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„Fighting planktic algae with benthic algae: A pilot study at the Heustadelwasser in Vienna“

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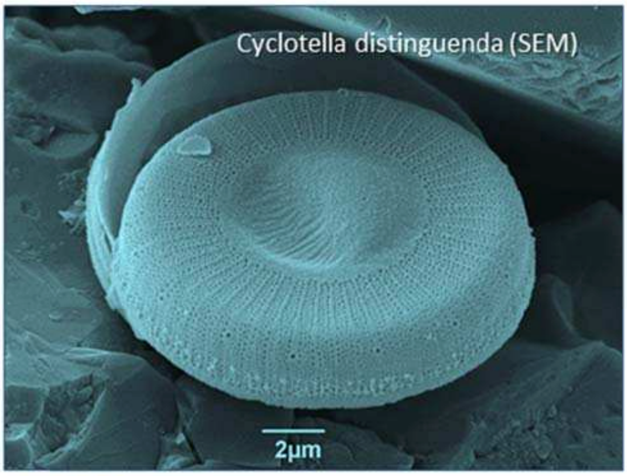

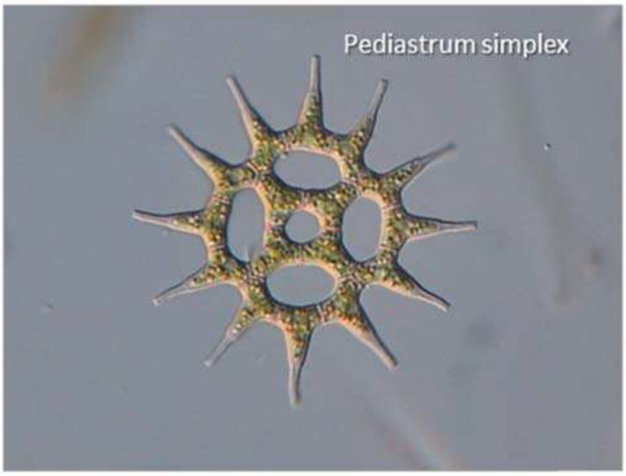

Algen – sie sind vielfältig, schön und nützlich zugleich – Eindrücke aus der Phykologie

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1 Allgemeine Einleitung

Algen sind eine heterogene Organismengruppe, die funktionell und nicht durch Verwandtschaftsbeziehungen definiert ist (Graham et al., 2009). Algen umfassen mikroskopisch kleine Bakterien und einzellige Eukaryoten, aber auch meterlange Seetange, welche dichte Bestände bilden können. Die meisten Algen betreiben Photosynthese, bei der Sauerstoff entsteht (=oxygene Photosynthese) und besitzen nicht den herkömmlichen Aufbau von Landpflanzen, die in Blätter, Stamm und Wurzel unterteilt sind. Ebenso fehlt ihnen ein Leitgewebe zum Wasser- und Stofftransport. Vor über 3 Milliarden Jahren besiedelten Cyanoprokaryota (= Blaualgen) als eine der ersten Lebensformen die Erde. Diese Organismen waren schließlich auch dafür verantwortlich, dass sich die Erdatmosphäre mit Sauerstoff anreicherte. Viele Algen leben aquatisch, sie kommen aber auch im Boden, auf Felsen oder als Aufwuchs auf Blättern und sogar in der Luft vor (Graham et al., 2009). Algen sind also überall zu finden. Innerhalb der Algengruppe Streptophyta werden neuerdings auch Gefäßpflanzen eingeordnet. Die Landpflanzen entwickelten sich aus einzelligen Grünalgen (Leliaert et al., 2011), daher werden Landpflanzen manchmal auch als „Höhere Algen“ bezeichnet.

Massenauftreten von Algen sind meist mit erhöhten Nährstoffeinträgen in Oberflächengewässern verknüpft (Heisler et al., 2008). Besonders anorganischer Phosphor, welcher in Gewässern oft nur in Spuren vorhanden ist, kann bei erhöhten Konzentrationen das Wachstum stark ankurbeln. Massenentwicklungen von Algen bestehen in nährstoffbelasteten Seen oft aus Cyanoprokaryoten, welche über längere Zeit dominieren können (Dokulil & Teubner, 2000). Viele Vertreter der Cyanoprokaryoten sind fähig, gefährliche Toxine zu synthetisieren, welche bei akuter und chronischer Aufnahme Tier und Mensch schädigen und bis zum Tod führen können (Landsberg, 2002). Weiters führen hohe pH-Werte - ausgelöst durch eine Kombination von schlechtem Puffervermögen und starker Algenproduktion - zu einer Verschiebung des chemischen Gleichgewichtes von harmlosem Ammonium hin zu stark giftigem Ammoniak. Ammoniak oder Sauerstoffzehrung - erzeugt durch den mikrobiellen Abbau von Organismen - können Fischsterben verursachen (Camargo & Alonso, 2006, Landsberg, 2002). Diese Konsequenzen der Eutrophierung (Nährstoffeintrag) wurden auch am Heustadelwasser in Wien beobachtet.

Das Heustadelwasser ist ein ehemaliger Altarm der Donau und befindet sich im grünen Prater in Wien. Starke hydrologische Veränderungen seit dem 19. Jahrhundert und letztendlich durch den Kraftwerksbau Freudenau führten zur Eutrophierung des Gewässers. Um die Nährstofffracht zu reduzieren, wurde als Sanierungsmaßnahme 2007 ein Kiesfilter, installiert; das System wird als Neptunanlage bezeichnet. Trotz einer leichten Verbesserung der Situation wurde 2010 immer noch ein Massenauftreten von Cyanoprokaryoten verzeichnet (Donabaum et al., 2011), das nach einer weiteren Reduzierung der Nährstoffe verlangte. Ist eine Verringerung der Nährstoffzufuhr nicht möglich, können verschiedene Maßnahmen gesetzt werden, unter anderem chemische Phosphorfällungen oder Pflanzenkläranlagen.

Im aktuellen Fall wurden Algal Turf Scrubbers (ATS) (Adey et al., 1993) eingesetzt, welche direkt auf dem Kiesfilter installiert wurden. ATSs sind künstliche Fließrinnen, die von dem zu reinigenden Wasser überströmt werden und mit der Zeit einen dicken Algenbiofilm entwickeln. Zwei Reinigungsprinzipien liegen hier zugrunde. Einerseits werden dem Wasser durch die Algen Nährstoffe entzogen, welche die Algen für ihr Wachstum brauchen und welche sie in ihrer Biomasse speichern. Andererseits wirkt der Algenbiofilm wie ein Filter, indem sich planktische Algen oder andere Partikel verfangen und so aus dem Wasser entfernt werden.

Für Aufwuchsalgen ergeben sich auf den ATSs perfekte Wachstumsbedingungen. Durch Kippschalen erzeugte Wellen verhindern die Bedeckung der Algen mit Sediment und die ständige Zufuhr mit neuem Wasser gewährleistet eine gute Nährstoffversorgung. Der Algenbewuchs ist nur wenige Zentimeter mit Wasser bedeckt und lässt ausreichend Licht und Kohlendioxid zu den Algen vordringen. Die ATSs werden nicht mit Algen beimpft, sondern von den natürlich aufkommenden Algen besiedelt. Um die Nährstoffe zu entfernen, wird die gesamte Algenbiomasse periodisch abgeerntet und steht dann als Rohstoff für weitere Anwendungen zur Verfügung. Die Algenbiomasse eignet sich als Dünger, Futter für Aquakulturen oder als Kraftstoff zur Energiegewinnung (Roeselers et al., 2008). Zudem benötigt die ATS-Technologie keine teuren und ökologisch bedenklichen Chemikalien und die Energieaufwendungen bleiben gering, da das Algenwachstum (=Nährstoffaufnahme, Reinigung) von der Sonne betrieben wird.

Für diese Pilotstudie wurden eigene ATSs entwickelt, welche unter realen Bedingungen in vier Replikaten getestet wurden. Drei Durchläufe wurden von Juni bis September 2011 durchgeführt, wobei wöchentlich Proben genommen wurden.

