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Synergy of wastewater treatment and microalgae cultivation

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Abstract

A synergy of wastewater treatment and microalgae cultivation might be an economic alternative for conventional sewage treatment by removing nutrients such as phosphorus and nitrogen from wastewater, while building up biomass. We focused on an additional treatment stage of wastewater by the use of microalgae, which is an environmentally friendly way to reduce nutrients and costs for wastewater treatment at the same time. Prior to main experiments, screening tests were carried out to decide of suitable wastewater dilutions, the application of unialgal or mixed algae cultures and autoclaved versus non-autoclaved wastewater. It turned out that unialgal cultures in 1:5 dilution and non-autoclaved wastewater performed best. In the main experiments, microalgae (a pure culture of *Chlorella emersonii* and a mixed algae culture for the flumes) were cultivated on three wastewaters originating from a municipal sewage plant, a dairy and a brewery using three different cultivation systems (Erlenmeyer flasks, Bubble Column Reactors, flumes). Dairy wastewater performed best (max Chl-a $104 \pm 33 \mu\text{g cm}^{-2}$ in flumes) in all cultivation systems. Phosphorus reduction rates of 96% for dairy- and 40% for brewery- and 43% for municipal wastewater could be achieved in the flumes. Flumes also proved best in production per irradiated area and therefore present a recommendable cultivation system for practical application.

Keywords: sewage treatment, phosphorus removal, biofilm, bubble column reactor, erlenmeyer flask, flumes

Introduction

Algae have come to the fore as potential source of energy and high-quality compounds (Chisti 2007, Mata et al. 2010, Stephens et al. 2010) because they show several advantages: contrary to land plants, microalgae do not need to invest their biomass into reinforcement structures: they can invest most of their energy into their offspring. Compared to land plants, this strategy results in a 5-fold increased biomass yield (Sastre, 2012). Today one of the most imminent challenges is to find new sustainable energy sources. As microalgae tend to have a higher oil yield (Chisti, 2007; Brennan & Owende, 2010; Guschina & Harwood, 2013) than traditional feedstock, e.g. soybean, sunflower or rapeseed, and require less land area for growth, they are an interesting alternative for biofuel production (Scott et al. 2010; Mata et al. 2010, Singh et al. 2013). Research into other applications such as hydrogen production (Doebbe et al. 2010) is ongoing. Finally, algae cultivation systems are not necessarily dependent on agricultural land – they can also be installed in hostile environments. Moreover, algae offer an enormous potential for high-quality compounds. Many products made from algae are already established, such as food (Nori, Wakame), carrageenans and agar (food additives), *Spirulina (Arthrospira)* as food supplement or beta-carotene from *Dunaliella* (Radmer, 1996; Sastre, 2012). Exploitation of algae grown in wastewater is restricted to certain applications such as fertilizers or biofuel production including biogas; utilization for pharmaceutical products or for food additives is to be excluded because of sanitary issues. Still a synergy of wastewater treatment and microalgae cultivation offers numerous advantages. Conventional growth media for algae mass cultivation are expensive, so wastewater for nutrient supply might be an economic alternative for some applications. Algae take up nutrients from the wastewater in order to build up biomass and are therefore cleaning the wastewater at the same time (Bogan, 1961, Hammouda, 1995; Hwang, 1998; Collos & Vaquer, 2005). Additionally, nutrients are eliminated through precipitating processes (Hartley et al. 1997) and volatilization. Moreover, metal ions are absorbed on the algae surface (Munoz, 2006) and removed together with the algae biomass. Conventional sewage purification is a three step process that involves mechanical, biological and chemical treatments. Phosphorus is usually eliminated by chemical precipitating agents (e.g. FeCl_2 , FeSO_4 , FeClSO_4) and together with nitrogen, difficult and expensive to remove from wastewater (Tschobanoglous & Burton, 1991). An EU-directive (91/271/EWG) claims that at least 75% of the nitrogen and phosphorus charge have to be eliminated from municipal

wastewater (Kommunale Abwasserrichtlinie 2012). Several studies about the combination of microalgae growth and sewage treatment already achieved these numbers with wastewater from various sources (Wei, 2008; Ruiz-Marin et al. 2009; Kothari et al. 2012; Zhu, et al. 2013).

