nutrients, the uptake of nutrients could have increased temporarily.

Another explanation could be given with regard to the initial ammonium concentration in the medium of 28 $mg \cdot l^{-1}$ and its increase over the investigated period. As nitrogen plays an particularly important role in the nutrition of duckweeds and their growth (Lüond, 1980), with optimal growth rates reported to be at 28 $mg \cdot l^{-1}$ for Lemna Minor (Caicedo, 2000) and for Spirodela Polyrhiza below 20 $mg \cdot l^{-1}$ (Wang, 2014b), the growth

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

rate and with it the ammonium uptake should decrease with increasing ammonium concentration, according to the statements of the two authors above. The experiment was stopped after 6 days. Only the two better performing species Spirodela Polyrhiza and Landoltia Punctata were investigated one additional day. For this purpose, the filtrate was changed by 100% and not only by 50% as was done previously. The ammonium concentration of the filtrate added on 12.10.2016 accounted for just 25.6 $mg \cdot l^{-1}$. The removal of 3.9 $mg \cdot l^{-1}$ of ammonium for Spirodela Polyrhiza was the second highest achieved removal during this experiment, as shown in Table 6 – a result in line with the estimated better performance under low ammonium concentrations.

Another parameter influencing the growth and nutrient uptake performance of duckweeds is the pH value of its growing media. For Lemna Punctata the initial pH value at the start of the experiment amounted to 7.638 and rose to 8.091 after 22 hours. The average pH of the daily added filtrate was 7.962 with an average daily increase of 0.347 (determined on basis of data presented in Table 6). Figure 15 displays the development of nutrient concentration and pH of Landoltia Punctata, exemplarily for the four tested duckweed species. The course of the ammonium concentration and pH value are shown as lines, whereas TN, TP, Nitrate and nitrite are shown as dots.

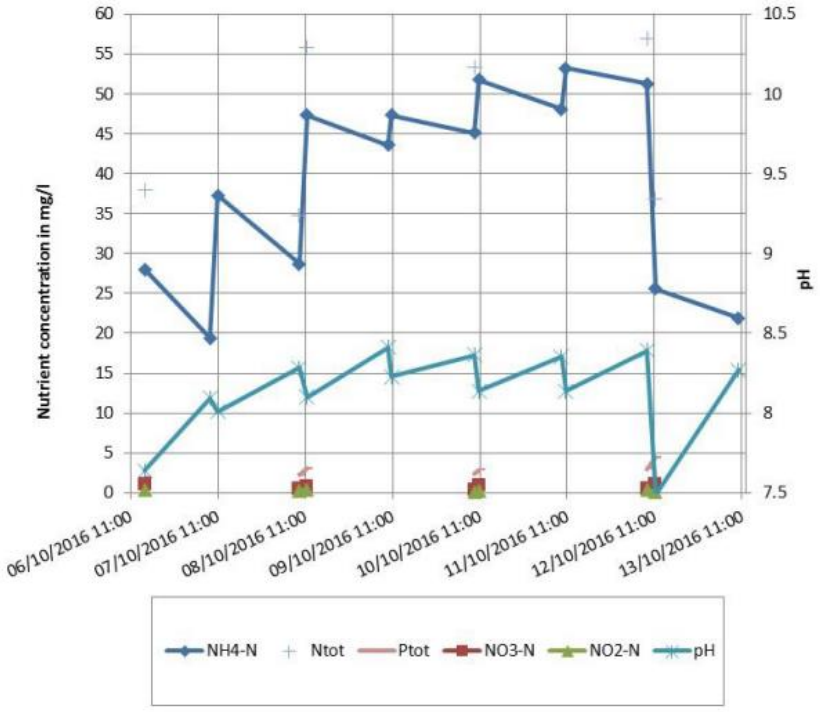


Figure 15: Nutrient degradation and pH development of nutrient media

The concentrations are indicated on the left vertical axis and the pH on the right vertical axis. The horizontal axis indicates the corresponding time. The previously mentioned increase in ammonium concentration is well visible. The ammonium removal per day within the media is indicated by the decline of concentration. It is clearly visible that the removal amounts only to a few mg/l. At the same time, a daily increase in the pH value is visible. The decline in pH is caused by extracting 50 % (20ml) of the media daily and replacing it with new filtrate. The daily produced filtrate had a pH of 7.8 on

average. The rise of the pH in the media can be caused by different reasons. In the presence of algae, CO_2 removal takes place through the uptake of CO_2 by algae causing the pH to increase. The photosynthetic CO_2 uptake by algae is reported to cause pH increase up to a level of 10.5 (Cohen, 2004) – Levels which are reported to be toxic for duckweeds (Landolt, 1987). TN, TP, NO_3 and NO_2 were only measured every next day for controlling purposes. As they were measured every next day, it is not possible to draw conclusions on the removal rates of these parameters. The average NO_3 and NO_2 concentrations measured in the filtrate were 1.14 and 0.3 $mg \cdot l^{-1}$ with standard deviations of 0.21 and 0.12 $mg \cdot l^{-1}$. The average NO_3 and NO_2 concentration in the media of Landoltia Punctata amounted to 0.5 and 0.26 $mg \cdot l^{-1}$. These values are not directly comparable but they allow an observation regarding the activity of nitrifying and denitrifying bacteria. The concentrations of NO_3 and NO_2 are constantly low with NO_3 concentrations in the media on the average 56% lower than in the filtrate. The lower nitrite concentrations in the media in comparison to the filtrate could be caused by the activity of nitrifying bacteria Nitrobacter – responsible for the conversion of nitrite to nitrate – and the activity of denitrifying bacteria reducing the nitrate concentration.

The physical appearance of the duckweed at the beginning and at the end of the experiment was also compared. The purpose was to determine the health condition of the duckweed grown in the wastewater. All four tested duckweed species showed a healthy green colour at the beginning of the experiment. Within the course of the experiment a yellowing colouring of the fronds of all four species was observed. At the end of the experiment run, a white coloration of most fronds of Spirodela Polyrhiza occurred (Figure 16) – a sign for dying and dead duckweed fronds. Spirodela Polyrhiza had the highest amount of white frond, followed by Lemna Minuta, while Lemna Minor had the least dead fronds.

Lemna Minor had the best physical appearance of all four duckweed types – in the sense of yellowish colouring and die-off. Because of this reason, Lemna Minor is observed to best withstand fluctuating ammonium concentrations of the wastewater at WWTP Westewitz, which makes it the most suitable duckweed type to treat the wastewater. The achieved average ammonium removal and efficiency of Lemna Minor of 1.55 $mg \cdot l^{-1}$ and 3.78 % efficiency is comparably smaller than the measured removal and efficiency of Landoltia Punctata of 4.64 $mg \cdot l^{-1}$ and 13.09 %. However, the determined removal rates and efficiencies do not take into account varying biomasses of the duckweeds at the beginning of the experiment.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

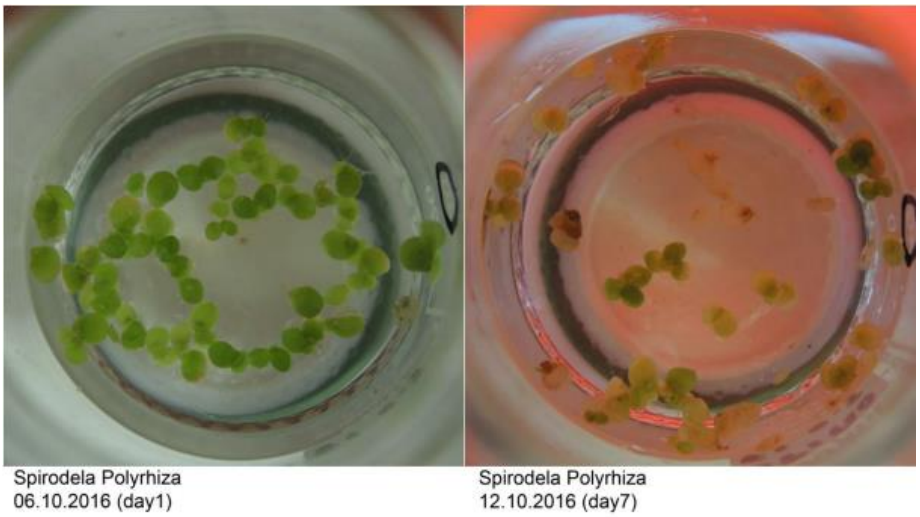


Figure 16: Spirodela Polyrhiza – appearance before and after carried out experiments

The duckweed species most suitable for the treatment of the wastewater at WWTP Westewitz were found to be Lemna Minor and Landoltia Punctata due to their resistance to fluctuations in ammonium concentrations. Lab experiment I revealed first insights of the performance of the four selected duckweed species on the filtered wastewater of WWTP Westewitz. Its main outcomes are:

- Average removal (and efficiency) of Lemna Minor 1.55 $mg \cdot l^{-1}$ (3.78%), Lemna Minuta 0.68 $mg \cdot l^{-1}$ (2.95%), Spirodela Polyrhiza 2.47 $mg \cdot l^{-1}$ (7.11%), Landoltia Punctata 4.64 $mg \cdot l^{-1}$ (13.09%);
- Lemna Minor identified as most suitable due to least yellowish discoloration of fronds which is an indication that Lemna Minor can thrive in the given wastewater constitution;
- Duckweed mat density insufficient (for Lemna Minor estimated to be 4.22% of the optimal mat density of 750 $g \cdot m^{-2}$), causing formation of algae and with-it pH increase Additional performance tests under higher duckweed mat densities required.

5.1.2. Growth and pH

The focus of these experiments was to determine the duckweed growth and observe the pH development within the growing media at different initial duckweed mat densities.

These have been determined for three differing experimental settings:

- 150 ml filtrated domestic wastewater, as described in chapter 4.3.2;
- 150 ml filtrated domestic wastewater with additional Blue Exit dosing to control cyanobacteria growth;
- 150 ml filtrated domestic wastewater with shading of the beaker sides to prevent the intrusion of other light sources to prevent the stimulation of growth of submerged algae.

In the first experimental setting, four test runs have been carried out, in the second experimental setting three test runs; and in the third experimental setting only one test

run. The relative growth age as well as the doubling time were calculated for the four different duckweed mat densities: 0.05 gFW·cm^{-2}; 0,075 gFW·cm^{-2}; 0.1 gFW·cm^{-2} and 0.15 gFW·cm^{-2}. Results are highlighted in Table 7.