Ziel der Studie war (1) das Wachstum, die Produktivität und die Zusammensetzung der Algenbiomasse im Detail zu untersuchen; (2) das Potenzial der ATSs für eine weitere Nährstoffreduzierung im Heustadelwasser zu bewerten und (3) die Biomasse in Hinblick auf weitere Anwendungen zu untersuchen. Die Besonderheiten dieser Studie sind der Fokus auf die Verknüpfung von Biomassegewinnung und Gewässersanierung an einem realen Standort und die neuartige Kombination eines Kiesfilters mit der ATS-Technologie. Diese Ideen verbunden mit der detaillierten Analyse der Algenbiomasse bilden das Grundkonzept dieser Pilotstudie.

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2 Fighting planktic algae with benthic algae: A pilot study at the Heustadelwasser in Vienna

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2.1 Abstract

Anthropogenic eutrophication in surface waters may cause serious problems, such as fish kills, mass development of algae including cyanoprokaryotes, which sometimes produce severe toxins. In the current case study, drastic hydrological changes initiated an eutrophication process of the former Danube backwater Heustadelwasser. As a restoration measure, a gravel filter, followed by a phosphate trap was installed in 2007. Additionally, we installed a biological treatment using the ability of self-purification in streams, particularly through biofilms: we used artificial stream beds (algae turf scrubbers = ATS) situated at the gravel filter surface, which were periodically supplied with water from the Heustadelwasser. For removing nutrients, the biomass was harvested from time to time. The study aimed to estimate growth, productivity and composition of the algal biomass, to investigate the potential of the ATSs for nutrient removal at the Heustadelwasser and to examine the biomass with regard to further applications. Weekly measurements of biomass, nutrients and fatty acids were done during three growth runs from June to September 2011. Maximal removal rates of phosphorus were observed in August with about 19 mg total phosphorus m^{-2} d^{-1}: peak biomass of approximately 250 g m^{-2} dry mass was achieved. In total, approximately 0.9 kg of phosphorus could be removed by a gravel filter sized ATSs within five summer months. Further use of the harvested biomass as fertilizer, fermentation feedstock or combustion fuel is possible. The pilot study showed that the ATS technology has great potential and provides an effective and ecologically sustainable way to remove nutrients from surface waters, with the positive effect of producing biomass.

Keywords: periphyton, productivity, water amelioration, biofuel, fatty acid, ecological engineering

2.2 Introduction

Restoration of eutrophicated surface waters is still a focus of applied limnology. Increased nutrient supply is connected to massive algal growth (Heisler et al., 2008), which degrades the water body in several ways. Highly eutrophicated water bodies are often dominated by cyanobacteria for long time periods (O'Neil et al., 2012, Dokulil & Teubner, 2000). Certain cyanobacteria are able to produce severe toxins, such as Microcystins, Anatoxin-a and Cylindrospermopsin (Patocka, 2001), which have a strong negative impact on the usability of the water. For instance, Microcystin is a hepatotoxin which harms humans as well as fish and birds leading to death when consumed in higher doses (Landsberg, 2002). Furthermore, high algal productivity causes an increase of the pH value, which changes the chemical equilibrium from ammonium towards toxic ammoniac (Camargo & Alonso, 2006). Ammoniac or depletion of oxygen due to algal decay processes may result in massive fish kills (Landsberg, 2002, Camargo & Alonso, 2006) and malodor. Some of these problems were found at the Heustadelwasser.

The Heustadelwasser is a former backwater of the Danube situated at the Prater area in the city of Vienna. Nutrient enrichment was caused by drastic hydrologic changes due to the construction of the power plant Freudenau at the river Danube. To reduce the nutrient load in the Lower Heustadelwasser, a gravel filter treatment was installed in 2007. Despite an overall decline of the planktic algal biomass, mass occurrence of algae was still observed in 2010 (Donabaum et al., 2011b), which called for further ecological engineering measures. Several options are available on the market, for example wetland treatments are used for nutrient reduction (Tang et al., 2012), if a prevention of nutrient enrichment is not possible. Besides, chemical phosphorus removal is widely performed (Lewandowski et al., 2003, Gibbs et al., 2011). Another idea for restoring eutrophicated water bodies is the application of autotrophic biofilms for nutrient removal. The biofilms can be harvested and used for various purposes, as there is an increasing demand for biofuels (Mata et al., 2010) and phosphorus recovery (Van Vuuren et al., 2010).

A combination of water treatment and biomass production is achieved with the algal turf scrubber technology (ATS^{TM}) (Adey et al., 1993). It has already been used for several types of waters such as industrially contaminated groundwater (Adey et al., 1996), waste water of tertiary treatment (Craggs et al., 1996), animal manure discharge (Mulbry et al., 2008) and riverine water (Mulbry et al., 2010). The ATS is an artificial stream bed with an attached biofilm, mainly consisting of algae and bacteria. An advancement of the ATS technology to achieve increased growth is the use of pulsed water movement. Waves are created by e.g., tipping buckets to prevent the biofilm from sediment covering and to ensure an optimal water exchange.

Based on the self-purification processes in streams (Sabater et al., 2002), the ATS technology uses two major strategies of nutrient removal from the water. First, the algal biofilm takes up nutrients, especially phosphorus and nitrogen, which are stored in the biomass. Therefore, “fighting planktic algae with benthic algae” is meant in terms of nutrient competition. Second, the microstructure of the biofilm allows an additional trapping of suspended solids from the supplied water, including planktic algae. The biomass is harvested periodically and is then available for further applications.

A major advantage can be seen by the attached growth of algae, as high densities are achieved and the harvest is facilitated in comparison to suspended algal production in ponds or photo-bioreactors (Pittman et al., 2011). The produced biomass can potentially be used for several purposes such as slow release fertilizers (Mulbry et al., 2005), aquaculture feed, or as raw material for energy generation or biofuel production (Mata et al., 2010). Moreover, no cost intensive and ecologically risky chemicals have to be applied. Energy requirements stay low, as the system is sunlight driven. Thus, the ATS technology presents an ecological and economical treatment to improve water quality and gain biomass.

For this study, ATSs were developed to conduct a pilot study under real conditions in a temperate region. Two ATS types, a horizontal and a vertical type were taken into consideration, with a focus on the horizontal type. Three growth runs were conducted during the sampling period, which lasted from June to September, 2011. Our study pursued several objectives: (1) to analyse the growth, productivity and composition of the biomass in detail, (2) to determine the potential of the ATS for a further reduction of nutrients in the Heustadelwasser and (3) to examine the biomass with regard to further applications by measuring the fatty acid content. To our knowledge, this study is the first one testing the ATS technology at a highly eutrophicated backwater in central Europe. Moreover, we provide a detailed analysis of the biomass growth and combined different biomass parameters to get targeted information about the algal biomass content.

2.3 Material and Methods

Operation site

The Heustadelwasser (Longitude: 48.1977898, Latitude: 16.4309468), a former backwater of the river Danube, is situated in the green Prater in Vienna, which is an important local recreation area. The water body is fed with Danube water to prevent desiccation. To counteract the severe eutrophication process, a gravel filter (area 478 m^2) with an attached phosphorus trap, the so-called Neptunanlage, was installed in 2007. Our experiments were conducted on top of the gravel filter.

Design of the algal turf scrubber

Preliminary experiments have been conducted to test two different approaches. The first approach was to use vertical ATSs. A submerged pump in a water tank created a steady water cycle. The water was pumped into a slotted horizontal tube, from where it was dispersed over a net with a width of 1 m and a mesh size of about 5 mm; an aluminum rail was used to fix the net within the tube. This provided an even dispersal of water and stabilized the net for a higher loading. In order to be able to increase the height of the vertical ATS, pumps with higher delivery rates were used. To test the vertical ATS under outdoor conditions, an aluminum frame had to be installed as a wind protection. To prevent water loss a plastic foil cover was fastened on the supporting rack.

The second approach dealt with horizontal ATSs. Again, a water cycle was created with a submerged pump in a water tank. The bottom of the flow channel was covered with a net to increase the surface area for the algae to attach. For the pulsed water supply, an acrylic glass tipping bucket was installed. To ensure frictionless moving, a ball bearing was used. As the water pressure on the tipping bucket was too high with only one outlet, an additional distributor was added. Water loss in the bucket area was minimized by a plastic cover fixed on the upper end of the ATS.

Both ATS prototypes were tested on the terrace of the Biozentrum, which is situated in the 9th district of Vienna. The water tanks were filled with tap water, which came from the Donaukanal, an artificial side canal of the Danube. Some nutrients and soil were added to facilitate the initiation of the algal growth.