As early as the 1950s, Ludwig (1951) and Oswald (1953) were studying the cultivation of different suspended algae taxa in wastewater. Algae were introduced in stabilization ponds to support bacterial degradation processes by producing oxygen (Oswald et al. 1957). In the 1990s, several studies about algae cultivation and wastewater treatment with attached algae systems were performed (Davis, 1990; Adey, 1995; Adey et al. 1996; Craggs et al. 1996, Hoffmann, 1998). Wastewater was taken from various sources such as municipal wastewater treatment plants, breweries, dairies, swine manure, industrial and agricultural sewage (Pittmann et al. 2011; Boelee et al. 2012; Simate et al. 2011; Pizarro et al. 2002; Kothari et al. 2012; Zhu et al. 2013) and significant nutrient removal rates regarding COD, N and TP could be achieved as well as biomass growth.

For mass cultivation of microalgae, open and closed systems are on the market (Griffiths, 2013, Chisti, 2007). Early systems include High-Rate Algal Ponds (HRAP), which are shallow oxidation ponds with suspended microalgae for wastewater treatment, as described by Oswald et al. (1957, 1988). These open pond systems are less expensive to build and to operate than closed photobioreactors, but they are more susceptible to weather conditions, contaminations and zooplankton grazing. Photobioreactors on the other hand are more flexible with much better control of growth conditions, but there are problems with overheating and biofouling (Chisti, 2007; Scott et al. 2010). Other studies focus on either immobilized algae in a polymeric matrix (Huntley, 1989; de la Noüe, 1990) or on attached algal systems like Algal Turf Scrubbers (ATS), where wastewater runs over inclined flow lanes with attached benthic algae (Adey, 1993; Craggs, 1996). Systems with benthic algae in biofilms make harvesting the accumulating biomass easier by simply scratching the biofilm off the surface. In pond systems or bioreactors, suspended microalgae have to be harvested with advanced techniques like continuous centrifuges, floating or coagulation.

In this study four wastewaters and three cultivation systems were tested for feasible microalgae cultivation. Chl-a was measured to detect biomass growth and Pt- and COD- removal rates were calculated for nutrient reduction in the wastewaters.

Most studies focus on microalgae cultivation in one type of wastewater and one cultivation system; however the goal of this study was not only a comparison of different cultivation systems, but also a comparison of different types of wastewaters available for microalgae cultivation in order to identify a suitable wastewater and an appropriate cultivation technique for microalgae.

Material and Methods

For selection of wastewaters, parameters such as chemical oxygen demand, pH, phosphorus, nitrogen and conductivity were considered. Four wastewaters were finally chosen: from a large municipal wastewater plant (K), a small treatment plant (Z), a brewery (B) and a dairy (M). Prior to main experiments, the wastewaters were screened with microplates in order to identify optimal dilutions (pure, 1:5, 1:50), to use either autoclaved or raw wastewater, and to decide if unialgal or mixed cultures are preferred. Based on screening results, main experiments were carried out with three wastewaters in Erlenmeyer flasks, Bubble Column Reactors with pure algae cultures and in flumes with a mixed algae culture.

Chemical analyses

Ion contents of wastewaters were measured through ion chromatography (DIN EN ISO 10204 for anions and DIN EN ISO 14911 for cations). Chemical oxygen demand (COD) analyses were performed for both the Erlenmeyer flasks and the Bubble Column Reactors. For COD measurements, potassium dichromate is used as an oxidizing agent which gets reduced to Cr^{3+} . With ferrous ammonium sulfate titration, the left over dichromate is measured. Phosphorus fractions were measured according to the European norm EN ISO 6878 (photometric method with ammonium-molybdate). Dissolved phosphorus and orthophosphate were filtered prior to analysis. For dissolved phosphorus and total phosphorus, samples were combusted prior to color reactions (oxidative cracking). Phosphorus removal rates for Erlenmeyer flasks and Bubble Column Reactors were calculated as follows: the amount of particulate phosphorus was calculated by subtraction of dissolved phosphorus from total phosphorus. For theoretical phosphorus removal rate the particulate phosphorus was divided through the total phosphorus. In the flumes total phosphorus was measured in the raw wastewater and at the end of the experiments. The P-reduction was calculated as the difference for both values.

Biological analyses

Chlorophyll-a (Chl-a) is a proxy for algae biomass. A certain volume of the suspension (depending on the culture density) was filtered on filters (BMC Ederol). The filters were then wrapped up in aluminum foil and put into a freezer at -20°C for at least 10 min to assist cell

burst. For pigment extraction, filters were cut into small pieces and homogenized with an ultrasonic mixer (Branson Sonifier 250) in 90% acetone and refrigerated at 5°C overnight. The extract was centrifuged and the supernatant measured in a spectral photometer (Hitachi U-2001) at 663nm (Lorenzen 1967). High performance liquid chromatography (HPLC) was used for quantitative pigment analysis (for preparation of extract see Chl-a; VWR Hitachi LA CHROM Elite®-System, consisting of an L-2130 pump, an L-2200 autosampler, an L-2300 column oven- temperature for analysis 35°C, an L-2455 Diode Array detector and an L-2485 FL- detector, column Supersphere RP-18, with a precolumn LiChrocart RP-18; gradient program according to Wright et al. 1991). Pigments were detected at 440nm and identified through co-chromatography of authentic standards (DHI, Denmark), with comparison of the retention times and spectra in VIS-range (Jeffrey et al. 1997; Roy et al. 2011).