Table 7: Test results for relative growth rate (RGR) and Doubling time at four different mat densities (0.05, 0.075, 0.1, 0.15) for three different settings;

Mat density	Test run	N° of Test runs	Mean RGR	RGR SD	RGR Min	RGR Max	Mean Doubling Time
[g·cm- ²]	[-]	[-]	[d $^{-1}$]	[d $^{-1}$]	[d $^{-1}$]	[d $^{-1}$]	[d]
0.05	Normal	4	0.086	0.026	0.044	0.115	9.22
0.075	Normal	4	0.072	0.012	0.056	0.085	9.97
0.1	Normal	4	0.045	0.008	0.032	0.051	16.00
0.15	Normal	4	0.019	0.011	0.000	0.029	21.13
0.05	Blue Exit	3	0.092	0.006	0.087	0.100	7.61
0.075	Blue Exit	3	0.091	0.026	0.061	0.125	8.29
0.1	Blue Exit	3	0.060	0.007	0.052	0.052	11.66
0.15	Blue Exit	3	0.033	0.010	0.047	0.047	23.14
0.05	Shading	1	0.092				7.55
0.075	Shading	1	0.077				9.04
0.1	Shading	1	0.061				11.92
0.15	Shading	1	0.041				15.58

The analysed duckweeds propagated better at lower mat densities of 0.05 and 0.075 gFW·cm^{-2} (Figure 18). At these two lower mat densities, the water surface area was not fully covered by the duckweeds, giving them space to spread out more (Figure 18). At the highest mat density of 0.15 gFW·cm^{-2}, a self-hindering effect occurred, as the water surface at the beginning of each test run was already fully covered with duckweed which limited the available space for daughter fronds. A decrease in growth rate with increasing mat density resulting in self-hindering due to overcrowding, as observed within the test runs, are in line with outcomes published by Driever et al. (2005), Frédéric et al. (2006) and Lasfar et al. (2007).

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

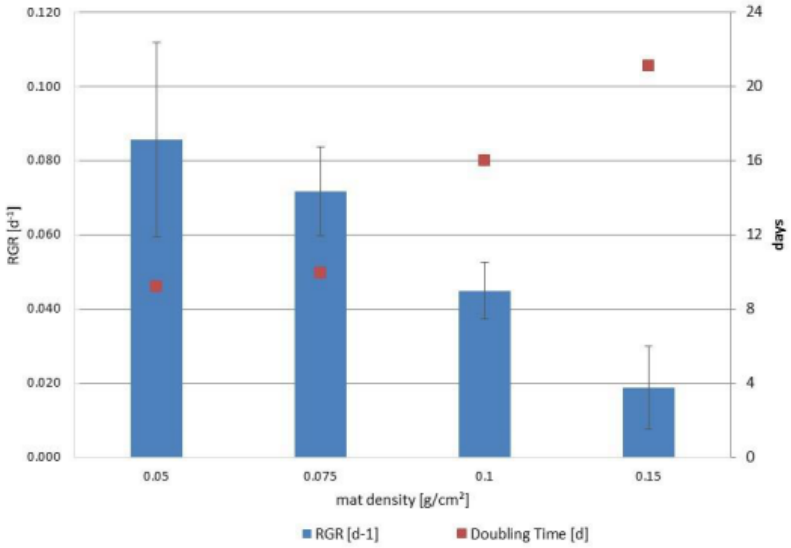


Figure 17: Relative growth rate (RGR) and doubling time of the first four test runs (first experimental setup) depending on mat density

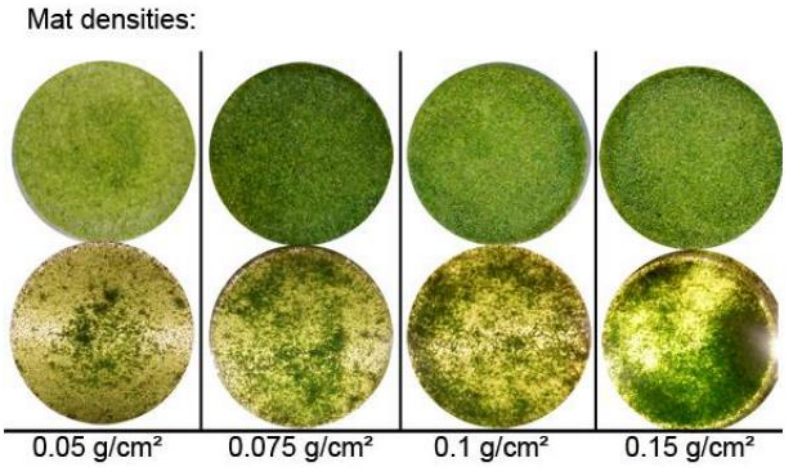


Figure 18: Correlation between mat densities light permeability

The achieved RGRs and doubling times differ noticeably from statements made in the literature. Most literatures indicate the RGR or doubling time under optimal environmental conditions. Ziegler et al. (2014) determined for clones of the duckweed species Lemna Minor under optimal growth conditions an average RGR of 0.4 d^{-1}, which corresponds to a doubling time of 1.7 days. Rejmánková (1975) observed an RGR for Lemna Minor of 0.24 d^{-1} in the laboratory and 0.20 d^{-1} in the field. An explanation for the lower RGRs measured within experiments, could be the given environmental conditions differing from the optimal growing conditions. The average temperature at the beginning and end of the test runs amounted to 10.82°C and 15.43°C. Highest growth rates are achieved between 25 and 31°C (Iqbal, 1999). The cultivation conditions in the experiments carried out by Ziegler et al. (2016) amounted 25 ± 1°C for instance.

Furthermore, the lighting conditions in these experiments amounted to only 40°µmol·m^{-2} ·s^{-1} which is below the recommended 100 µmol·m^{-2} ·s^{-1} (Appenroth, 2016a). Also, the

average pH at the beginning of the test runs amounted to 7.967 – a value right at the edge of the stated range for optimal growth. At the end of the test runs (after 7 days), the average pH values at the different mat densities differed greatly. The highest pH rise, to an average value of 8.804, was observed in the control beaker which was solely filled with filtrate and did not contain any duckweed (Table 8).

Table 8: Test results for dissolved oxygen content and pH at the end of each test run at different mat densities and at different test settings (normal, Blue Exit and shading)

Mat density	Test run	N° of test runs	DO	DO SD	pH	pH SD
[g·cm^{-2}]	[-]	[-]	[mg·l^{-1}]	[mg·l^{-1}]	[-]	[-]
Filtrate	All	6	4.50	4.03	8.804	0.520
0.05	Normal	4	12.81	4.03	8.359	0.484
0.075	Normal	4	7.24	5.53	8.165	0.253
0.1	Normal	3	8.58	2.64	8.333	0.417
0.15	Normal	3	0.49	0.67	7.541	0.052
0.05	Blue Exit	3	7.27	8.66	8.688	0.266
0.075	Blue Exit	3	4.63	5.01	8.160	0.072
0.1	Blue Exit	3	4.79	6.16	7.935	0.136
0.15	Blue Exit	3	0.39	0.55	7.545	0.116
0.05	Shading	1	1.55			
0.075	Shading	1	0.00			
0.1	Shading	1	0.00			
0.15	Shading	1	0.00			

An increase in pH can be an indicator for the activity of submerged algae. In natural ponds the pH varies throughout the day due to photosynthesis and respiration of aquatic organisms (Wurts, 1992). Wurts and Durborow (1992) state that during periods of high photosynthesis at algae blooms the pH value climbs dramatically above 9 and higher due to the high photosynthetic activity which increases the dissolved oxygen concentration in the water. High pH values inhibit duckweed grow and can even lead to their death (Landolt, 1987).

Submerged algae, the duckweeds' main competitors in nutrient uptake (Skillicorn, 1993), can be effectively suppressed by a dense duckweed mat density. Figure 19 displays the average DO concentration measured in the beakers of different mat densities at the end of each test run for the first test run setting. Highest average DO concentrations were measured in the beaker with the lowest mat density and the lowest average at the highest mat density. Especially at the lowest duckweed mat density of 0.05 gFW·cm $^{-2}$ gaps between the duckweed fronds allowed light to penetrate into deeper water layers, stimulating submerged algae growth. The growing

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

media was significantly greener than the other media at higher mat densities – an indication for algae activity (Figure 19).

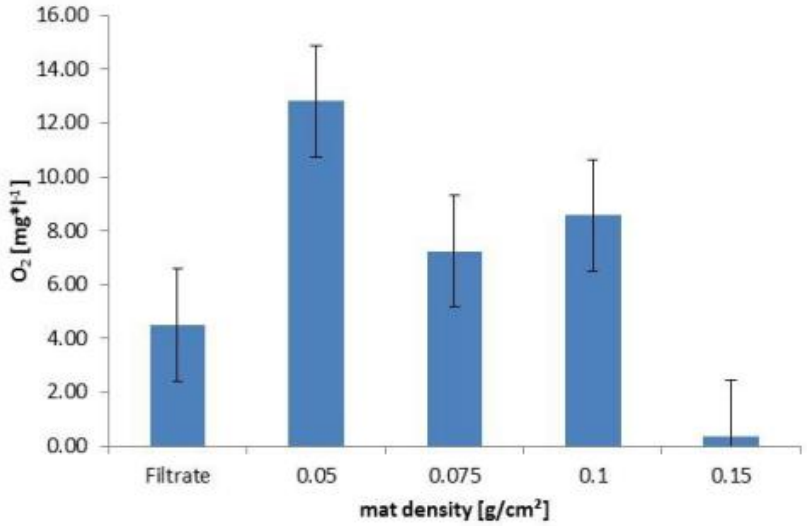


Figure 19: Development of oxygen content in the first four test runs (first experimental setting) in depending on mat density

Figure 20 shows as an example of the occurrence of submerged algae during the test run during a seven-day test run in the beakers with a duckweed mat density of 0.05 gFW·cm^{-2} and of 0.15 gFW·cm^{-2}. The beaker with the lower duckweed mat density showed a noticeable higher green coloration, perceived as turbidity, and a DO concentration of 7.95 mg·l^{-1}. At the highest duckweed mat density, the water was considerably clear and the DO concentration amounted nearly 0 mg·l^{-1}. This observation during all test runs led to the conclusion that a completely closed duckweed mat on the water surface prevents light to penetrate in deeper water layers which consequently inhibits submerged algae growth.