For the main experiment, the ATS had to be adapted to the structural conditions at the study site. First, the water pressure provided a maximal height of about 40 cm. Second, water supply was given in intervals of approximately two minutes by the Neptunanlage, which had to be considered for the setup.

Also, the vertical ATS had to be adapted to the field situation. A mesh wire was arranged around one of the spray valves of the gravel filter and again a net was used as a substrate for the algae. To retain the water, a fabric belt was added. Due to the fact that the water supply was not continuous, the net desiccated during the gaps and therefore, the algal growth was strongly inhibited. Therefore, the vertical ATS were no longer followed up and we focused on horizontal ATS.

Four replicas of the horizontal ATSs, each of about 1.6 m^2, were built for the main experiment (Figure 1). The basis was a wooden construction with a pond foil cover. To ensure adequate stability, the tipping buckets were made of stainless steel. A plastic net with 5 mm mesh size was added to assist algal growth. Furthermore, a 20 mm barrier was fixed at the end of the flow channel to protect the algae against desiccation. The 4 replicas were situated on the gravel filter and each was supplied with water by an adapted spray valve of the Neptunanlage at a flow rate of approximately 26 $l\ min^{-1}$. The ATSs were adjusted to approximately 1% of slope and they were not inoculated with algae prior to the growth period.

Operation and sample preparation

Three runs were conducted during the sampling period lasting from June to September 2011. Samples were taken weekly and a complete harvest was done every 4 to 6 weeks (duration of the first run: June 3rd- July 12th, second run: July 12th - August 9th and third run: August 16th - September 20th, 2011). The weekly biomass sampling was done with a modified Douglas sampler (Douglas, 1958). Two to four circular areas (13.6 or 17 cm^2 respectively) were randomly defined from the biofilm, scraped off with a brush and removed with a suction unit. The raw sample was then homogenized and partitioned for several analyses. Samples of the water supply were also collected weekly.

Irradiance

For each ATS, the solar radiation was measured with Hobo^{TM} Data Loggers (logging interval 5 min); data were converted from Lux to photosynthetically active radiation (µmol photons m^{-2} s^{-1}) by a factor of 0.0139, which was derived from a calibration with a Li – Cor LI1000 Datalogger.

Analysis of ATS biomass and water supply

To determine fresh-, dry- and ash mass, glass microfiber filters GF/C were first combusted and preweighed. Additionally, the wet mass of the filters was measured. A known volume of the raw sample or water sample was filtered by vacuum filtration and weighed (fresh mass). Then the filters were dried at 90°C and reweighed (dry mass). For ash mass the filters were combusted for 2h at 450°C and weighed. The weight loss from dry- to ash mass represents the ash-free dry mass (organic part of the biomass).

For spectrophotometric chlorophyll-a analysis, a defined volume was filtrated on a GF/C filter. The filter was then frozen in the dark at -20°C to assist algal cell bursts. The filters were ground with a homogenizer (Polytron) and stored in the dark at 5°C over night with 9 ml of 90% acetone for pigment extraction. After centrifugation, the clear supernatant was measured with a U-2001 spectrophotometer (Hitachi) at a wavelength of 663 nm (Lorenzen, 1967), (Jeffrey & Humphrey, 1975).

Measurements of the total phosphorus (TP) were done according to the European standard EN ISO 6878.

Organic carbon was analysed as non purgeable organic carbon (NPOC) with a TOC-VCPH total organic carbon analyser. This analysis was combined with a total nitrogen analyser (TMN-1 Shimadzu). Samples were either measured immediately after transport to the laboratory or acidified by 2 molar HCl and stored at 5°C until analysis. If necessary, the samples were diluted and homogenized by means of an ultrasonic apparatus (Branson Sonifier 250) prior to analysis. The samples were acidified to a pH of about 2 with HCl to remove the inorganic carbon and were then sparged with carrier gas five minutes before combustion. To prevent particles from sedimentation, the samples were mixed continuously with a magnetic stirrer. For each sample, four replicas were analysed and the three with the lowest deviation were taken for the calculation of a mean value. The concentration of elements was calculated from calibration curves (correlation factor > 0.999) performed with potassium hydrogen phthalate for carbon and potassium nitrate for nitrogen.

The fatty acid analysis was done once for each run. The sample preparation was done according to a modified protocol of Krienitz and Wirth, (2006). A defined volume of raw sample was frozen at -20°C, lyophilized (VaCo2-E, Zirbus technology) and transferred into Falcon tubes. After adding 5 ml of $CHCL_3$:MeOH 2:1 v/v and 1 ml internal standard (0.4 mg tricosan acid), a 90min ultrasonic bath was applied for lipid extraction. Afterwards, the samples were centrifuged at 4,000 rpm; evaporation took place over night in an exhaust hood. After adding tertiary butyl-methyl-ether, the samples were filtrated by Phenex – NY 17 mm syringe filters 0.2 µm or 25 mm PTFE 0.2 µm syringe filters into a GC/MS vial. Then 250 µl TMSH (tri-methyl-sulfonium-hydroxide) was added. The detection of the fatty acids was done with GC/MS (Clarus 600, Perkin Elmer, column: EILITE-5MS, 30 m, 25 µm, 0.25 m) with parameters set as follows: injection temperature 260°C, helium flow 1.3 ml min^{-1}; temperature gradient started at 80°C with an increase of 4°C until 240°C, which was kept for 19 minutes (total time of 60 min). For the MS, a scan (m/z 30-600) and a single-ion-mode were applied. The masses 74, 55, 67 and 79 are characteristic fractions for fatty acids and were used for detection. To identify fatty acids, the retention times in comparison with a standard mixture of fatty acid methyl esters (F.A.M.E. Mix 14-22, Fluka) were used. Furthermore, the mass spectra were compared to the NIST data base (NIST Standard Reference Database). To do a first quantification, four different concentrations of heptadecanoic acid were used and a calibration with integrated areas of the single-ion-mode with the ion m/z = 74 was developed.

Calculation of growth and removal rates

Calculation of the net growth rate r [d^{-1}] was done by plotting the logarithmic chlorophyll-a results against time. Here, the exponential growth phase forms a line and a linear regression of this provides r, which is represented by the slope. For each of the three runs, the net growth rate was calculated. Furthermore the doubling time of algae dt = ln(2)/r was calculated (Graham et al., 2009). To calculate the maximal areal removal rate of phosphorus [mg m^{-2} d^{-1}], the growth rate of the dry weight was multiplied by the mean phosphorus content of the dry mass (Davis et al., 1990).

Non-invasive measurement of oxygen respiration and production

To measure the oxygen, 5 x 5 cm pond foil slices and plastic net were placed randomly on the ATS flow channels. After exposition over a certain time period, slices were taken to the laboratory. Slices were then cut to a dimension of 3 to 14 cm^2 and transferred into a glass chamber, which was filled with ATS water. A magnetic stirrer was added and the chamber was sealed gas proof and thermostated at 20°C in a water bath (Thermostatbad F3, Haake). The oxygen respiration in the darkness and the net oxygen production in light at about 192 µmol photons m^{-2} s^{-1} (converted from Lux by a factor of 0.0141) were measured with a non-invasive oxy meter (OXY-4-Mini, Presens). After the measurement, algal biomass was scraped off, followed by chlorophyll-a (chl-a) analysis.

Fluorescence analysis – pulse amplitude modulated fluorometry (PAM-fluorometry)

Outdoor measurements were done with the Diving-PAM Underwater Fluorometer (Walz GmbH, Germany). The glass fiber for measurements was fixed just below the water surface. During the whole sampling period, three daily courses of the actual fluorescence yield were done with a measuring interval of five minutes. The quantum yield of Photosystem II is given as $\phi_{PSII} = (F'_m - F_t)/F'_m$, where F'_m is the maximal fluorescence measured with a saturating flashlight during light conditions under ambient light. F_t is measured immediately before this flashlight and gives the steady state of the fluorescence (Maxwell & Johnson, 2000).

Statistics

The statistical evaluation was done with the software packages R 2.12.1, Sigma Plot 12.0 and Excel 2010. Spearman rank correlations were used to investigate correlations between different parameters. One-way analyses of variance (ANOVA) combined with a Tukey post-hoc test were conducted to compare different oxygen measurements.