For cell density, optical density (OD) was measured as a proxy at 550nm against wastewater for color/turbidity correction (Hitachi U-2001). For dry mass (DM), dried filters were pre-weighted (BMC Ederol) and a defined volume of suspension filtered. Afterwards, filters were dried for 24h at 95°C and re-weighted. For ash mass (AM), the dried filters were combusted for 2h at 450°C in a muffle furnace and weighed again. Loss of ignition (organic matter or ash free dry mass AFDM= DM-AM). Gravimetric measurements were done at the beginning and the end of the experiments.

In order to compare algal growth, doubling time (DT) per d was calculated from Chl-a: $T_d \text{ d}^{-1} = 0.69 \times (t_2 - t_1) \times (\ln N_2 - \ln N_1)^{-1}$. Where N_1 is Chl-a at time t_1 and N_2 Chl-a at time t_2 ; t_1 and t_2 are day of harvest 1 or 2, respectively.

By means of PAM (Pulse Amplitude Modulated) - fluorescence measurements (Diving PAM, Walz Mess- und Regeltechnik), the actual physiological state of algae was estimated. The dark yield was measured after 10 min dark acclimation: first, minimal fluorescence yield (F_o) is measured in the dark with a weak modulated measuring beam, which is followed by measurement of the maximal fluorescence (F_m) during a short, strong light flash, which fully reduces the electron transport chain of photosystem II (PSII). The fluorescence yield is a relative parameter, which measures the efficiency PSII and is calculated as $\Phi_{PSII} = (F_m - F_o)/F_m$ (Maxwell and Johnson 2000).

A pure culture of *Chlorella emersonii* (ACOI 516) was used for the experiments. Precultures were cultivated in 3NBBM (with 7.5g NaNO₃, 0.25g CaCl₂·2H₂O, 0.75g MgSO₄·7H₂O, 0.75g K₂HPO₄·3H₂O, 1.75g KH₂PO₄, 0.25g NaCl/100ml H₂O_{dest.} (Bischoff, 1963)) with constant irra-

diance at 22°C. For preparing the mixed algae culture, various samples from the area of the large municipal wastewater plant Wieselburg were taken (aeration tanks, discharge system) and cultivated in 3NBBM. The mixed culture consisted mainly of *Coenochloris hindakii*, *Chlorolobion braunii* and *Scenedesmus sp.*. Identification of algae taxa was done with a light microscope (Axio Imager M1, Zeiss company) and by consulting the relevant literature: Ettl (1983); Ettl und Gärtner (1988); Kadłubowska (1984); Mrozińska (1985); Pascher (1930).

Screening series

The four wastewaters were put into 12 microplates (Corning 24 Flat Bottom Transparent Polystyrol) with 6 replicas each. For each wastewater, 3 concentrations (undiluted, 1:5, 1:50 with MilliQ water) were tested, three approaches (unialgal culture + autoclaved wastewater, unialgal culture + raw wastewater, mixed algae culture + raw wastewater) were performed and fluorescence (excitation wavelength 410nm, emission wavelength 670 nm, Gain 100, number of flashes 3 and integration time 40 μ s) and absorption (wavelength 550nm, number of flashes 3) were measured with a multiplatereader (Tecan i-control infinite 200).

Main experiments

For algae cultivation in 250 ml Erlenmeyer flasks, B, K and M were chosen (duration of experiment 21 d, constant irradiance at 75 μ mol photons $m^{-2} s^{-1}$, air temperature 22°C, placed on a shaker). The experiment was carried out with 1: 5 diluted, raw wastewater and *Chlorella emersonii* in 4 replicates (control *Chlorella emersonii* in 3NBBM).

For the experiments in Bubble Column reactors (duration 21 d, constant lighting), three non-autoclaved wastewaters (B, K, M) were used with 1:5 dilution and 3 replicates (control *Chlorella emersonii* in 3NBBM) in three replicates (12 Bubble columns in total). The cultures were aerated with sterile air (constant irradiance at 75 μ mol photons $m^{-2} s^{-1}$, air temperature 22°C).