Figure 20: Comparison of algae formation at different mat densities from 03.03.-09.03.2017: Left beaker: Mat density 0.05 g·cm^{-2}; Right beaker: Mat density 0.15 g·cm^{-2}

The average DO concentration in the filtrate is significantly lower than the average DO concentrations at duckweed mat densities of 0.05, 0.075 and 0.15 gFW·cm^{-2} as shown in Figure 20. An explanation can be given due to the cleaning and harvesting procedure at the end of every test run. The beakers were cleaned and the duckweed harvested

with a sieve prior to the weight determination. The excess in duckweed was extracted and lowered to its initial weight with respect to its mat density of 0.05, or 0.075, 0.1 and 0.15 gFW·cm^{-2}. Algae could easily stick to the fronds and roots of duckweeds which were utilized in the following test run before the beakers containing duckweed had algae from the beginning of each test run. Algae which had formed in the beaker containing only the filtrate (no duckweed) were removed entirely from the beaker during the cleaning procedure. The contamination with new algae could have happened by the means of the new filtrate, which might had been carrying algae. Due to this uncontrollable parameter of potential algae in the daily added filtrate, the contamination of the beaker containing only filtrate with algae differed greatly from one test run to another.

The aim of the analysis of the pH value was to examine the influence of the mat density on the stability of the pH. As explained before – the higher the DO concentration, the higher the pH of the growing media. Lowest pH values with the smallest SD, which serves as indicator for stable pH conditions, were observed for the highest duckweed mat density of 0.15 gFW·cm^{-2} (Figure 27). Surprisingly, low duckweed growth was observed at the highest mat density. At a mat density of 0.075 gFW·cm^{-2} for the first test run setting, the DO concentration and average pH was lower than at mat densities of 0.05 and 0.1 gFW·cm^{-2}. An explanation cannot fully be given for the circumstance that the pH seemed to be more stable at the lower mat density of 0.075 than at 0.1gFW·cm^{-2}. Logically, the DO concentration at the lower mat density should be higher due to higher light penetration into deeper layers.

Next to the activity of submerged algae, the formation of adhering microorganisms on the roots and fronds was observed. The occurrence of microorganisms on the roots of duckweed is often observed on duckweeds thriving on natural ponds, of which some are reported to have positive effect on duckweed growth (Appenroth et al., 2015).

Additionally, within the experiments carried out in Westewitz, the formation of a biofilm which was attached to the surface of duckweeds' fronds has been recognized. This biofilm mainly consisted of cyanobacteria hindering the duckweed fronds from thriving on the water surface. The occurrence of the biofilm was varying in degrees of infestation. The infestation of this biofilm was the reason to examine the effect of Blue Exit – a commercially available agent to combat cyanobacteria.

Duckweeds contaminated with cyanobacteria from the laboratory pilot plant were used for the second test run setting with Blue Exit. Within three test runs, each lasting for 7 days, a positive effect of the Blue Exit onto cyanobacteria suppression, duckweed growth and pH stability was observed. The DO concentration and the pH were lower than under the normal conditions. Same applies for the corresponding standard deviations (Table 8). The RGR of the duckweed for all four investigated duckweed mat densities were, with the addition of Blue Exit, higher than at the test runs carried out in the beginning (Figure 19). Additionally, the biofilm on top of the fronds disappeared but microorganisms were still visible on the roots of the duckweeds. It has to be taken into account that the water surface and with it the duckweeds were stirred up daily during the dosing of new filtrate. This surface turbulence was observed to have a positive effect against adhesion of biofilm and especially cyanobacteria on the duckweeds'

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

surface. It is reported that some cyanobacteria release compounds which inhibit the growth of duckweed (Szabó, 1999).

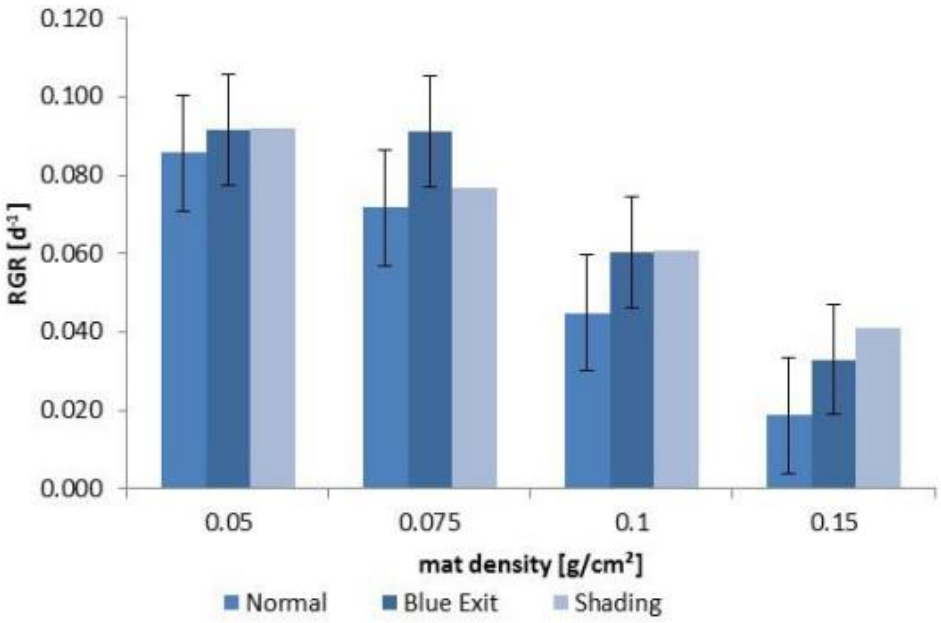


Figure 21: Comparison of RGR of three different settings; normal; with Blue Exit to prevent the formation of cyanobacteria; shading of the side to prevent light intrusion from the side

The suppression of cyanobacteria activity by Blue Exit could be the reason for the measured higher RGRs, which would indicate the functionality of Blue Exit as cyanobacteria growth inhibitor. The test results demonstrate as well, that the application of Blue Exit did not influence the growth of duckweed in a negative way; it only had a negative impact on cyanobacteria. An influencing factor on the cyanobacteria growth was the water and surrounding temperature. Cyanobacteria growth increases with temperature and reaches its maximum at 37°C (Zhang, 2015). The water temperature in the duckweed laboratory plant amounted to 22.59°C on average, meanwhile the water temperature within experiment II amounted to 15.45°C. Indoors, the formation of a biofilm was considerably smaller. An explanation can be given by the water temperature. Nevertheless, the biofilm formation occurred within experiment II as well, and with the dosing of Blue Exit a positive effect of cyanobacteria inhibition was observed. In the last test run setting with papers covering the sides of the beakers (Figure 22), the measured RGRs were surprisingly higher than the average of the normal set up, and even higher than the RGRs of the Blue Exit, except at a mat density of 0.075 $gFW \cdot cm^{-2}$ (Figure 21). The greatest difference was achieved at the highest mat density with a reduction in doubling time from 21.128 days to 16.93 days, in comparison to the average doubling time of the first test run setting.

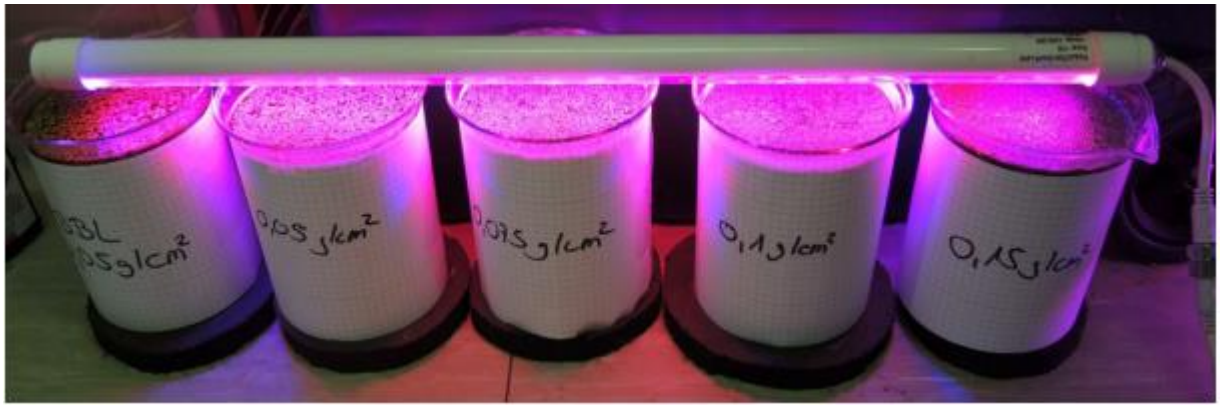


Figure 22: Beaker with additional side cover to prevent light irradiation from sides to prohibit growth of submerged algae

The measured DO concentration revealed a total lack of a photosynthetic activity of submerged algae at mat densities of 0.075, 0.1 and 0.15 gFW·cm^{-2}. A DO concentration of 1.55 mg·l^{-1} was only measured at the lowest mat density of 0.5 gFW·cm^{-2} which indicates only a small photosynthetic activity. Figure 23 shows the greenish turbidity of the media in the beaker of a mat density of 0.5 gFW·cm^{-2} without side cover, within the first test run setting, and a significant clearer media at the same mat density with side cover. This comparison illustrates the influence of diffused light through the beakers' sides onto submerged algae growth and activity. These results were very surprising as the diffusive light intensity due to natural light through windows and artificial light could not be measured with the light intensity measurement device PAR. For this reason, the light source causing algae growth was assumed to originate from the LED lamp penetrating into deeper water layers due to a not fully covered surface. The test results of the highest mat density of 0. 15 gFW·cm^{-2} – very low DO concentration and stable pH values – support this assumption.