2.4 Results

Water analysis and environmental factors

Chlorophyll-a amounts of the surface water ranged from 21.1 ± 1.7 to 69.1 ± 2.2 µg l^{-1} (mean ± standard deviation) with two peaks in June and July, respectively (Figure 2). TP concentration was between 33.8 ± 1.5 and 84 ± 0.3 µg l^{-1} (Figure 2) with the maximum recorded in the beginning of July. Dry mass in the water supply ranged from 6.1 ± 0.2 to 15.6 ± 2.1 mg l^{-1} (Figure 2). The ash-free dry mass accounted for 89% ± 9% of the dry mass. Dry mass, TP and chl-a concentration show similar fluctuation patterns over the sampling period, which was supported by a Spearman rank correlation analysis (Table 1).

The mean daily irradiance ranged from 5 to 35 mol photons m^{-2} d^{-1} (PAR) and decreased during the sampling period.

Biomass growth and algae composition of the ATS

At the beginning of the first run, a colonization phase occurred, followed by an exponential growth phase. The colonization phase was distinct in the first run. The peak biomass determined by dry mass, ash-free dry mass and chl-a was attained after 3 to 4 weeks of growth. Subsequently the biomass stagnated or decreased (Figure 3).

The maximal areal removal rate of total phosphorus (MRP) of the first run with 9.7 ± 2.6 mg m^{-2} d^{-1} was similar to the third run (Figure 4). The second run showed a considerably higher removal rate with 19.1 ± 2.5 mg m^{-2} d^{-1}. At the same period, the doubling time of algae was lowest in the second run (2.7 ± 0.2 d). The first and third run showed higher doubling times (3.9 ± 0.5, 4.6 ± 0.4 d).

Dry mass reached its maximum during the first run on June 28th with 251.9 ± 44.4 g m^{-2} (Figure 3) with almost comparable yields during the second and third run. The maximal ash-free dry mass was attained in the second run on August 2nd with 68.9 ± 10.70 g m^{-2}, the maximum values of the first and third run were in the same range. Interestingly, the ATS biomass consisted of a substantial portion of inorganic compounds. The ash mass accounted for around 74% ± 4% (*n=60*) of the dry mass.

The chlorophyll-a was highest in the second run on August 2nd, when it reached a maximum of 700.5 ± 141.1 mg m^{-2} (Figure 3). During the first and third run the chl-a maxima were lower. A similar pattern was shown with TP, which also peaked on Aug. 2nd with 441.8 ± 44.4 mg m^{-2}. The correlation between chl-a and TP was particularly strong (Table 2; Spearman rank correlation coefficient = 0.908, p <0.001). The phosphorus content of the dry mass averaged 0.13% ± 0.05% (*n=60*) throughout the sampling period.

The ratio of chl-a to ash-free dry mass provides an insight into the composition of the biofilm (Figure 5). During the first run the chl-a portion increased, in the second run it also increased and stayed high; the third run showed a general lower chl-a portion compared to the second run. The organic carbon to total nitrogen ratio ranged from 6.1 ± 0.2 to 18.2 ± 1.7 (Figure 6).

For a further characterization of the biofilm, a fatty acid analysis was conducted. In 10 of 12 samples hexadecanoic acid (trivial name = palmitic acid) and octadecanoic acid (trivial name = stearic acid) could be detected (Table 3). A quantitative analysis of the octadecanoic acid was not possible due to small bands and a bad signal to noise ratio.

Physiology of the biofilm

Oxygen respiration and production

The oxygen respiration didn’t show a significant difference between the first and the second measurement, the third date showed a significantly higher respiration per area (One-way ANOVA, F=51.5, d.f.1=2, d.f.2=8, p<0.001; Tukey test p<0.001). Net production with an overall mean of 38.6 ± 33.4 mg m^2 h^{-1} and gross production with an overall mean of 54 ± 36.3 mg m^2 h^{-1} did not vary significantly between the three sampling dates (Figure 7).

Quantum yield of photosystem II

The first series of measurements of the quantum yield of photosystem II was carried out on July 27th (Figure 8). During this day a notably high solar irradiance with a short drop between 13 and 14 o’clock was recorded. The pattern of the incoming irradiance was reflected by an inverse pattern of the quantum yield (ϕ_{PSII}). Although less strongly developed, this effect was also noticed in the second and third series.

2.5 Discussion

One aim of the study was estimating the biomass growth on the algal turf scrubber. We found a characteristic pattern of three growth phases during a run starting with the colonization phase, which was followed by the exponential phase and after reaching the peak biomass the autogenic sloughing phase began, in which the biomass stagnated or decreased. This pattern is in agreement with other studies (e.g., Biggs, 1996). The colonization phase was especially evident in the first run, because no algae had been grown on the substrate before, and it was also evident in the third run, probably because the substrate had to be recolonized after a complete desiccation.

The exponential phase was shortest in the second run with only two weeks and the doubling time of algae was very fast with about 2.7 ± 0.2 days. The fast growth of the second run could be due to remaining algal cells on the substrate after harvesting, which may have boosted the recolonization of the substrate within a short time. The slower growth rate of the third run is possibly due to the strongly decreasing solar irradiance. Obtained algal growth rates were comparable to a study conducted in a wastewater treatment plant, where doubling times of about 3 days were found (Table 4).

The biomass peaked after three weeks in the second run and after four weeks in the first and third run. Mean peak biomass varied between 221.1 ± 46.8 g m^{-2} dry mass in the second and 251.9 ± 44.4 g m^{-2} dry mass in the first run, with the third run lying in between. The maximum was followed by a period of stagnation or decrease. The biomass removal was also observed by blank eyes, as brownish agglomerations of bacteria and algae were formed, which sloughed off easily.

The amount of grazers on the ATSs stayed very low during the whole sampling period, only a few ciliates and nematodes were observed. Grazing might cause a net decline of phosphorus uptake of the biofilm (Sabater et al., 2002).

Another focus of interest was the biofilm composition. The dry mass consisted of about 74% ash mass, which was probably caused by the high amount of diatom frustules and trapped inorganic sediments in the biofilm. Comparable ash mass proportions were found in the study at the Chesapeake Bay tributaries by Mulbry et al. (2010). The phosphorus content accounted for approximately 0.13% ± 0.05% of the dry mass.

Phosphorus contents in the biomass were sometimes higher in other studies, mostly due to higher nutrient levels in the water supply (Adey et al., 2011). The water supply in the present study contained 0.6 to 1.6 mg l^{-1} total nitrogen (Donabaum et al., 2011a) and 34 to 84 µg l^{-1} total phosphorus, higher loads would probably promote the biomass growth and increase the nutrient content of the biomass (Adey et al., 2011).

Correlations between the parameters were generally very strong. Dry mass and ash-free dry mass were highly correlated. The good correlation of chl-a and ash-free dry mass indicates that the fluctuation of the organic biomass was mainly algae-driven. A closer look at the ratio chl-a to ash-free dry mass showed that the chl-a content was around 0.5% most of the time, which indicates a high contribution of photoautotrophs to the biofilm (various algal groups hold a percentage of 0.5 to 1.5%; (Donabaum, 1992). TP and chl-a also showed a strong correlation, which implies that phosphorus in the biofilm was taken up by photoautotrophs. A study conducted at the Chesapeake Bay revealed that about 65% of the total N and about 50% of the total P in the biomass originated from the algae (Mulbry et al., 2010).

Chlorophyll-a amounts per area were very high compared to natural streams (Dodds, 2006). The peak chl-a was reached in the second run with approximately 0.7 g m^{-2}. Organic carbon to total nitrogen ratios showed some fluctuations during the sampling period. The ratio was always above the Redfield ratio of 5.68 (mass specific). The maximal value was about 3 times higher than the minimum value. Other studies however showed even higher C:N ratios (e.g., Dodds et al., 2004).

To gain more insight into respiration and production of the biofilm, three primary productivity measurements were conducted during the third run. Interestingly, both the net production and the gross production did not differ significantly between the three dates. Unlike the oxygen production, the oxygen respiration was significantly higher at the third date in comparison to the first and second. The higher respiration was probably due to an increased microbial activity in the biofilm at the third date, which could be connected to the priming effect as labile organic matter, for instance the cell content of algae, could enhance the decomposition of recalcitrant organic matter and therefore cause an elevated respiration (Guenet et al., 2010). Compared to other studies the oxygen gross production per area was rather low (e.g., Dodds et al., 1999).