Because flumes are open systems, the mixed algae culture was used. Duration of the experiment was 23 d with constant irradiance supply at 100 μ mol photons $m^{-2} s^{-1}$ and 22°C air temperature. This approach used recirculating wastewater: from an elevated tank, the wastewater circulates via plexiglas flumes (length 1.30m, width 0.02m) in a collecting tank. A pump (Ocean Runner 3500) in the collecting tank pumps the wastewater back to the headertank (Fig.1). Non glazed clay tiles (length 9.2 ± 0.3 mm, width 18.2 ± 0.3 mm, height

2.5 ± 0.1mm) were used as a substrate for the phototrophic biofilms (Singer et al., 2006). For each wastewater, four flumes were used in parallel. The flow rate was adjusted with the help of clips at the tubes between headertank and flumes to 50mL s⁻¹. Straws (length 10cm) were placed at the upper end of the flumes as flow rectifiers.

Statistics

Statistical analysis for group comparisons (ANOVA) and plots were performed with Sigma Plot 12.0, PAST 2.17 and MS Excel 2010.

Results

Wastewater analysis

Highest total ion contents were found in B with 38.78 meq L⁻¹; Z showed the least amounts with 18.83 meq L⁻¹ (Tab.1). HCO₃⁻ was generally predominating followed by Na⁺, Cl⁻ and Ca²⁺. Z had the largest amount of NH₄⁺ (77.1 mg L⁻¹), for M no NH₄⁺ could be detected. COD values for the pure wastewaters ranged from 468 mg L⁻¹ for K to 4215 mg L⁻¹ for M. Total phosphorus varied from 1.6 mg L⁻¹ (K) to 51.4 mg L⁻¹ (M). The pH-value of the raw wastewaters varied between 7.1 and 10.6 (M). For M, pH had to be adjusted to 7.0 with HCl (37%) prior to the experiments.

Screening

Generally, raw wastewater with 1:5 dilution performed best. The shortest DT was observed for sample Bb1_5 (brewery, unialgal, not autoclaved, 1:5 dilution). Longer DTs were calculated with higher dilutions and with autoclaved wastewater (Fig. 2). The results of the screening led to the exclusion of Z, which resulted in generally long DTs. Based on the screening results, unialgal *Chlorella emersonii* and raw wastewater with 1:5 dilution were selected for main experiments in Bubble Column Reactors and Erlenmeyer flasks. For the experiments with the flumes, a mixed algae culture in raw wastewater with a 1:5 dilution was chosen.

Erlenmeyerflasks

Algal biomass in Erlenmeyer flasks had the highest increase in M; in the second week, maxima of 2.19 ± 0.35 mg L⁻¹ Chl-a were measured (Fig.3). Chl-a values for M at the end of the experiments were significantly different to C, B and K (One-way ANOVA: $F = 18.32$ d.f.₁=3, d.f.₂=12, $p < 0.001$; Tukey test, $p < 0.05$). During the third week, algal biomass already dropped in M and K, for B maximum values of 0.47 ± 0.38 mg L⁻¹ Chl-a were obtained in this period. DM was highest in M at the end of the experiments (783.0 ± 97.7 mg L⁻¹). OD was likewise increasing most in M. A phosphorus removal rate of $53\% \pm 10\%$ for B, $52\% \pm 7\%$ for K and $75\% \pm 12\%$ for M could be calculated for the Erlenmeyer flasks (Tab.2).

Bubble Column Reactors

The highest increase in Chl-a showed the control ($9.0 \pm 2.8 \text{ mg L}^{-1}$) and M ($4.7 \pm 1.8 \text{ mg L}^{-1}$), followed by K ($3.1 \pm 0.5 \text{ mg L}^{-1}$, Fig.4). At the end of the experiments the control varied significantly from B and K, but not from M (One-way ANOVA: $F= 12.24$ d.f.₁=3, d.f.₂=8, $p<0.002$, Tukey test $p<0.05$). There was no significant difference between B, K and M. OD was highest in M, followed by C and K. DM was highest in M at the end of the experiments at $1.42 \pm 0.12 \text{ g L}^{-1}$, followed by K with $0.73 \pm 0.12 \text{ g L}^{-1}$. Phosphorus removal rates from $35\% \pm 19\%$ in K to $41\% \pm 5\%$ in B and $44\% \pm 9\%$ in M were calculated (Tab.2).

The COD measurement showed a reduction in both the Erlenmeyer flasks and Bubble Column Reactor between 63% (Bubble Columns K) and 95% (Bubble Columns B, Tab.3).