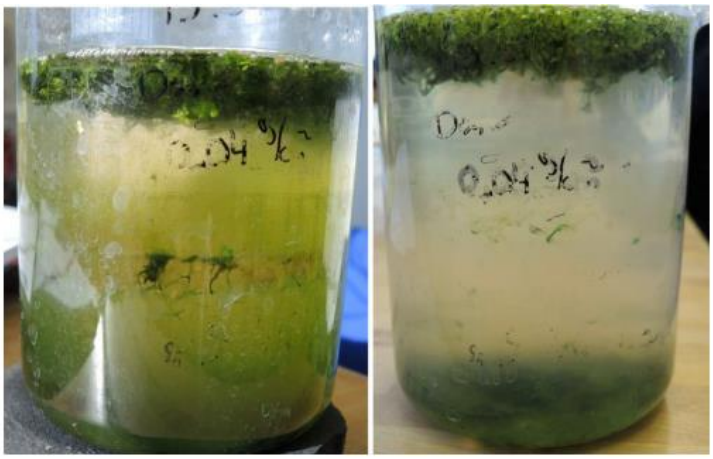


Figure 23: Formation of submerged algae in beaker with duckweed mat density of 0.05 g·m^{-2} without side cover (left picture; 16.02.2017) and with side cover (right picture; 10.03.2017)

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

For the APS duckweed plant within POWERSTEP, the influence of diffused light onto algae activity is especially of importance as the trays are made of transparent perspex sheets and the supporting structure is of a translucent material. Diffused light could trigger submerged algae growth from underneath. The influence of diffused light onto the APS duckweed plant has to be examined especially on sunny days during sunrise and sunset. It might be necessary to mount additional shading components. Main outcomes of the experiments:

- The highest average RGRs at all three tests were always at the lowest mat density of 0.05 gFW·cm^{-2} with RGR 0.086 d $^{-1}$ (doubling time 8.06 days), 0.092 d $^{-1}$ (7.53 days) ,0.092 d $^{-1}$ (7.53 days);
- Measured RGRs are significantly smaller than statements in the literature (e.g. Lemna Minor: 0.4 d $^{-1}$ (1.73 days) and 0.2 d $^{-1}$ (3.47 days). Reasons for lower RGRs could be due to several factors differing form optimal growing conditions:
- Temperature (14,6°C) below optimum (25-31°C); light (40 µmol·m^{-2} ·s $^{-1}$) below optimum (100 µmol·m^{-2} ·s $^{-1}$); pH value of filtrate with 7.98 on the border of what is considered to good growing conditions and the activity of cyanobacteria which is stated to have negative effects on duckweed growth;
- Best mat density to prevent extensive submerged algae growth as well as reduced growth of duckweed through overcrowding was identified to be 0.075 gFW·cm^{-2}. Most stable pH values at highest duckweed mat density, highest pH rises up to 8.8 (average) for beaker containing only filtrate;
- Positive effect of cyanobacteria inhibitor Blue Exit on small scale in beakers indoor. Vanishing of cyanobacteria mat and higher duckweed RGRs at all mat densities;
- Discovery of the great influence of diffused light onto submerged algae growth via beaker sides. Identification as problem in APS duckweed plant due to utilized tray material and its supporting structure.

5.2. Results Laboratory plant

The evaluation of the experiments carried out in the laboratory pilot plant is divided into two main chapters. Chapter 5.2.1 analyzes the functionality of the technical components and the distribution of light, temperature and pH within the plant. The second chapter 5.2.2 contains the evaluation of the whole system with regard to its performance to remediate wastewater components. This chapter includes explanation of problems which occurred during the operation and approaches to solve these problems.

5.2.1. Performance & Functionality of the laboratory pilot plant

Peristaltic Pump

The peristaltic pump in combination with two automatic timers enabled an hourly operation regime consisting of 50-minute feeding with filtrate and 10-minute break. With a 1 mm diameter tube, the pump capacity amounted to 9.5 ml·min^{-1}during the first two test runs. After the replacement with a 2-mm diameter tube and adjustments on the

stepper motor, the pump capacity amounted to 10 ml·min^{-1}. The pump capacity of 10 ml·min^{-1} comply with a daily feeding volume of 12 liters with respect to the set feeding interval. Several samplings verified a constant operation of the pump.

Gate Valve

The automatic gate valve, operated by an electric motor and driven by the Arduino Nano microcontroller, was put together of several components to enable the functional principle as intended for the harvest pit of the APS duckweed pilot plant. Difficulties, caused corrosion of the electrical wires preventing the activation of the electric motor operating the gate valve. Reasons for the corrosion are seen to have happened due to higher air temperature inside the box compared to the temperatures outside and the heated filtrate up to 25°C increased the humidity and caused the formation of water condensation inside the box, which softened the plastic protection around the wires making it permeable to water. New wires and a permanently open box cover which enabled a better air circulation remedied this problem.

Temperature

The box of the laboratory pilot plant showed good insulation qualities. The temperature profile in the greenhouse and inside the laboratory pilot plant was measured by two EASYLOG temperature loggers recording the temperature every five minutes. The blue line in Figure 24 and **Fehler! Verweisquelle konnte nicht gefunden werden.** shows the daily temperature development within the greenhouse and the red line the temperature within the laboratory pilot plant. During the period presented in Figure 24, the laboratory pilot plant was closed and only opened for operational purposes. The temperature amplitude in the greenhouse was quite high with minimum temperatures of minus 10°C and maximum temperatures around 24°C. During this period, the temperature within the box dropped under 10°C only once. The temperature within the box dropped considerably slower than outside the greenhouse and a higher average temperature could be achieved. The temperature profile within the laboratory pilot plant in comparison with the temperature profile within the greenhouse proves the insulation effect of the box (Figure 24).

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

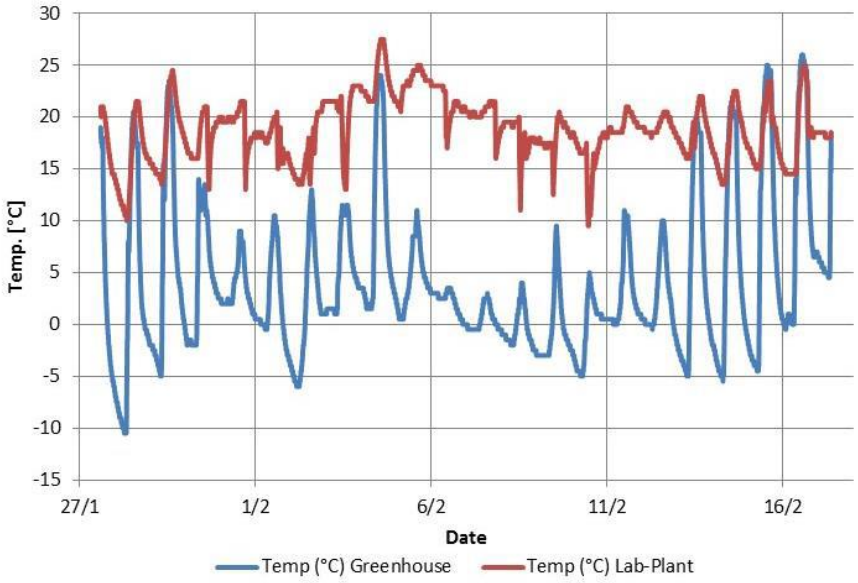
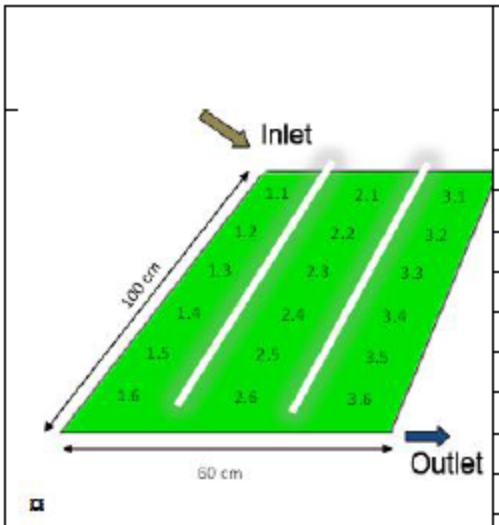


Figure 24: Daily temperature inside the greenhouse and in the laboratory plant

The actual water temperature within the laboratory pilot plant was measured on two runs; on the 27th of January 2017 and on the 3rd of February 2017. In addition to the temperature, the pH value was measured too (Figure 25). On each run 18 measurement points were determined. It was observed that the lowest temperatures were measured near the inlet and at the outlet. An explanation for the low temperature at the inlet can be given by the temperature of the filtrate which amounted, in January and February, on average to 8°C. Entering through the inlet, the filtrate had to be heated up, wherefore the water temperature around the inlet is lower. The lower temperature at the outlet (point 1.6, 2.6, 3.6) can be explained by the placement of the heating cable underneath the tray which did not include the last groove (harvesting groove).

The lower average temperatures measured on the 27th of January were affected by the duration that the cover of the box was left open, and the temperatures within the greenhouse. Low air temperatures caused a rapid cooling of the water temperature. During the measurements on the 27th of January the cover was left open approximately for 20 minutes before the measurements were carried out.

The results from the 3rd of February and the overall measured average water temperature of 22.59°C in the tray show that the heating cable and thermostat effectively heated up the water in the tray close to the reported temperature optimum of 25°C. The target value was set to 25°C but could not be achieved constantly due to heat losses to the ambient temperature.



Points of measurement	27/01/2017		03/02/2017	
	°C	PH	°C	pH
1.1	14.3	8.08	13.4	8.152
1.2	17.5	8.094	24.6	7.909
1.3	18.4	8.129	25	8.029
1.4	20	8.126	27.1	8.005
1.5	19.4	8.223	25.5	7.939
1.6	15.5	8.328	20	8.453
2.1	16.1	7.97	19.3	7.911
2.2	19.1	8.076	24.7	7.802
2.3	19.6	8.119	26.5	7.954
2.4	19.9	8.122	25.8	7.966
2.5	19.1	8.179	25.6	8.037
2.6	14.7	8.303	21.2	8.212
3.1	16.8	7.901	21.3	7.871
3.2	17.6	8.016	23.8	7.841
3.3	18.5	8.099	25.1	7.951
3.4	19.1	8.148	24.1	8.042
3.5	19.6	8.164	24.7	8.008
3.6	14.5	8.286	19.6	8.317

Figure 25: Water temperature and pH values within the laboratory plant at different points

Harvest

The actual determined duckweed harvest amount fell behind its target value. Reasons for this were:

1. The barrier separating the harvest groove from the rest of the reactor needed a supernatant of more than one to two millimeters to prevent duckweed roots from getting tangled up.
2. The infestation of the biofilm by cyanobacteria resulted in the total immobility of duckweed fronds.

The harvest regimes (opening of the gate valve), was set every six hours for two minutes. During this time three liters were pumped into the laboratory pilot plant, causing a supernatant of approximately 5 mm. The time interval of six hours in between each harvest process was required to achieve a supernatant of 5 mm at the set pump flow of 10 ml·min^{-1}. During the opening time of the gate valve of 2 minutes, the contents in the harvest groove as well as the supernatant were obtained from the plant. It was observed that especially the duckweed at the end of the harvest groove (60 cm), the furthest away from the gate valve, did not get flushed out during the harvest process.

The estimated doubling time within the laboratory pilot plant was expected to be 9 days. This value is determined due to the outcomes of lab experiment which revealed at a mat density of 0.075 g·cm^{-1} a doubling time of 9.966 days at an average temperature of 14.7°C. The higher average temperature of 22.59°C in the plant was

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

expected to increase the growth and decrease the doubling time, wherefore a doubling time of 9 days was estimated. Due to the low water depth in the laboratory pilot plant at the high points of the trapezoidal sheet (1 cm), a determination of the mat density was comparably difficult. The placement of the sieve underneath the duckweed surface always caused the duckweed to move away, delivering a falsified result.