In agreement to other studies (e.g., Kurzbaum et al., 2010), the quantum yield (ϕ_{PSII}) was negatively linked to solar irradiance. In addition to photosynthesis and non-photochemical quenching, the decrease of quantum yield can be caused by photoinhibition (Maxwell & Johnson, 2000). During night, the quantum yield recovered to approximately 0.72, this is indicating that the biofilm was in a healthy condition (Baker, 2008). The dark yield is very close to a study of *Cladophora* sp. with about 0.71 (Hiriart-Baer et al., 2008).

A further goal of the study was to assess the potential of the ATS technology for a further reduction of nutrients in the Heustadelwasser. The MRP had a mean of 13.14 mg m^{-2} d^{-1} for the whole sampling period. Optimal harvesting intervals will enable a removal of approximately 0.9 kg of phosphorus in total from May to September from the surface of an upscaled ATS (478 m^2). The gravel filter removed about 3.3 kg of phosphorus in 2010 and about 2.9 kg in 2011 (Donabaum et al., 2011b, Donabaum et al., 2011a), so the ATS will enhance purification significantly. Additionally, the sediment retention of the ATS prevents clogging of the gravel filter and acts also as a trap for the cyanoprocaryote *Microcystis*, which formerly passed the gravel filter. The proposed harvesting interval from May to September is three weeks, for other months the optimal harvesting period has to be investigated. The combination of the ATS with the gravel filter provides several advantages. First, the area is used for two treatment steps and therefore saves space. Second, the ATS acts as a filter for suspended solids, which would otherwise clog the filters with time and third, the ATS shades the gravel and thus prevents the agglutination of the gravel by algal mats.

The fatty acid content was examined to evaluate the potential for industrial applications of the biofilm. Hexadecanoic acid contents ranged from approximately 0.2% to 0.8% of the ash-free dry mass, other fatty acids were below the detection limit. In the study of Mulbry et al., (2010), comparably low fatty acid contents of 0.35% to 0.65% dry mass were found. In order to exploit the fatty acid content of the biomass, the content would have to be higher to compete with other resources (Mata et al., 2010). Nevertheless other applications are conceivable like biogas production (Lakaniemi et al., 2011) or combustion for heat or electricity generation (Cantrell et al., 2008). Furthermore, the biomass could be used as a soil amendment (Mulbry et al., 2005).

The vertical type of ATS should be considered for further studies, although it was not followed up in this study, because required conditions were not present. According to our pilot studies vertical ATSs need a continuous water supply to avoid desiccation. Experimental setups with different substrates and constructions, secure against wind, would be essential. The major advantage would be the space-saving.

The present pilot study provides a detailed description of the development of the biofilm on the ATS. According to this pilot study, the horizontal ATS technology is ready for upscaling even in this climatic region. It seems that the combination of different treatment steps would result in a significantly higher nutrient and particle removal. The ATS act as a first cleaning step and retain nutrients and inorganic particles; they prevent clogging of the gravel filter, in which the organic load is decomposed by heterotrophs in a second step. Lastly, a phosphorus trap will further reduce the nutrient load. An additional positive side-effect of the ATS is the biofilm harvest, which can be used for other purposes. The results allowed us to suggest optimal harvesting intervals, which is crucial for effective future operations. Further investigations are needed to assess the growth rates and optimal harvesting intervals for spring and autumn. Winter temperatures are often below 0°C in this region; therefore outdoor algal production is not possible during wintertime. To save space, further studies could include vertical ATSs. This type however needs some adaptations, as wind and desiccation problems have to be sorted out.

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2.8 List of tables and figures

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2.9 Tables and figures

Table 1 Spearman rank correlation coefficient of the water supply parameters. The test was done for the whole data set of the sampling period.

	Chlorophyll-a (*n=56*)	Total phosphorus (*n=56*)
Dry mass (*n=56*)	Rs=0.643, p<0.001	Rs=0.815, p<0.001
Chlorophyll-a (*n=56*)		Rs=0.790, p<0.001

Table 2 Spearman rank correlation coefficient of the ATS biomass parameters. The test was done for the whole data set of the sampling period, AFDM = ash-free dry mass.

	AFDM (n=60)	Chlorophyll-a (n=60)	Total phosphorus (n=60)
Dry mass (*n=60*)	Rs=0.930, p<0.001	Rs=0.732, p<0.001	Rs=0.804, p<0.001
AFDM (*n=60*)		Rs=0.861, p<0.001	Rs=0.872, p<0.001
Chlorophyll-a (n=60)			Rs=0.908, p<0.001

Table 3 Fatty acid content of the ash-free dry mass (AFDM) at three different dates.

Date	Hexadecanoic acid in % of ash-free dry mass	Standard deviation
12.7.2011 (*n=4*)	0.290	0.096
9.8.2011 (*n=3*)	0.583	0.246
13.9.2011 (*n=3*)	0.610	0.184

Table 4 Comparison of TP in the water supply, maximal P-removal, doubling time and maximum production of selected studies.

System	Water supply [mg TP l^{-1}]	Maximal P-removal [mg m^{-2} d^{-1}]	Doubling time (chl-a) [d]	Maximum production [g dry mass m^{-2} d^{-1}]	References
Present study - ATS at the Heustadelwasser	Lake water 0.03 – 0.05	19	2.7 – 4.6	9.2 – 11	
Periphyton on tiles in secondary clarifier	Waste water 0.07 – 4.13	157	2.9 – 3.5	4 – 22	(Davis et al., 1990)
ATS at Chesapeake Bay tributaries	Riverine water 0.1 – 0.2	7 – 45		6 – 21	(Mulbry et al., 2010)
ATS at Everglades Agricultural Area	Agricultural run off 0.03 – 0.08	79 – 92		19 – 27	(Adey et al., 1993)

Figure 1 Schematic and photographic illustration of the algal turf scrubber construction.

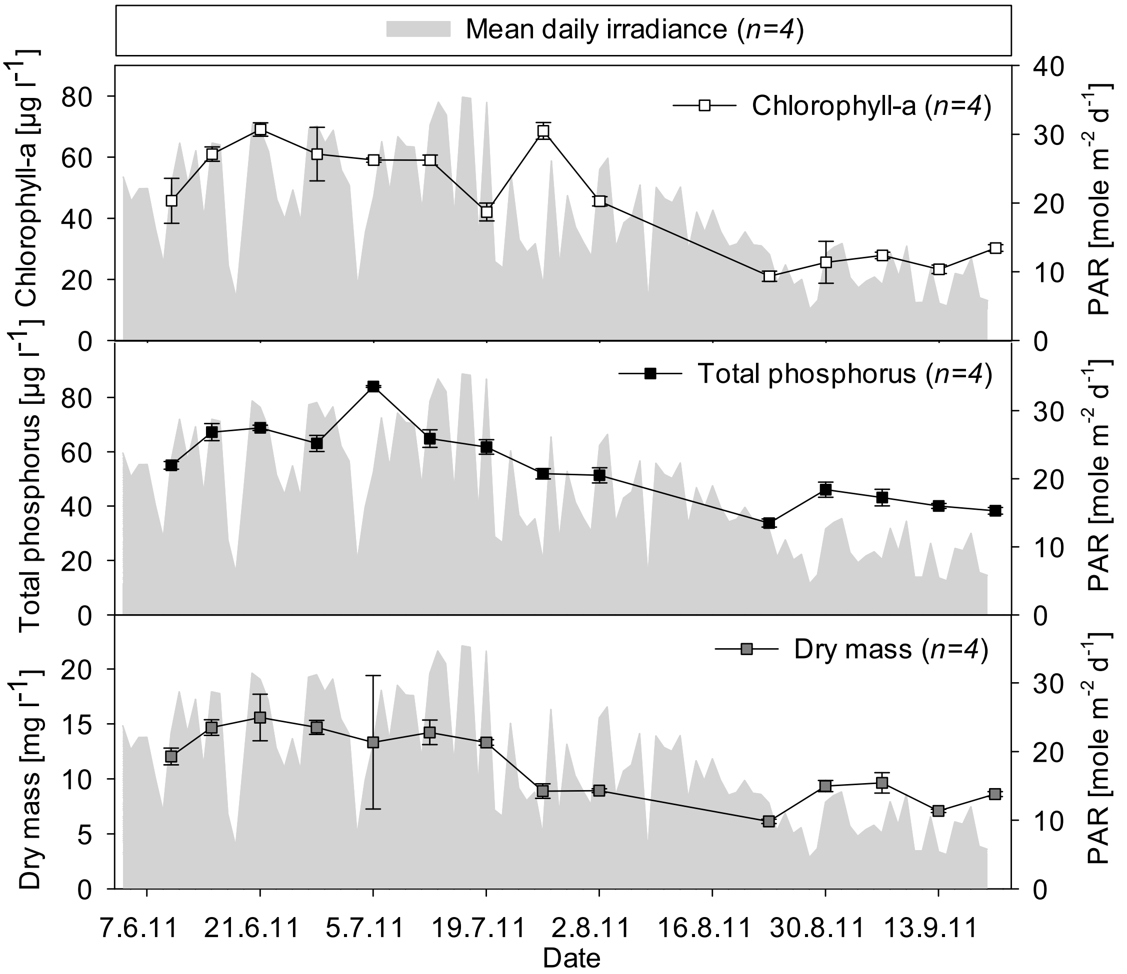

Figure 2 Solar irradiance and analyses of the water supply for the whole sampling period (arithmetic means with standard deviations).