Flumes

Chl-a analysis of the biofilms in the flumes showed maximum values in M ($104 \pm 33 \text{ } \mu\text{g cm}^{-2}$) and K ($75 \pm 6 \text{ } \mu\text{g cm}^{-2}$) at the end of the experiment. Chl-a maxima of B were much lower with around $23 \pm 18 \text{ } \mu\text{g cm}^{-2}$ after 6 d and $2 \pm 1 \text{ } \mu\text{g cm}^{-2}$ at end of the experiments (Fig.5). The third sampling date for B (after 6d) was significant different to the other sampling dates (ANOVA on ranks: $H=15.6$, d.f.= 4, $p<0.004$; Tukey test $p<0.05$). Chl-a values for M and K were significantly different to B at the end of the experiments (One-way ANOVA: $F= 20.43$, d.f.₁= 2, d.f.₂= 9, $p<0.001$; Tukey test $p<0.05$). DM was generally highest at the end of the experiments with $34 \pm 10 \text{ mg cm}^{-2}$ for M, $13 \pm 9 \text{ mg cm}^{-2}$ for K and $2.0 \pm 0.3 \text{ mg cm}^{-2}$ for B. AFDM was around 29% of DM in K and 27% in M. B had the highest AFDM proportion of DM with 77% of DM (Fig.6). A Chl-a/AFDM ratio of $0.45\% \pm 0.02\%$ for M, $0.71\% \pm 0.05\%$ for K and $0.1\% \pm 0.03\%$ for B was calculated. The DTs times of algae were longest in K with $1.54 \pm 0.16 \text{ d}$ and shortest in M with $0.21 \pm 0.02 \text{ d}$. Phosphorus removal rates were 40% for B, 43% for K and 96% for M with initial values of total phosphorus in the raw wastewaters of 2.04 mg L^{-1} in B, 1.62 mg L^{-1} in K and 51.35 mg L^{-1} in M, respectively (Fig.7).

Microscopical analysis of the biofilms showed no difference in algae composition over time. The green algae *Coenochloris hindakii*, *Chlorolobion braunii* and *Scenedesmus sp.* were predominant. Towards the end of the experiment, some rotifers were recognized. HPLC analysis detected solely pigments characteristic of green algae (Chl-a, Chl-b, lutein, violaxanthin, antheraxanthin, neoxanthin, luteoxanthin, beta-carotene). Additionally, breakdown products of Chl such as phaeophytine, phaeophorbide and chlorophyllide were detected indicating

grazing activities. The proportion of decomposition products in relation to Chl-a did not change over time.

Discussion

Elevated pH of M was caused by detergents used in the dairy; pH needed to be adjusted prior to the experiments to enable optimal algae growth, which is usually in the range between 6 and 9 (Jerney, 2012). We could not detect any ammonium in the dairy wastewater, which is advantageous because high pH-values combined with ammonium would lead to reduced efficiency in photosynthesis (Gonzales, 2008) or even be responsible for the complete breakdown of the culture due to conversion of NH_4^+ into NH_3 at alkaline conditions. Also increased photosynthetic activity may cause a pH rise (Lampert & Sommer, 1999) especially in weakly buffered systems. For Z, the highest NH_4 amounts (77 mg L^{-1} in pure wastewater) were found, which might be one cause for the low growth rates.

In accordance to other studies (Gonzalez, 2008; Abeliovich & Azov, 1976), the screening tests clearly revealed that non-autoclaved wastewater and a 1:5 dilution are to be favored. In this way, NH_4 concentration and turbidity can be reduced thus facilitating algal growth. In some microplates, algae tended to attach to wells, which was the case especially for Z., which had also the highest degree of turbidity. Turbidity and impaired translucence have a negative effect on algae growth (Kebede-Westhead et al. 2003; Wang et al. 2009).

The Erlenmeyer approach can be seen as small pond system without aeration and only limited turbulence. Except B, the Erlenmeyer experiments showed for all wastewaters a decline in Chl-a towards the end. In contrast to the Bubble Column Reactors, the irradiated area is smaller; moreover no extra aeration was provided in the Erlenmeyer flasks, so the algae suspension were possibly carbon and/or light limited. The overall performance regarding biomass growth was higher in the Bubble Column Reactors than in the Erlenmeyer flasks, but no significant difference in Chl-a values among the different wastewaters could be detected in the Bubble Column Reactor. A production comparison per irradiated area also shows a better performance of the Bubble Column Reactors compared to Erlenmeyer flasks. The composition of the biofilm of M and K indicated that it consisted to around two-thirds of AM, with a Chl-a/AFDM ratio of $0.45\% \pm 0.02\%$ for M and $0.71\% \pm 0.05\%$ for K. This implies that the majority of phosphorus reduction is not directly linked to algae, but to the biofilm matrix, a process that is also reported by Adey et al. (1993). A phosphorus reduction of over 90% in M and about 40% in K and B could be achieved. Several studies about wastewater treatment through immobilized algae and biofilms showed phosphorus reduction rates of more than 90% (Yan, 1996; Ruiz-Marin et al. 2009; Wei, 2008, Kothari et al.