The estimated RGR of 0.077 (doubling time 9 days) in the laboratory pilot plant resulted in a daily produced duckweed biomass of 60 $g \cdot m^{-2}$ at a mat density of 0.075 $g \cdot cm^{-1}$. For the laboratory pilot plant with a surface area of 0.6m², this will result in 35 grams of fresh duckweed. This amount had to be harvested every day, to keep the initial mat density of 0.075 $g \cdot cm^{-1}$.

The actual harvested duckweed biomass per day was significantly lower on an average value. During the first test run, the average duckweed biomass harvested amounted to 3.14 g per day (Table 9) – less than a tenth of the targeted harvest rate.

Table 9: Mean value of the daily harvested duckweed, Standard deviation of daily harvest and the target value during the test runs.

Test run	Mean daily harvest	SD daily harvest	Target value	Achievement of target value
	[g]	[g]	[g]	[%]
1st run	3.14	4.06	35	9.0
2nd run	10.34	6.77	35	29.5
3rd run	4.73	4.47	35	13.5
4th run	20.33	12.85	35	58.1
5th run	11.4	18.17	35	32.6
6th run	36.97	44.56	35	105.6
7th run	No data	No data	35	

In the second run, it amounted to 10.34 g with decreasing amounts towards the end. Within the fourth and fifth runs the harvest rates amounted to 20.33 and 11.4 g per day. During the sixth run the average duckweed biomass harvested amounted to 36.96 g per day. The higher average value for this test run is influenced by two events of gate valve failure. Due to the opening failure of the gate valve, the filling level within the reactor rose approximately three centimeters above the normal maximal level.

The main cause for the low harvest rates is seen to be caused by the infestation of the cyanobacteria mat. The installation of additional flush equipment at the end of the harvest groove which flushes through the harvest groove during the harvest process could be an effective measure to achieve a complete harvest/flush of duckweed in the harvest groove. Measures to combat the cyanobacteria infestation are described in the following chapter.

5.2.2. Ammonia removal during the laboratory pilot plant trials

The main task within these experiments was the examination of the ammonium removal rate achieved by the duckweed-based system. The seven test runs were carried out in the period from the 4th of January 2017 until the 21st of April 2017 with varying test durations of four to 17 days (Table 10). Reasons for the irregular test durations were due to a recurring biofilm mat on and between duckweed fronds, the failure of the peristaltic pump once and minor operational changes on the plant. The formation of the biofilm and its effect on duckweed performance will be also discussed in this chapter.

The filtrate fed daily to the laboratory pilot plant from Monday to Friday, showed an average pH value of 7.91 (±0.15) and average concentrations of NH_4-N, COD, TP, TN, TKj, NO_2-N and NO_3-N of 41.28 (±8.8), 371 (±104.76), 7.52 (±2.00), 70.09 (±16.11), 68.01 (±16.15), 0.33 (±0.1) and 1.76 (±0.65) $mg \cdot l^{-1}$. The average and SD are formed from 63 values each. The maximum concentration measured for NH_4-N amounted to 63.5 and the minimum to 24.5 $mg \cdot l^{-1}$. As the filtrate was directly obtained from the effluent of the Hydrotech drum filter, its temperature was dependent on the temperature of the raw sewage reaching WWTP Westewitz. In January, the measured temperature of the filtrate fed to the laboratory pilot plant was 8.3°C which increased up to 11.8°C in April due to increasing outside temperatures.

The ammonium removal performance of the laboratory pilot plant within the 7 test runs yielded the following average results (in detail see Table 10):

- NH_4-N effluent of concentration of 12.26 (±8.98) $mg \cdot l^{-1}$;
- NH_4-N degradation of 29.24 (±6.66) $mg \cdot l^{-1} \cdot d^{-1}$;
- NH_4-N removal of 0.56 (±0.14) $g \cdot m^{-2} \cdot d^{-1}$;
- NH_4-N degradation efficiency of 72.68 (±18.85)%.

The high standard deviations of the average results indicate great differing performances among the test runs. Table 10 shows the mean results of each test run.

Table 10: Laboratory pilot plant ammonium removal performance within the 7 test runs

Test run	NH4-N effluent	NH4-N degradation	NH4-N removal	NH4-N degradation efficiency	Start date	End date	Duration
	[$mg \cdot l^{-1}$]	[$mg \cdot l^{-1} d^{-1}$]	[$g \cdot m^{-2} d^{-1}$]	[%]	[-]	[-]	[d]
1st run	9.06	33.31	0.55	78.85	04.01.2017	16.01.2017	12
2nd run	21.38	22.43	0.36	50.75	16.01.2017	20.01.2017	4
3rd run	10.01	28.99	0.58	77.51	25.01.2017	10.02.2017	16
4th run	29.53	17.72	0.37	38.47	10.02.2017	22.02.2017	12
5th run	1.87	31.40	0.63	93.88	02.03.2017	17.03.2017	15
6th run	8.82	31.32	0.63	80.23	20.03.2017	31.03.2017	11

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

7th run	6.00	39.54	0.79	89.04	04.04.2017	21.04.2017	17
Mean	12.26	29.24	0.56	72.56			
SD	8.98	6.66	0.14	18.58			

Ammonium degradation efficiency, degradation and removal rate

The lowest mean NH_4-N effluent concentration of 1.87mg·l^{-1} was achieved during the fifth test which resulted in a degradation efficiency of 93% of the inflow concentration (Table 10 and Figure 27). The high degradation efficiency could lead to the presumption that during the fifth test run, the laboratory pilot plant showed the best performance – a false conclusion. A high degradation efficiency and low effluent concentration do not necessarily indicate a high removal performance of the duckweed-based system; it only shows the reduction in concentration to its initial value. As Körner and Vermaat (1998) state, the HRT, water depth and initial nutrient concentration are among factors which have to be considered prior to judging the actual performance of a duckweed-based system. The ammonium degradation, stated as mg·l^{-1}·d^{-1}, specifies the reduction in concentration per day within the media but does not take into consideration the depth of a pond or a container. The ammonium degradation in duckweed-based systems with equal performance is lower at greater pond depth than in a shallow pond, as the ammonium degradation takes place on the water surface where the duckweeds are situated.

For the comparison of the performance of the laboratory pilot plant in the seven test runs, the ammonium degradation is more meaningful than degradation efficiency and effluent concentration as it shows the reduction in concentration (see Table 10 and Figure 26).

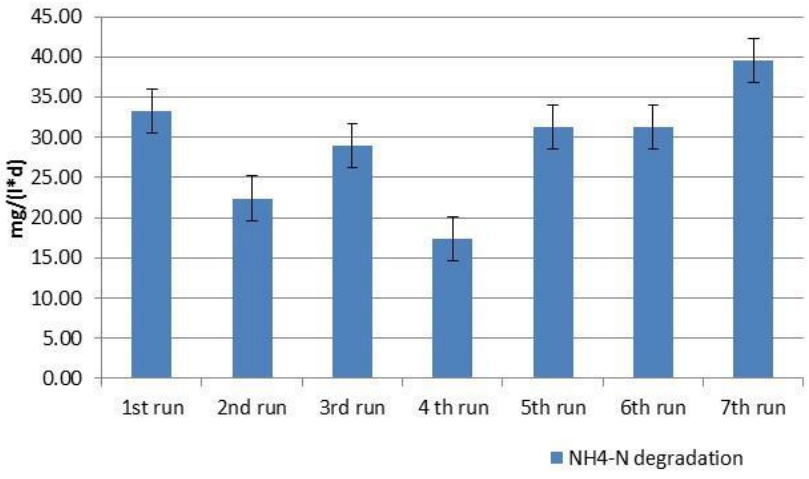


Figure 26: Average ammonium degradation [mg/l*d] during each test run

In the seventh test run the average degradation amounted to 39.54 mg·l^{-1}·d^{-1}, the highest achieved mean degradation of the seven test runs. The degradation efficiency reaching 89.04%, with an effluent concentration of 6 mg·l^{-1}. Degradation efficiency and effluent concentration of the seventh test run are lower than during the fifth, but the

actual amount of ammonium removed by the duckweed-based system was higher in the seventh run, as displayed in Figure 27. The reason for the lower degradation efficiency at higher degradation is the initial ammonium concentration of the filtrate. During the fifth test run, the ammonium concentrations of the filtrate was 8.96 mg·l^{-1} lower than at the seventh run (45.43 mg·l^{-1}) which explains the higher ammonium effluent concentration by simultaneous higher degradation.

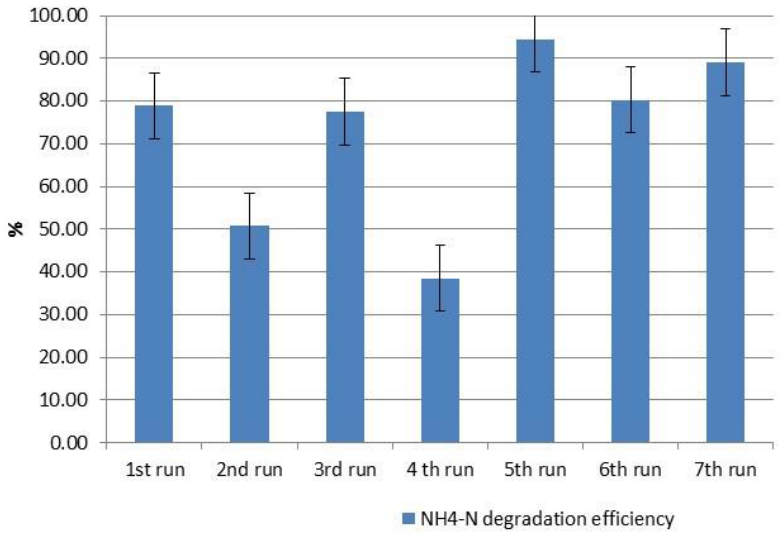


Figure 27: Average ammonium degradation efficiency [%] during each test run

Nevertheless, the degradation efficiency is an important parameter, indicating the percentage in removal of the influent concentration. But, a more reliable and informative measure regarding the evaluation of the performance of a system is the removal rate which states the amount removed per surface area and day (e.g. g·m^{-2}·d^{-1}). This removal rate makes it easier to compare outcomes of different studies with each other. Performance influencing variables like mat density and environmental conditions (e.g. temperature, light and nutrient load) still must be considered.