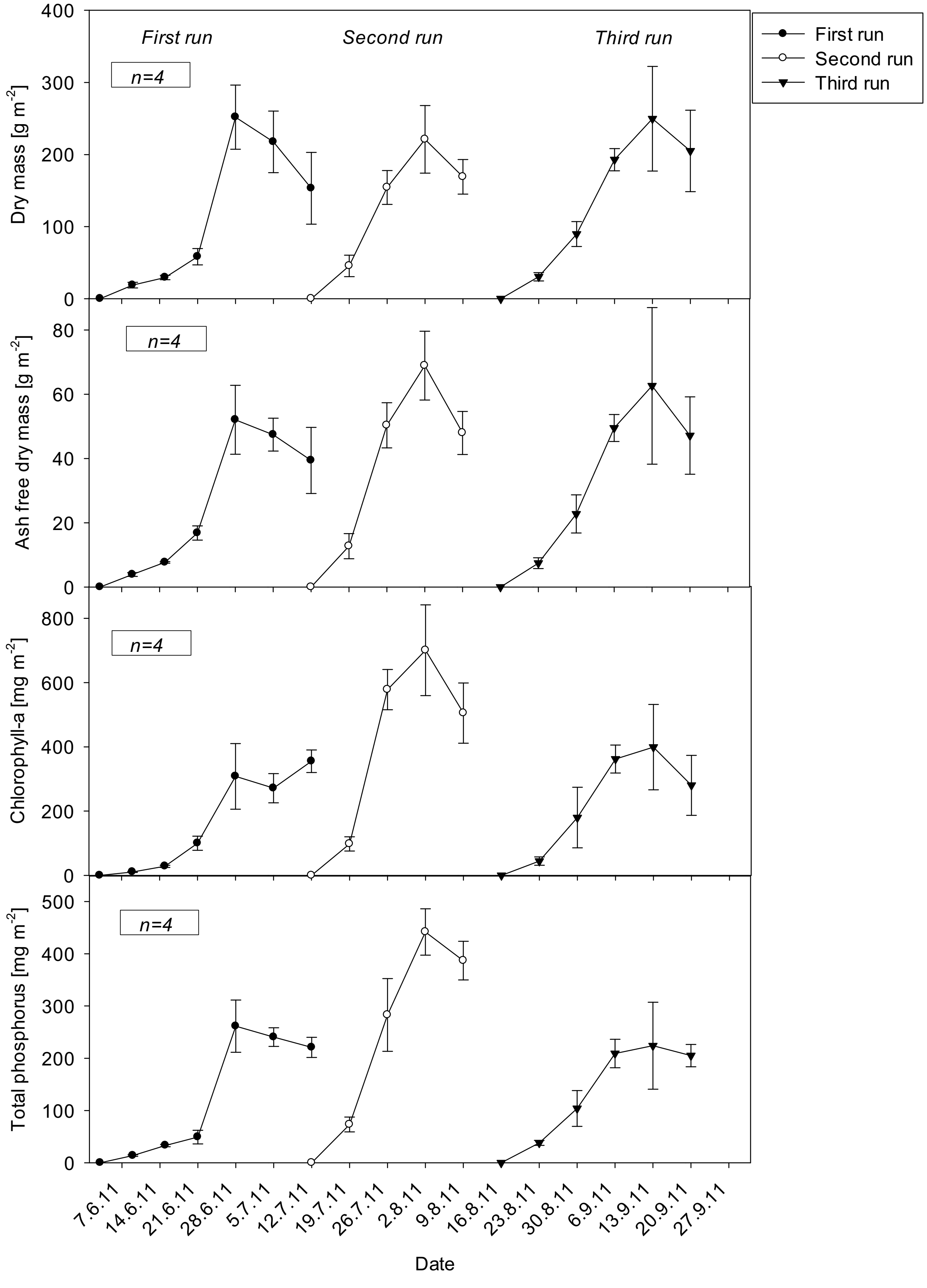

Figure 3 Selected biomass parameters (arithmetic means with standard deviations).

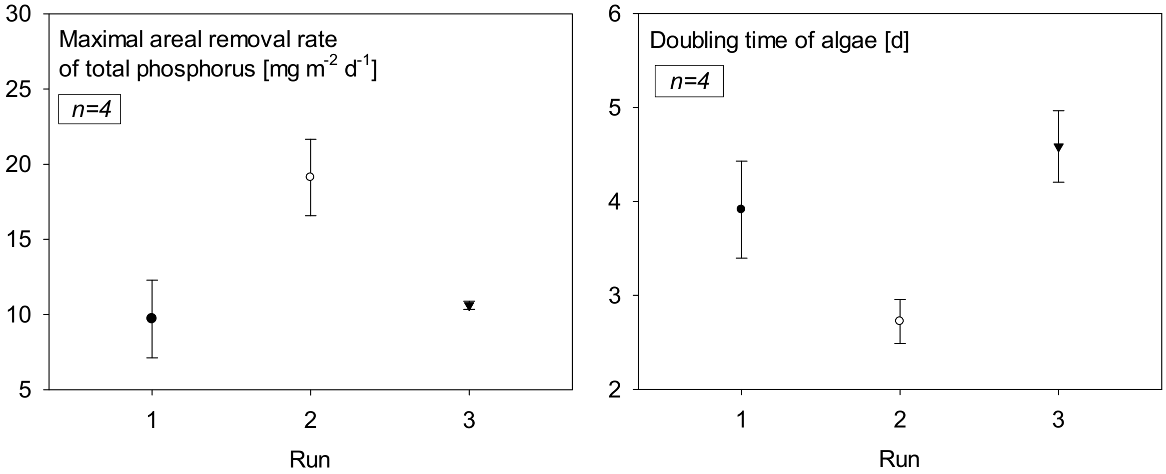

Figure 4 Maximal areal removal rate of total phosphorus and the doubling time of algae based on the chlorophyll-a analysis are shown as arithmetic means with standard deviations for each run.

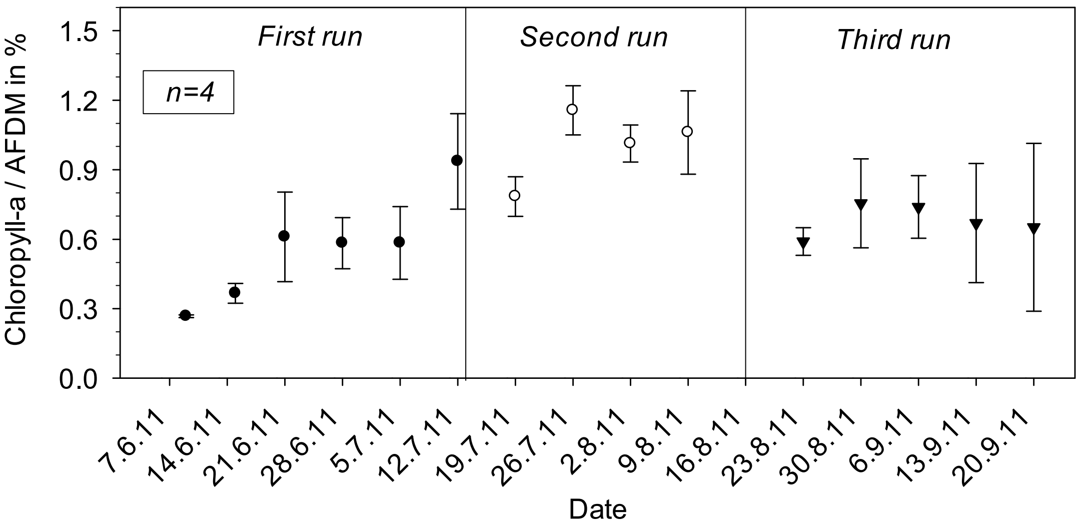

Figure 5 Ratio of chlorophyll-a to ash-free dry mass (AFDM) in % for each sampling date (arithmetic means with standard deviations).