2012). In both K and B, much lower initial phosphorus values (1.62 mg L^{-1} in K; 2.04 mg L^{-1} in B) than in M (51.35 mg L^{-1}) were measured in the raw wastewaters and their biofilms were less developed than in M (DM of K = 13.0 mg cm^{-2} , B = 2.5 mg cm^{-2} , M = 34.3 mg cm^{-2}). The biofilm in B had reached its maximum after 6 d and declined afterwards possibly releasing phosphorus into the water. In Austria, about 80% of nitrogen and about 89% of phosphorus of the effluent are removed from the wastewater (Kommunale Abwasserrichtlinie 2012), a rate that could be exceeded for M in the flume system.

Although B showed less DM increase compared to K and M, it had relatively short DTs, which was in accordance to microplate screenings. This biofilm was less thick, but the proportion of AFDM to DM was highest with around 75% and a Chl-a/AFDM ratio of $0.1\% \pm 0.03\%$, suggesting that the biofilm in B mostly consisted of algae.

A direct comparison of the productivity of the different cultivation systems is difficult to accomplish, as Erlenmeyer flasks and Bubble Column Reactors operated with pure suspended algae cultures and flumes worked with a mixed algae culture in a biofilm. To compare the three culture systems, Chl-a production per irradiated area was calculated for each cultivation system. In comparison with the Erlenmeyer flasks and Bubble Column Reactors, the flumes showed a much better performance regarding biomass growth per area, indicating a better performance of mixed algae cultures opposed to pure algae cultures (Fig.9 flumes: max Chl-a $104 \text{ } \mu\text{g cm}^{-2}$, Erlenmeyer flasks: max. Chl-a $0.9 \pm 0.15 \text{ } \mu\text{g cm}^{-2}$, Bubble Columns Reactor: max. Chl-a $33.6 \pm 12.8 \text{ } \mu\text{g cm}^{-2}$). The productivity of the flumes can possibly also be explained by size of the irradiated area, which was largest in the flumes (Erlenmeyer flasks 35.26 cm^2 , Bubble Columns 141 cm^2 , flumes 260 cm^2).

Complex algae communities which we used in the flumes or ATS are less susceptible and more stable than pure algae cultures (Adey, 1991). Some authors suggest that a combination of different algae strains or algae-bacteria associations leads to better performance regarding nutrient removal (Obgonna et al. 2000; Singh et al. 2011). Although in this study algae-bacteria associations in the biofilm were not investigated, a mix of different algae was used in the flumes. Systems that are working with biofilms also have the advantage that harvesting the biomass is easier and cheaper than harvesting suspended microalgae and they need less energy than the operation of photobioreactors (Boelee et al. 2011), especially if sunlight can be utilized instead of artificial illumination. Depending on location reduced light and temperature during winter at higher latitudes could also be a limiting factor to

open systems. Nonetheless additional wastewater cleaning with microalgae could reduce costs and energy in the wastewater treatment process and therefore open cultivation systems with biofilms like ATS display the most promising prospects in microalgae cultivation in wastewaters.

Many valuable products, such as food supplements or pharmaceuticals cannot be produced from algae grown on wastewater, as these algae would be considered waste by law and therefore are labelled as unsuitable for most applications. Instead, algal biomass could be used as fertilizer or for biofuel or biogas production. Close monitoring of several abiotic factors, such as pH, nitrogen, COD, BOD and heavy metals or light availability and temperature effects would be helpful for further studies. As most studies in this area were done at laboratory scale, upscaling of microalgae cultivation and wastewater treatment will be a challenge for future experiments. In conclusion, the results of the study show that wastewaters, especially dairy wastewater are suitable for microalgae cultivation and those algae are capable of removing nutrients from the wastewater. A cultivation system using a mixed algae culture in biofilms and a dilution of wastewater can be recommended for further experiments. A synergy of microalgae cultivation and wastewater treatment therefore could be a reasonable addition to conventional wastewater treatment systems.