Figure 28 shows the achieved mean ammonium removal rates in the laboratory pilot plant of the seven test runs, showing that it operated poorest in the 2nd and 4th runs with removal rates of 0.36 and 0.37 g·m^{-2}·d^{-1} and in the fifth, sixth and seventh with 0.63, 0.63 and 0.79 above the average of 0.56 g·m^{-2}·d^{-1} (Table 10). Other authors have measured ammonium uptake rates of a duckweed-based system of 0.083 – 0.453 and 1.332 g·m^{-2}·d^{-1} (Körner, 1998; Cheng, 2002). The higher uptake rate achieved within this experiment in comparison to the results presented by Körner and Vermaat (1998) could be due to a higher duckweed mat density in the laboratory pilot plant. Körner and Vermaat (1998) carried out their experiments over a length of three days with an initial mat density of 0.558 gFW·m^{-2} whereof the initial mat density in the laboratory pilot plant was 0.075 gFW·m^{-2} and due to the longer test duration and the insufficient harvest operation, the actual mat density within the plant amounted to 1 to 1.5 gFW·m^{-2}. The daily ammonium uptake rate as stated by Cheng et al. (2002) is determined by the authors on the basis of the highest measured hourly degradation rate of 0.955 mg·l^{-1}·h^{-1}. The actual average daily uptake rate should be considerably lower.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

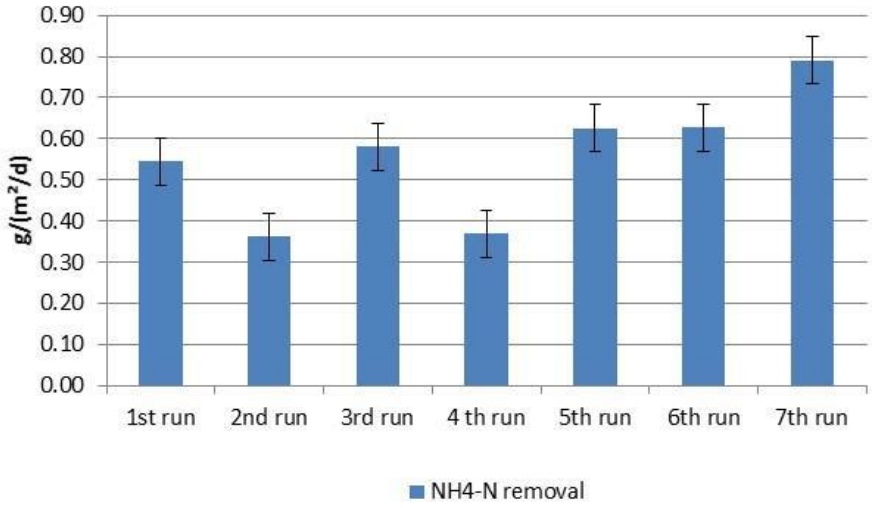


Figure 28: Average ammonium removal (g/m²*d) during each test run

Ammonium Removal by Duckweed as well as the contribution of Bacteria and Algae

The presented ammonium removal rates correspond to the rates achieved in the duckweed-based system. It is very important for the interpretation of the determined removal rates that these ammonium removal rates comprise of the removal activities of duckweed and non-duckweed related components. Few research results have been published on the ammonium and total nitrogen removal activities within a duckweed-based system, whereof Körner and Vermaat and Zimmo (2003) published informative studies on this topic. These two authors state that in duckweed-based wastewater treatment systems, duckweeds are not the only ones responsible for the total nutrient removal. Nutrient removal influencing variables are algae, bacteria and NH3-volatilization. An enclosed duckweed mat density inhibits the growth of the competitive submerged algae (Skillicorn, 1993). But algae and bacteria activity can occur in biofilms attached to duckweed roots and fronds, attached to walls or in the sediment. These contribute to the overall removal of nutrients (Körner, 1998). Zimmo et al. (2003) stated for the examined duckweed-based system an average NH3-votalisation of only 1.1% of the influent nitrogen concentration. This is said to be achieved due to the stable pH conditions caused by the surface covering duckweed mat density which suppressed the growth of submerged algae and with it suppressing a rise in pH. Körner and Vermaat (1998) identified a direct ammonium removal by duckweed uptake of 79 to 99% of the initial ammonium, which complied with a total N-loss of 30 to 47% in their experiment. The direct and the indirect contribution of duckweed due to nitrifying and denitrifying bacteria and algae in the attached biofilm were stated to come up for approximately ¾ of the total nitrogen loss. The last quarter occurs due to algae and bacteria attached to walls and in the sediment.

Throughout the experiments carried out within the laboratory pilot plant, great influences of bacterial activities have been observed. The most severe activity was the infestation of a dense biofilm mainly consisting of cyanobacteria which developed to an enormous problem for the operation of the duckweed-based system. Next to the cyanobacteria, the formation of a biofilm on the roots of the duckweeds on the walls of

the reactor volume and sedimentation of particulate matter was described by Körner and Vermaat (1998). Except for the cyanobacteria, these bacteria were not thought to affect the performance of the duckweeds negatively but rather enhance their growth and performance (Appenroth, 2016b).

5.3. Results Pilot plant

5.3.1. General performance

Finally experiments in the pilot plant were carried out with the focus to learn more about the general performance of the duckweed reactor in large scale as well as how nutrient removal (not only ammonium but also TP, TN as well as enrichment of NO_3-N) is working under real conditions. Results carried out in the pilot plant are highlighted Table 11. The average elimination rate based on the day load was 61.2% of ammonium. But there were differences in the elimination rates during the different seasons as described in chapter 5.3.2.

Table 11: Elimination rate of nutrients in Pilot plant

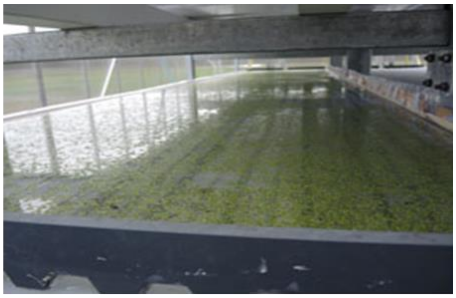
Parameter	Average load Inflow	Standard deviation Inflow	Average load Outflow	Standard deviation Outflow	Average Elimination Rate	Standard deviation
	[g/d]	[g/d]	[g/d]	[g/d]	[%]	[%]
NH_4-N	5.00	1.47	1.80	0.96	61.2	20.4
TP	7.06	1.97	4.07	1.40	28.3	12.7
TN	0.71	0.21	0.51	0.13	42.5	11.7

5.3.2. Biological performance based on seasons

Spring Period

Measurements to remove nutrients from duckweed during spring were carried out between April and May 2017. Temperatures in the tubes were between 12.4°C and 15.2°C, and measured pH values between 7.5 to 8.2. The average removal rates are presented in Figure 30. These low elimination rates resulted due to the moderate solar radiation and temperatures but also low mat density of duckweed because of the start of phase.

Parameter	Removal Rate [%]
NH_4-N	22-50
TN	11-40
TP	22-50



The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Figure 29: Nutrient removal efficiency and duckweed mat density during spring period

Summer Period

The measurements during the summer period were carried out between June and August 2017. At this time, the wastewater in the trays had a temperature of 18°C to 25.8°C and a pH value of between 7.8 and 9.5. While total phosphorus had a fluctuating reduction of between 7% and 60%, the TN reduction was up to 70% and ammonium removal up to 99% (Figure 30). During this period, the duckweed has been able to evolve very well due to the optimal temperature and sun exposure, resulting in an efficient reduction of nutrients in the wastewater. This was also noticeable visually on the duckweed by their strong green color and their noticeably high density.

Parameter	Removal Rate [%]
NH_4-N	60-99
TN	40-70
TP	7-60

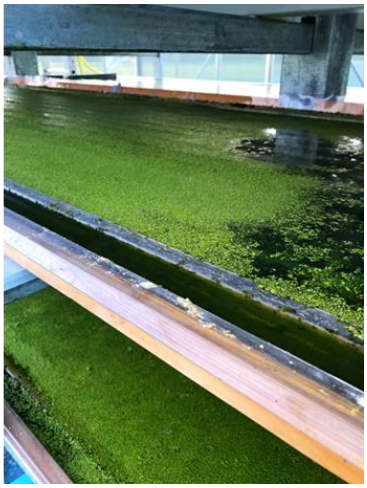


Figure 30: Nutrient removal efficiency and duckweed mat density during summer period

Autumn Period

The measurements during the autumn period were carried out in September and October 2017. During the period, wastewater temperature was between 14.0°C and 20.2°C and pH was measured between 8.1 and 9.5. Compared to the summer period a decrease of removal efficiency in all three parameters was shown. As highlighted in Figure 31 it can be seen that the number of living duckweed has started to decline (weaker intensity in color). This can be caused by the lower temperature as well as the short day periods.

Parameter	Removal Rate [%]
NH_4-N	45-70
TN	25-50
TP	20-30

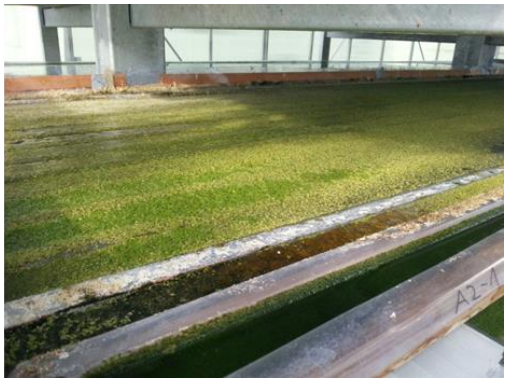


Figure 31: Nutrient removal efficiency and duckweed mat density during autumn period

Winter Period

Measurements in the winter period were carried out between November and December 2017. The wastewater temperature was between 8.3°C and 17.0°C and pH values between 8.0 and 8.8. No efficient wastewater treatment could be carried out during the winter period and elimination rates were between 37-50% for ammonia, 17-40% for TN and 2-27% for TP. A reason for the low elimination rate is the amount of active duckweeds. As shown in Figure 32 most of the duckweeds are already dead. The dying of duckweeds and the resulting lack of removal efficiency could have different reasons: on the one hand the duckweed was exposed to too low temperatures and reduced sunlight over the winter, and on the other hand in the beginning of November there was a storm, which tore many windows of the greenhouse which resulted in less protection of the duckweed during the night, resulting in temperatures below 0°C in the greenhouse. Due to the storm, there was also a power failure which resulted in harvesting difficulties.