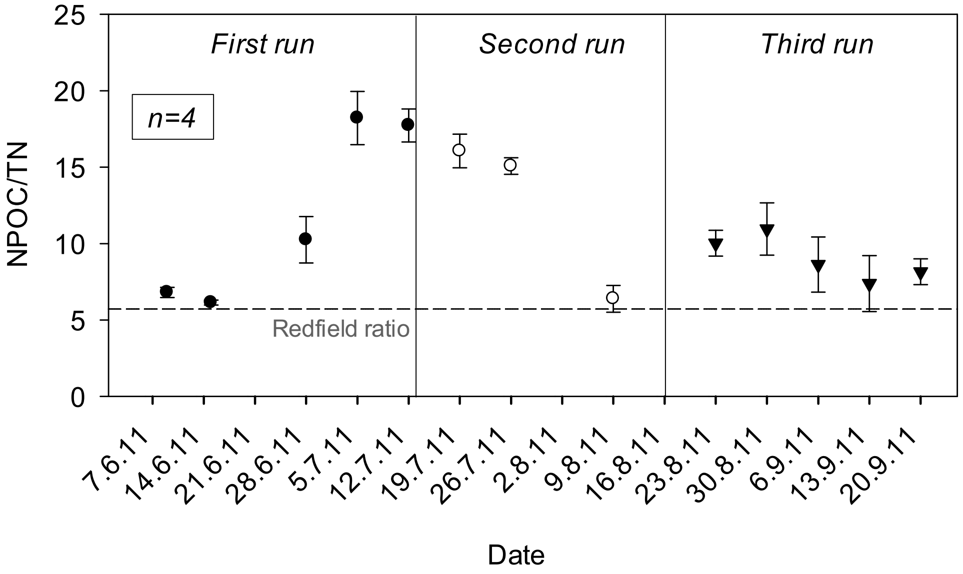

Figure 6 Ratio of organic carbon and total nitrogen for each sampling date (arithmetic means with standard deviations). Dashed line indicates the mass specific Redfield ratio of 5.68.

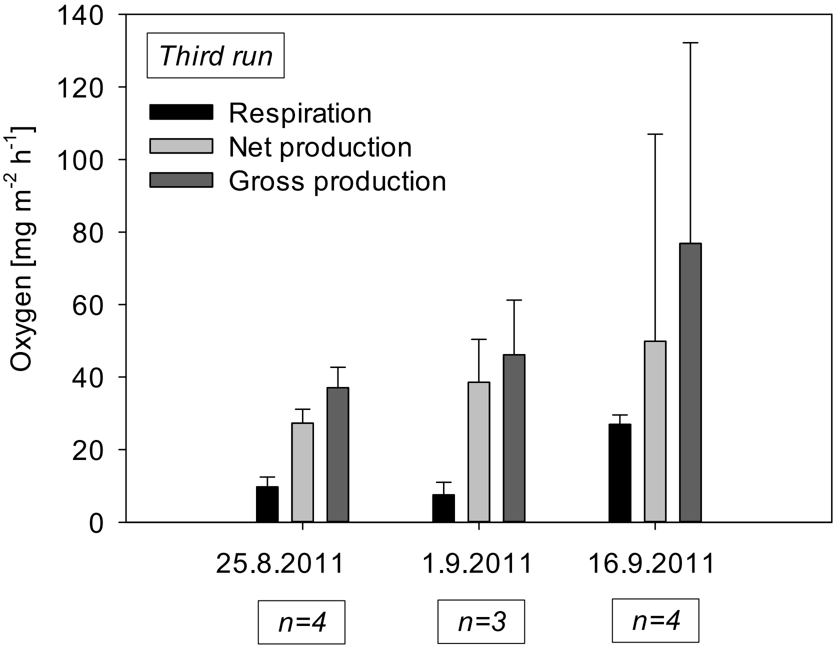

Figure 7 Respiration, net and gross production of oxygen at three dates of the third run (means and standard deviations).

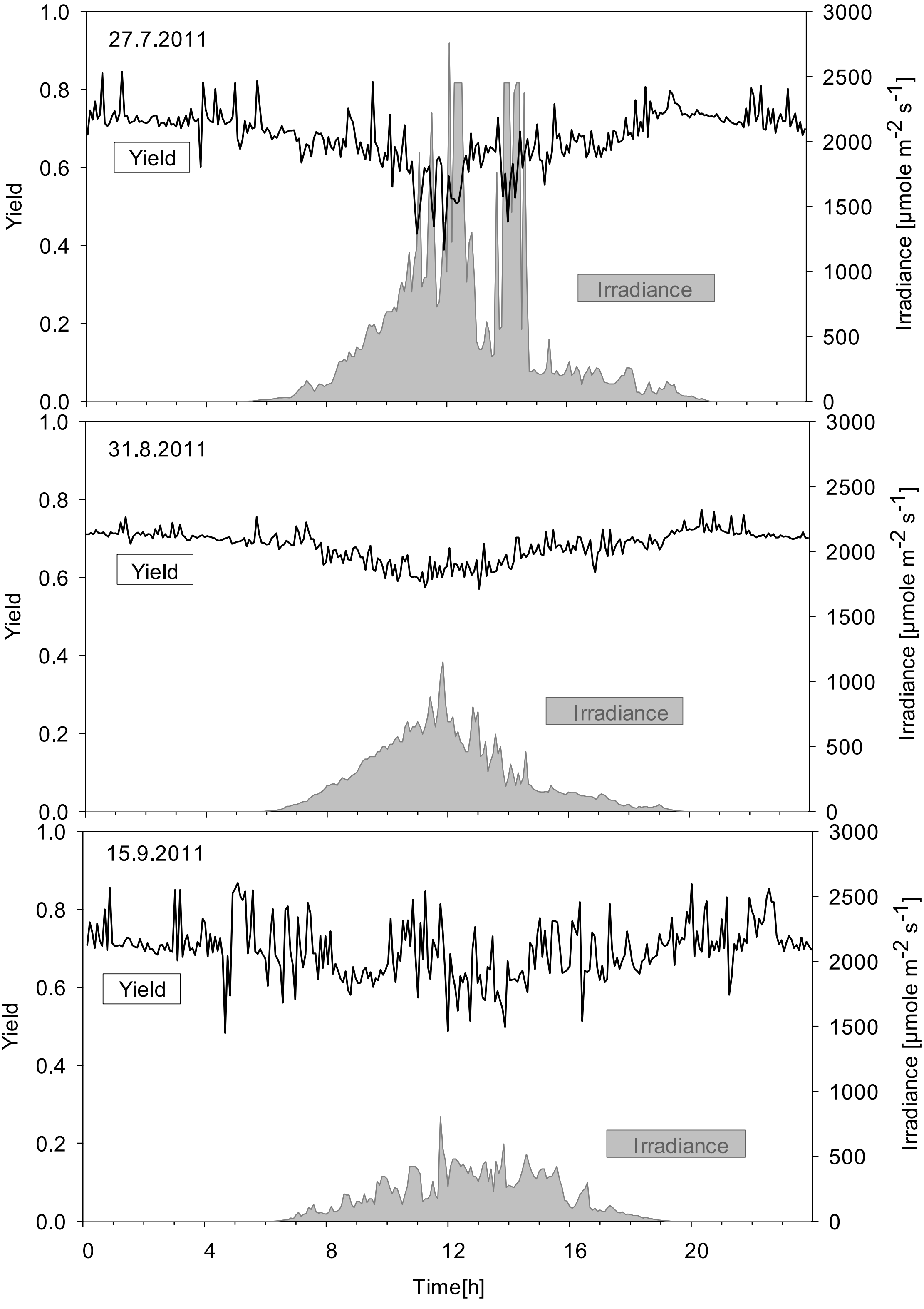

Figure 8 Daily course of the quantum yield of the photosystem II and the solar irradiance at three different dates in 5 minute steps.