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Table 1 pH and conductivity of the raw wastewaters (K= large municipal wastewater plant Wieselburg, B= brewery Wieselburg, M=dairy, Z= small municipal wastewater plant Zwettl: n.m. = not measured).

	pH	Conductivity [mS cm ⁻¹]	Total Ions [meq L ⁻¹]	COD [mg L ⁻¹]	TP [mg L ⁻¹]
K	7,2	1,4	20.3	468.0	1.6
B	7,1	2,8	38.8	2498.0	2.0
M	10,6	1,4	23.2	4215.0	51.4
Z	7,7	1,4	18.8	n.m.	n.m.

Table 2 Theoretical P-removal in Erlenmeyerflasks and Bubble Column Reactor, mean and standard deviation.

Erlenmeyerflasks	theoretical P-removal % MW	SD
B	52.77	9.67
K	52.39	6.52
M	75.37	12.25

Bubble Column Reactor	theoretical P-removal % MW	SD
B	41.07	5.14
K	34.95	19.11
M	43.54	8.54

Table 3 COD reduction [%] in Bubble Columns and Erlenmeyerflasks compared to COD reduction in wastewater treatment plants in Austria >50EW₆₀ in 2012 (Lagebericht, 2012), ww=wastewater.

wastewater	COD reduction [%]	COD reduction [%]	COD reduction [%]
	Bubble columns	Erlenmeyerflasks	in Austria > 50 EW ₆₀
K	63	75	
B	95	89	
M	88	90	
ww in Austria > 50 EW₆₀			95

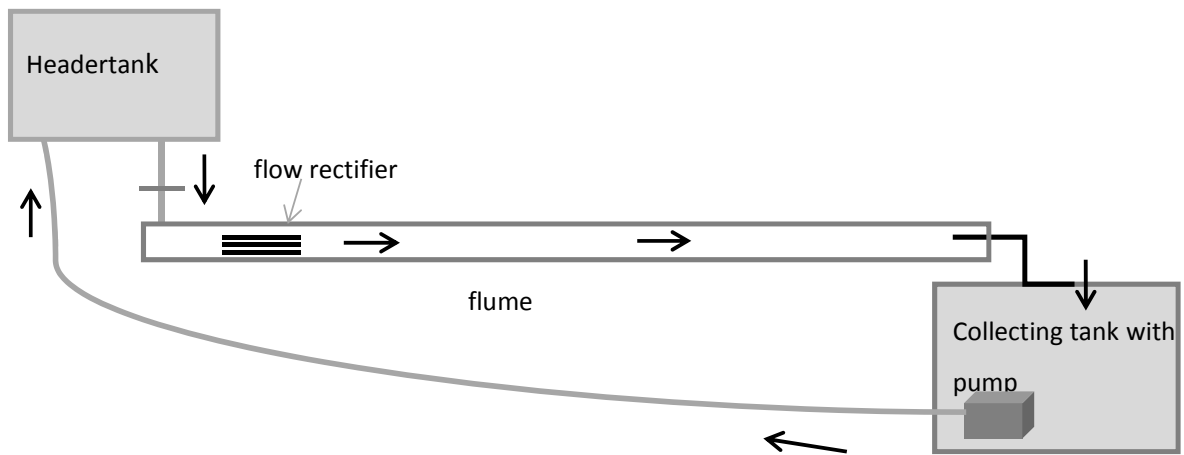
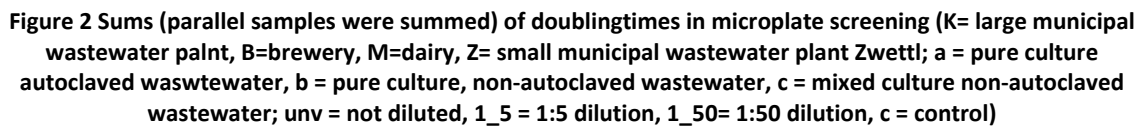


Figure 1 Sketch flume set up, lateral view.