Parameter	Removal Rate [%]
NH_4-N	37-50
TN	17-40
TP	2-27

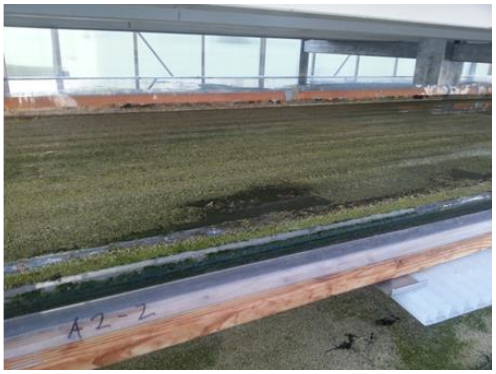


Figure 32: Nutrient removal efficiency and duckweed mat density during winter period

5.3.3. Biogas production using duckweeds

Within the duckweed trials also the potential of biogas production compared to other substrates were measured. The method used was described in Chapter 4.2.5. In Figure 33 results of the 3 batch tests using duckweeds as substrate for biogas production are presented. As highlighted in the figure a specific methane yield by carrying out 3 batches between 191and 217 NL kg oTs-1 could be achieved.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

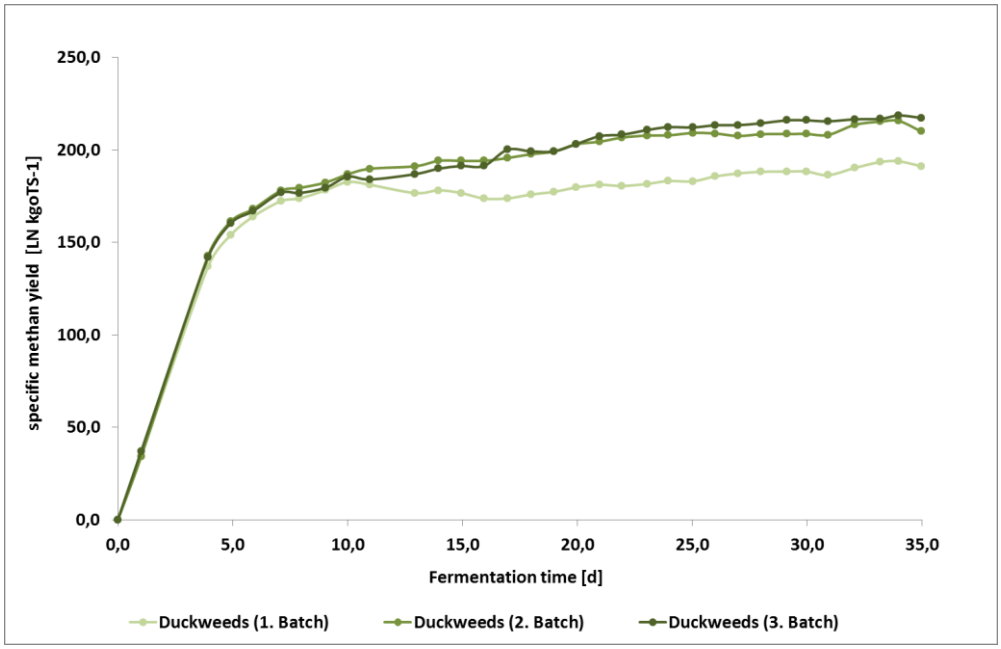


Figure 33: Specific methane yields testing duckweeds using a BMP (biomethanpotenzial test)

Furthermore a comparison using different other substrates was carried out in a separate test. Therefore primary activated sludge from the WWTP Westewitz right after the micro-screen was used as well as waste activated sludge (WAS) from the WWTP Waßmannsdorf in Berlin. Compared a reference (cellulose) was used too. Results are shown in Figure 34. As shown below the specific methane yield of primary sludge is three times higher compared to waste activated sludge as well as duckweeds. The mean value (of three batches) of primary sludge is around 704 NL kg oTs-1 compared to the 184 NL kg oTs-1 using waste activated sludge and 206 NL kg oTs-1 using duckweeds. Nevertheless without any pre-treatment of the duckweeds similar contents as WAS could be achieved.

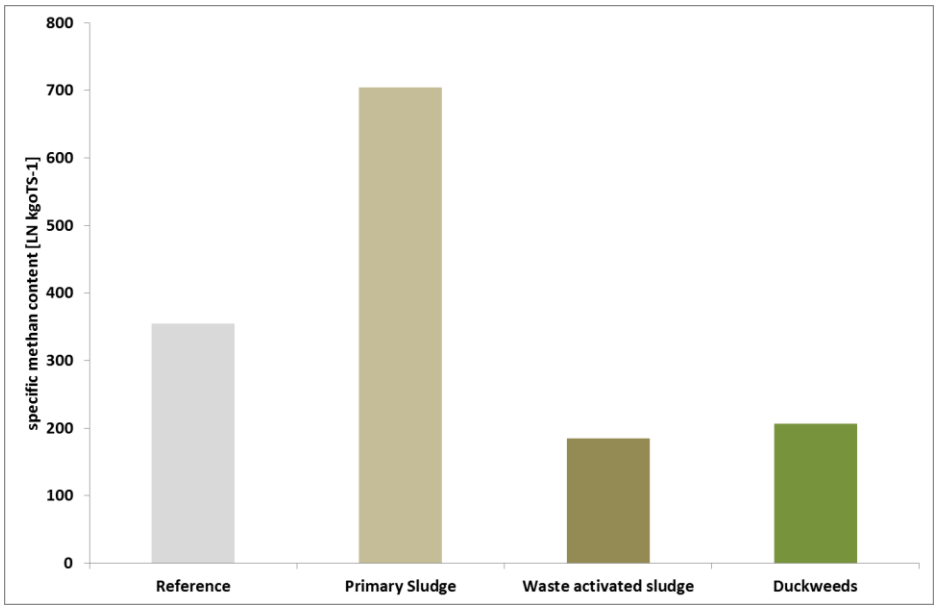


Figure 34: Comparison of specific methane yields using different substrates.

5.3.4. Problems that occurred during the pilot plant experiments

Construction of the greenhouse

Due to two strong thunderstorms during the testing period, the greenhouse was destroyed (see Figure 34) which also resulted in maintenance activities requiring a plant operation stop. When constructing a greenhouse for a duckweed reactor at a WWTP, additional attention must be paid to metrological conditions and the location itself. The right position to catch most of the daylight is important was well as a wind-protected area.

Figure 35: Duckweed greenhouse after a heavy thunderstorm

Construction of the tray and harvesting problems

The design of the duckweed tubing required further optimization, as its current configuration still caused problems during the operation as well as harvesting. The wrong design can lead to less turbulence in the tubes which results in duckweed death. Figure 36 shows the actual flow of filtrate (blue) versus how it should be carried out (orange). One optimization approach would be to add small corrugations in the "transition bar" to ensure earlier transfer of the wastewater, or to provide an initially broader flow by employing multiple inflows distributed across the entire width.

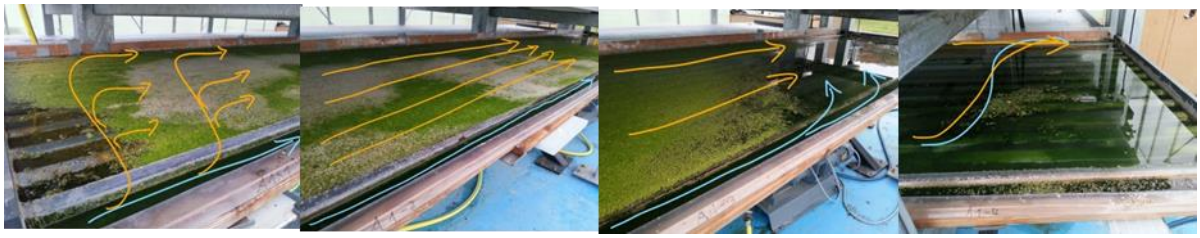


Figure 36: Flow behaviour of the filter as it is (blue) and how it should be (orange)

Intermediate storage of filtration before pilot plant operation

Operation had to be carried out with a totally full IBC as a storage tank because of semi-automatic operation lasting up to five days. By running the 22 trays of the duckweed plant the IBC would be empty after only one day. For automatic operation of the duckweed plant connected with an enhanced primary treatment tank (e.g. drum filter), it is necessary to design a control system for the two systems.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

6. Conclusion

In conclusion, running a nitrogen removal stage with a full-scale implementation of a duckweed reactor still needs further research and optimization. During the experiments from lab to pilot it could be highlighted that ammonia removal rates up to 99% could be reached depending on the circumstances (wastewater temperature and surrounding temperatures, pH value as well as the right mixture of duckweed culture). It could also be shown that these elimination rates could be reached without any additional lighting system in the pilot plant, which would be an additional cost factor. Nevertheless, optimization must be carried out in the reactor design as well as the harvesting system.

7. References

ANSARI, A. A., GILL, S. S., LANZA, G. R. AND RAST, W 2011. Eutrophication: causes, consequences and control: Plant Nutrient Phytoremediation using Duckweed.

APPENROTH, K.-J., SREE, K. S., FAKHOORIAN, T. AND LAM, E. 2015. Resurgence of duckweed research and applications: report from the 3rd International Duckweed Conference. *Plant molecular biology,* vol. 89, pp. 647-654.

APPENROTH, K. A. L., E. 2016a. Discussion corner: Standardization of protocols for duckweed research: Duckweed growth rate determination. *Newsletter of the Community of Duckweed Research and Applications,* vol. 3, pp. 276–279.

APPENROTH, K. J., NIKOLAI, B. AND ERIC, L. 2013. Telling Duckweed Apart: Genotyping Technologies for the Lemnaceae. *Chinese Journal of Appplied Environmental Biology,* vol. 19, pp. 1-10.

APPENROTH, K. J., ZIEGLER, P. AND SREE, K. 2016b. Duckweed as a modern organism for investigating plant - micro interactions in an aquatic enviroment and its applications. *Endocatobiosis and cell Research,* vol. 27, pp. 94-106.

ARMSTRONG, W. P. 2011. *Wayne's World: The Lemanceae (Duckweed Family)* [Online]. Available: http://waynesword.palomar.edu/1wayindx.htm [Accessed 9 January 2017 2017].

BACHMANN, L. 2014. Masterarbeit - Entwicklung einer unternehmensinternen Handlungsempfehlung zur Wirtschaftlichen Instandhaltung von Belüfterelementen in Kläranlagen.

BEIER, M., SANDER, M., SCHNEIDER, Y. AND ROSENWINKEL, K. 2008. Energieeffiziente Stickstoffelimination. *KA Korrespondenz Abwasser,* Abfall 55, pp. 671–678.