3 Zusammenfassung

Die Eutrophierung des Heustadelwassers, ein ehemaliger Altarm der Donau im Prater bei Wien, führte zu schwerwiegenden Algenproblemen. Massenauftreten von Cyanoprokaryoten, welche fakultativ stark toxische Substanzen bilden können, Fischsterben und üble Gerüche waren Ausdruck starker Verschmutzung. Ein Weg, die Situation zu verbessern, besteht darin, die Nährstoffe im Gewässer zu verringern. Dazu wurde 2007 ein Kiesfilter installiert. Wir testeten zusätzlich einen selbst entwickelten Algal Turf Scrubber (ATS), welcher auf dem Kiesfilter platziert wurden. Der ATS besteht aus einer künstlichen Fließrinne, die mit Wasser des Heustadelwassers versorgt wird und in der sich mit der Zeit ein dicker Algenbiofilm entwickelt. Der Algenbiofilm nimmt Nährstoffe auf, welche durch Ernten der Biomasse entfernt werden. Die ATSs kombinieren die Steigerung der Wasserqualität mit Biomasseproduktion; dies ist insofern wünschenswert, als es momentan einen großen Bedarf an Biorohstoffen gibt. Ziel der Studie war es, das Wachstum, die Produktivität und die Zusammensetzung der Algenbiomasse zu beschreiben. Zusätzlich wurde das Potenzial der ATS-Technologie, Nährstoffe aus dem Heustadelwasser zu entfernen, evaluiert und die Biomasse im Hinblick auf Weiterverwertung untersucht.

Drei Experimente wurden im Sommer 2011 durchgeführt. Biomassemessungen und Nährstoffmessungen wurden dabei wöchentlich vorgenommen. Zusätzlich wurden einige Male die Nettoproduktion und Respiration gemessen und der physiologische Zustand der Algen untersucht. Das eingebrachte Wasser enthielt 34 bis 84 µg l^{-1} Totalphosphor und die mittleren täglichen Lufttemperaturen bewegten sich zwischen 11 und 27°C. Je nach Durchlauf wurde die höchste Biomasse nach drei oder vier Wochen erreicht, welche zwischen 221,1 und 251,9 g m^{-2} Trockenmasse lag. Die maximale Phosphorentfernung lag im Mittel bei 13,14 mg m^{-2} d^{-1}.

Interessanterweise bestand die Biomasse zu ungefähr 74% aus Aschgewicht, wofür hauptsächlich Diatomeenschalen und andere anorganischen Partikel verantwortlich waren. ATSs im größeren Maßstab würden am Heustadelwasser ungefähr 0,9 kg Phosphor innerhalb von fünf Sommermonaten entfernen, bei optimalen Ernteintervallen von drei Wochen und bei einer Fläche, die der Größe des Kiesfilters

entspricht (478 m²). Die geerntete Biomasse ist beispielsweise als Bodendünger verwendbar, da sie einen hohen Nährstoffgehalt besitzt. Diese Studie zeigte, dass ATSs auch in unseren Breitengraden durchaus sinnvoll zur Reinigung von belasteten Gewässern verwendet werden könnten.

4 Summary

Intense eutrophication of the Heustadelwasser, a former backwater of the Danube located in the Prater area in Vienna, has led to serious problems. Mass development of cyanoprokaryotes occurred, which may lead to malodor formation and fish kills because of cyanotoxin synthesis. One way to overcome these problems is removing nutrients from the water. For this purpose, a gravel filter was installed in 2007. To increase the efficiency of this system, we tested a specially developed pilot scale algal turf scrubber (ATS) on top of the gravel filter. The ATS is an artificial stream bed supplied with water from the Heustadelwasser, onto which an autotrophic biofilm is developing with time. The algal biofilm takes up nutrients, which are then removed by harvesting the biomass. With the ATS water amelioration is combined with biomass production, which is favorable due to the current need for organic raw materials for various industrial purposes. The study aimed to characterize growth, productivity and composition of the biomass, to investigate the potential of the ATS for nutrient removal at the Heustadelwasser and to examine the biomass with regard to further applications.

Three runs were conducted in summer 2011. Weekly measurements of biomass and nutrients were conducted and furthermore, some gross production measurements were conducted and the physiological condition of the algae was investigated. The water supply contained 34 to 84 µg l^{-1} total phosphorus. The peak biomass was reached after three to four weeks and was between 221.1 and 251.9 g m^{-2} dry mass. The maximal areal removal rate of phosphorus was 13.14 mg m^{-2} d^{-1} on an average.

The removed biofilm consisted to about 74% of ash mass, mainly due to diatom frustules and trapped inorganic sediments in the dry mass. An up-scaled ATS at the

Heustadelwasser with the area of the gravel filter (478 m²) would remove approximately 0.9 kg of phosphorus within five months with optimal harvesting intervals of three weeks during summer. The harvested biomass could, for instance, be used as a soil amendment because of its high nutrient content. According to this pilot study, the algal turf scrubber is ready for upscaling, even in our latitudes.

5 Curriculum vitae

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Staatsangehörigkeit	Österreich
Geburtsdatum	2. August 1987
STUDIUM	
Datum	**2006-2012 Biologie/Ökologie**
Name und Art der Bildungseinrichtung	Diplomstudium der Biologe/Ökologie an der Universität Wien Spezialisierung: Limnologie/Phykologie
SCHULBILDUNG	
Datum	**2002-2006 Oberstufe**
Name und Art der Bildungseinrichtung	ORG Stifterstraße in Linz Schwerpunkt Musik Juni 2006: Matura (mit gutem Erfolg)
PRAXIS	
Datum	**Science Day des Fakultätszentrums für Ökologie (Universität Wien) am 12.3.12**
	Universität Wien
Tätigkeit	Vortrag „Fighting planktic algae with benthic algae: A pilot study at the Heustadelwasser in Vienna”
Datum	**Kongress der Sektion Phykologie der Deutschen Botanischen Gesellschaft in Wuppertal (26. - 29.2.2012)**
	Deutsche Botanische Gesellschaft
Tätigkeit	Vortrag „Fighting planktic algae with benthic algae: A pilot study at the Heustadelwasser in Vienna”
Datum	**Projektmitarbeit März 2011 – Dezember 2011**
	Universität Wien
Tätigkeit	Projektmitarbeiterin bei Algenkult Algenbestimmung und Berichtlegung

Datum	**Tutorin Sommersemester 2012**
	Universität Wien
Tätigkeit	Tutorin beim Projektpraktikum „Algen-eine Einführung in den Mikrokosmos“ bei Prof. Michael Schagerl
Datum	**Tutorin Wintersemester 2011**
	Universität Wien
Tätigkeit	Tutorin bei „Diversität und Systematik der Niederen Pflanzen“ bei Prof. Harald Zechmeister
Datum	**Tutorin Sommersemester 2010**
	Universität Wien
Tätigkeit	Tutorin beim Projektpraktikum „Ökologie von Flussauen" Bei Prof. Peter Peduzzi und Mag. Martin Gruber
Datum	**Praktikum September 2009, 2 Wochen**
	Konrad Lorenz Institut für Vergleichende Verhaltensforschung(KLIVV)
Tätigkeit	Praktikum: Vogelberingung in Illmitz
Datum	**Praktikum Juli 2009**
	Land Oberösterreich
Tätigkeit	Praktikum in der Abteilung Oberflächengewässer Chemische und biologische Probennahme
Datum	**Praktikum August 2008**
	Verein Auring
Tätigkeit	Praktikum Vogelberingung in Hohenau
STIPENDIEN	
Leistungsstipendium	Studienjahr 10/11 (Studienförderungsgesetz) Studienjahr 09/10 (Studienförderungsgesetz) Studienjahr 08/09 (Studienförderungsgesetz) Studienjahr 2010 (Stiftungen und Stipendienfonds der Universität Wien)
FÄHIGKEITEN UND KOMPETENZEN	
MUTTERSPRACHE	Deutsch
FREMDSPRACHE	Englisch
PC-KENNTNISSE	MS Office (Word, Excel, PowerPoint) EndNote, Sigma Plot, R, ArcGIS
PERSÖNLICHE INTERESSEN	Musizieren, Wandern, Vogelbeobachtung
VOLONTARIAT	Vogelzählung für BirdLife Kassier Stellvertreterin im Verein „Chorgemeinschaft Coro Siamo“
FÜHRERSCHEIN	B