CAICEDO, J., VAN DER STEEN, N., ARCE, O. AND GIJZEN, H. 2000. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (Spirodela polyrrhiza). *Water Research,* vol. 34, pp. 3829–3835.

CHENG, J., BERGMANN, B. A., CLASSEN, J. J., STOMP, A. M. AND HOWARD, J. W. 2002. Nutrient recovery from swine lagoon water by Spirodela punctata. *Bioresource Technology,* vol. 81, pp. 81-85.

CHENG, J. J. A. S., A.-M. 2009. Growing Duckweed to Recover Nutrients from Wastewaters and for Production of Fuel Ethanol and Animal Feed. *CLEAN - Soil, Air, Water,* vol. 37, pp. 17-26.

COHEN, Y. A. K., H. 2004. Increasing the pH of Wastewater to High Levels with Different Gases - CO2 Stripping. *Water, Air and Soil Pollution*, pp. 265-275.

CROSS, J. W. 2006. *The Charms of Duckweed: Duckweed Geography* [Online]. MIssouri Botanical Garden. Available: www.mobot.org/jwcross/duckweed/Habitat/geography.html.

CUI, W., XU, J., CHENG, J. J. AND STOMP, A. M. 2011. Starch Accumulation in Duckweed for Bioethanol Production'. *Biological Engineering,* vol. 3, pp. 187-197.

CUI, W. A. C., J. J. 2015. Growing duckweed for biofuel production: a review. pp. 16-23.

EDWARDS, P., HASSAN, M. S., CHAO, C. H. AND PACHARAPRAKITI, C. 1992. Cultivation of duckweeds in septage-loaded earthen ponds. *Bioresource Technology,* vol. 40, pp. 109-117.

FANG, Y. Y., BABOURINA, O., RENGEL, Z., YANG, X. E. AND PU, P. M. 2007. Ammonium and Nitrate Uptake by the Floating Plant Landoltia punctata. *Annals of Botany*, pp. 365-370.

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

FRÉDÉRIC, M., SAMIR, L., LOUISE, M. AND ABDELKRIM, A. 2006. Comprehensive modeling of mat density effect on duckweed (Lemna minor) growth under controlled eutrophication. *Water Research,* vol. 40**,** pp. 2901-2910.

GOOPY, J. P. A. M., P. J. 2003. A Review on the Role of Duckweed in Nutrient Reclamation and as a Source of Animal Feed. *Asian-Australasian Journal of Animal Sciences,* vol. 16**,** pp. 297-305.

GRADY, C. P. L. 2011. *Biological wastewater treatment*.

HASAN, M. R. A. C., R 2009. Use of algae and aquatic macrophytes as feed in small-scale aquaculture: A review. Rome: Food and Agriculture Organization of the United Nations.

HASTIE, B. A. 1992. *The use of aquatic plants in wastewater treatment: a literature review*. Dudley Knox Library.

HAUCK, M., MAALCKE-LUESKEN, F. A., JETTEN, M. S. AND HUIJBREGTS, M. A. 2016. Removing nitrogen from wastewater with side stream anammox: What are the trade-offs between environmental impacts? *Conservation and Recycling,* vol. 107.

HENZE, M., HARREMOËS, P., COUR JANSEN, J. AND ARVIN, E. 1997. *Wastewater Treatment: Biological and Chemical Processes* [Online]. Berlin, Heidelberg: Springer. Available: http://dx.doi.org/10.

HORN, H., RAPP-FIEGLE, S., GÜNTHER, W., HOLTORFF, M., BISCHOF, F. AND CHWISTEK, M. 2009. Schlussbericht - Endversion Forschungsvorhaben "Kläranlage derZukunft" - Themenbereiche Faulgasproduktion, Anaerobtechnik und Deammonifikation.

HU, Z., LOTTI, T., KREUK, M. DE, KLEEREBEZEM, R., VAN LOOSDRECHT, M., KRUIT, J., JETTEN, M. S. M. AND KARTAL, B. 2013. Nitrogen removal by a nitritation-anammox bioreactor at low temperature. *Applied and environmental microbiology,,* vol. 79**,** pp. 2807-2812.

IQBAL, S. 1999. *Duckweed Aquaculture: Potentials, Possibilities and Limitations for Combined Wastewater Treatment and Animal Feed Production in Developing Countries,* Duebendorf.

KÖRNER, S. A. V. 1998. The relative importance of Lemna gibba L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Water Research,* vol. 32**,** pp. 3651-3661.

LANDOLT, E. 1992. Lemnaceae: Duckweed Family. *Journal of the arizona-nevada academy of science,* vol. 26**,** pp. 10-14.

LANDOLT, E. A. K., R. 1987. Biosystematic investigations in the family of duckweeds ("Lemnaceae"). *The family of "Lemnaceaea" : a monographic study.,* vol. 2.

LENG, R. A., BELL, R. AND STAMBLIE, J. R. 1995. Duckweed - a potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development,* vol. 7.

LES, D. H., CRAWFORD, D., LANDOLT, E. AND KIMBALL, R. 2002. Phylogeny and systematics of Lemnaceae Dumortier, the duckweed family. *Systematic Botany***,** pp. 221-240.

LÜOND, A. 1980. Effects of nitrogen and phosphorus upon the growth of some Lemnaceae.

MENGEL, J., KIRKBY, E. A., KOSEGARTEN, H. AND APPEL, T. 2001. Principles of Plant Nutrition: 5th Edition. *Dordrecht.* Kluwer Academic Publishers.

MITCHELL, D. S. 1974. Aquatic vegetation and its use and control: A contribution to the International Hydrological Decade. Paris: UNESCO.

NULTSCH, W. 2012. Allgemeine Botanik: 12. Auflage. Stuttgart: Georg Thieme Verlag.

O'SHAUGHNESSY, M. 2016. *Mainstream Deammonification.*

OEWA 2012. Betriebshandbuch OEWA.

ORON, G., DEVEGT, A. AND PORATH, D. 1988. Nitrogen removal and conversion by duckweed grown on waste-water. *Water Research,* vol. 22**,** pp. 179-184.

PINNEKAMP, J. A. A. 2011. *Abwasserreinigung,* Aachen.

POORTER, H. 2013. *Relative growth rate and its components* [Online]. [Accessed Accessed 7 February 2017.

PORATH, D. A. P., J. 1982. Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquatic Botany,* vol. 13**,** pp. 125-131.

SKILLICORN, P., SPIRA, W. AND JOURNEY, W. 1993. *Duckweed Aquaculture: A New Aquatic Farming System For Developing Countries,* Washington D.C., The World Bank.

SREE, K. S., MAHESHWARI, S. C., BOKA, K., KHURANA, J. P., KERESZTES, Á. AND APPENROTH, K.-J. 2015. The duckweed Wolffia microscopica: A unique aquatic monocot. *Flora - Morphology, Distribution, Functional Ecology of Plants,* vol. 210**,** pp. 31-39.

SZABÓ, S., BRAUN, M. AND BORICS, G. 1999. Elemental flux between algae and duckweed. *Arch Hydrobiol.,* vol. 3**,** pp. 355-367.

TAYLOR, C. D., CLIVE, G., LONGLEY, E., MELLETT, P., REDFERN, M., SCHIELE, T., STOTT, C., WALKER, R. AND WILLIAMS, B. 2000. *The Kingfisher Science Encyclopedia,* Boston, Houghton Mifflin Company imprint.

VAN DEN BERG, H., VAN LIDTH DE JEUDE, E., HAK, A., MOOREN, C., DEN UIJL, R. L., GIOMI, T. AND KRAAIJEVELD, S. 2015. *Duckweed, a tiny aquatic plant with growing potential: A research on the potential applications of duckweed in urban water systems in The Netherlands,* Utrecht.

VERMA, R. A. S., S. 2015a. Impact of density loads on performance of duckweed bioreactor: A potential system for synchronized wastewater treatment and energy biomass production. *Environmental Progress & Sustainable Energy,* vol. 34**,** pp. 1596-1604.

VERMA, R. A. S., S. 2015b. Utility of Duckweeds as Source of Biomass Energy: A Review. *BioEnergy Research,* vol. 8**,** pp. 1589-1597.

VIDAKOVIĆ-CIFREK, Ž., SORIĆ, S. AND BABIĆ, M. 2013. Growth and photosynthesis of Lemna minor L. exposed to different light conditions and sucrose supplies. *Acta Botanica Croatica,* vol. 72.

WANG, W., HABERER, G., GUNDLACH, H., GLÄßER, C., NUSSBAUMER, T., LUO, M. C., LOMSADZE, A., BORODOVSKY, M., KERSTETTER, R. A., SHANKLIN, J., BYRANT, D. W., MOCKLER, T. C., APPENROTH, K. J., GRIMWOOD, J., JENKINS, J., CHOW, J., CHOI, C., ADAM, C., CAO, X.-H., FUCHS, J., SCHUBERT, I., ROKHSAR, D., SCHMUTZ, J., MICHAEL, T. P., MAYER, K. AND MESSING, J. 2014a. The Spirodela polyrhiza genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. *Nature Communications,* vol. 5.

WANG, W., LI, R., ZHU, Q., TANG, X. AND ZHAO, Q. 2016. Transcriptomic and physiological analysis of common duckweed Lemna minor responses to NH4(+) toxicity. *BMC plant biology,* vol. 16**,** pp. 92.

WANG, W., YANG, C., TANG, X., GU, X., ZHU, Q., PAN, K., HU, Q. AND MA, D. 2014b. Effects of high ammonium level on biomass accumulation of common duckweed

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Lemna minor L. *Environmental science and pollution research international,* vol. 21, pp. 14202-14210.

WETT, B. A. H., M. 2007. *Betriebserfahrungen mit dem DEMON-Verfahren zur Deammonifikation von Prozesswasser,* Innsbruck.

WILLET, D. 2005. Duckweed-based Wastewater Treatment Systems: Design Effects and Integrated Reuse Options for Queensland Conditions. Brisbane Qld.

WURTS, W. A. A. D., R. M. 1992. Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds.

ZHANG, D., DECHATIWONGSE, P., DEL RIO-CHANONA, E. A., MAITLAND, G. C., HELLGARDT, K. AND VASSILIADIS, V. S. 2015. Modelling of light and temperature influences on cyanobacterial growth and biohydrogen production. *Algal Research,* vol. 9, pp. 263-274.

ZIMMO, O. 2003. Nitrogen transformations and removal mechanisms in algal and duckweed waste stabilization ponds: PhD Thesis. Delft: UNESCO-IHE.