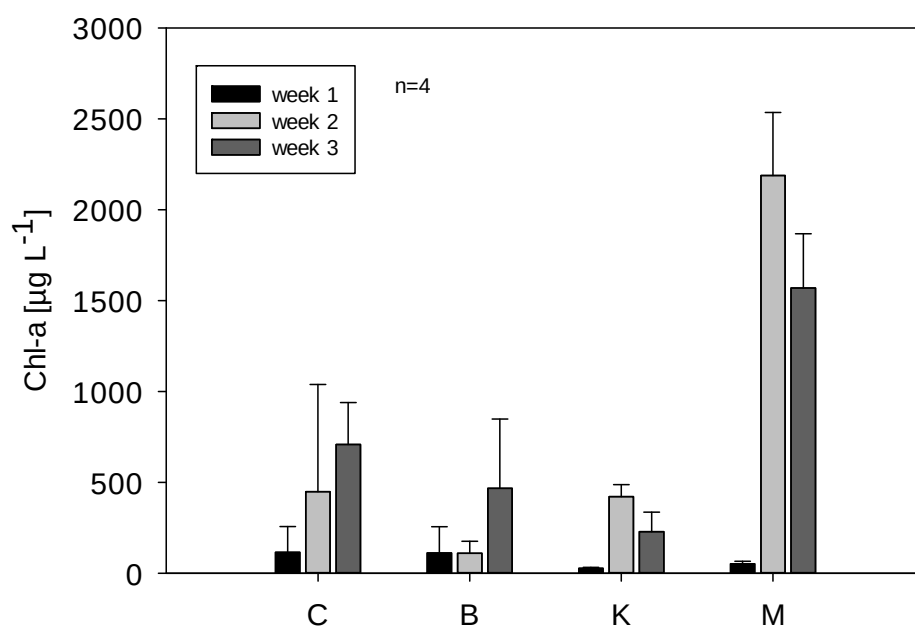


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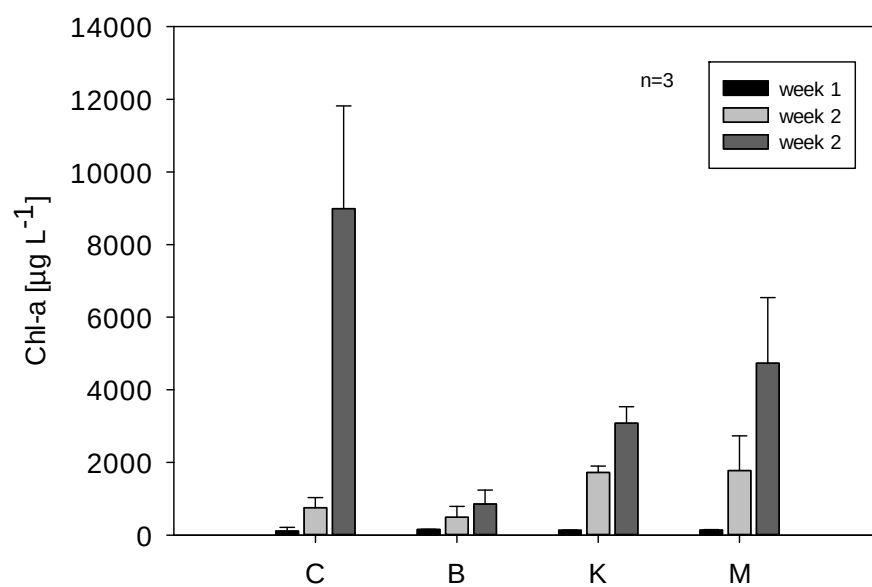


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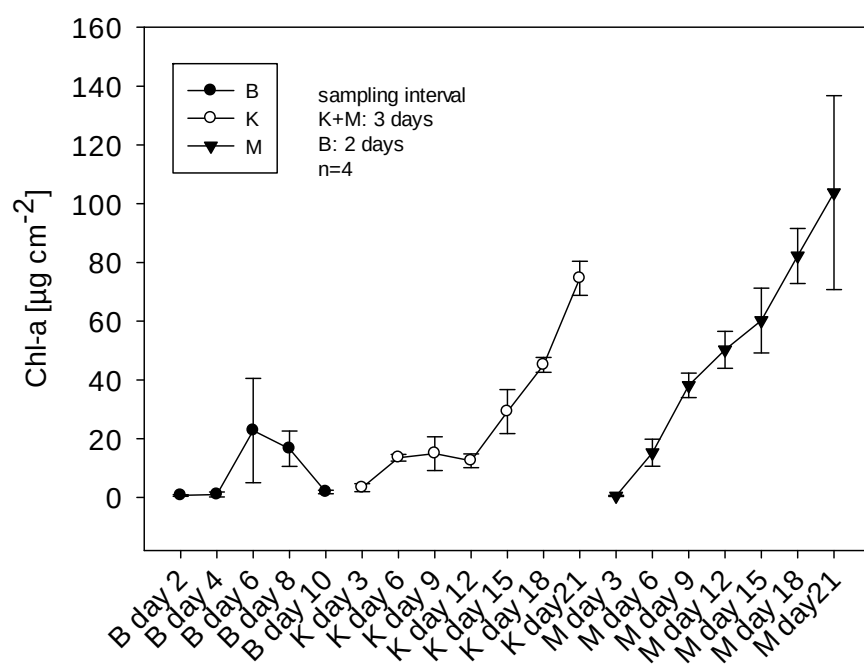


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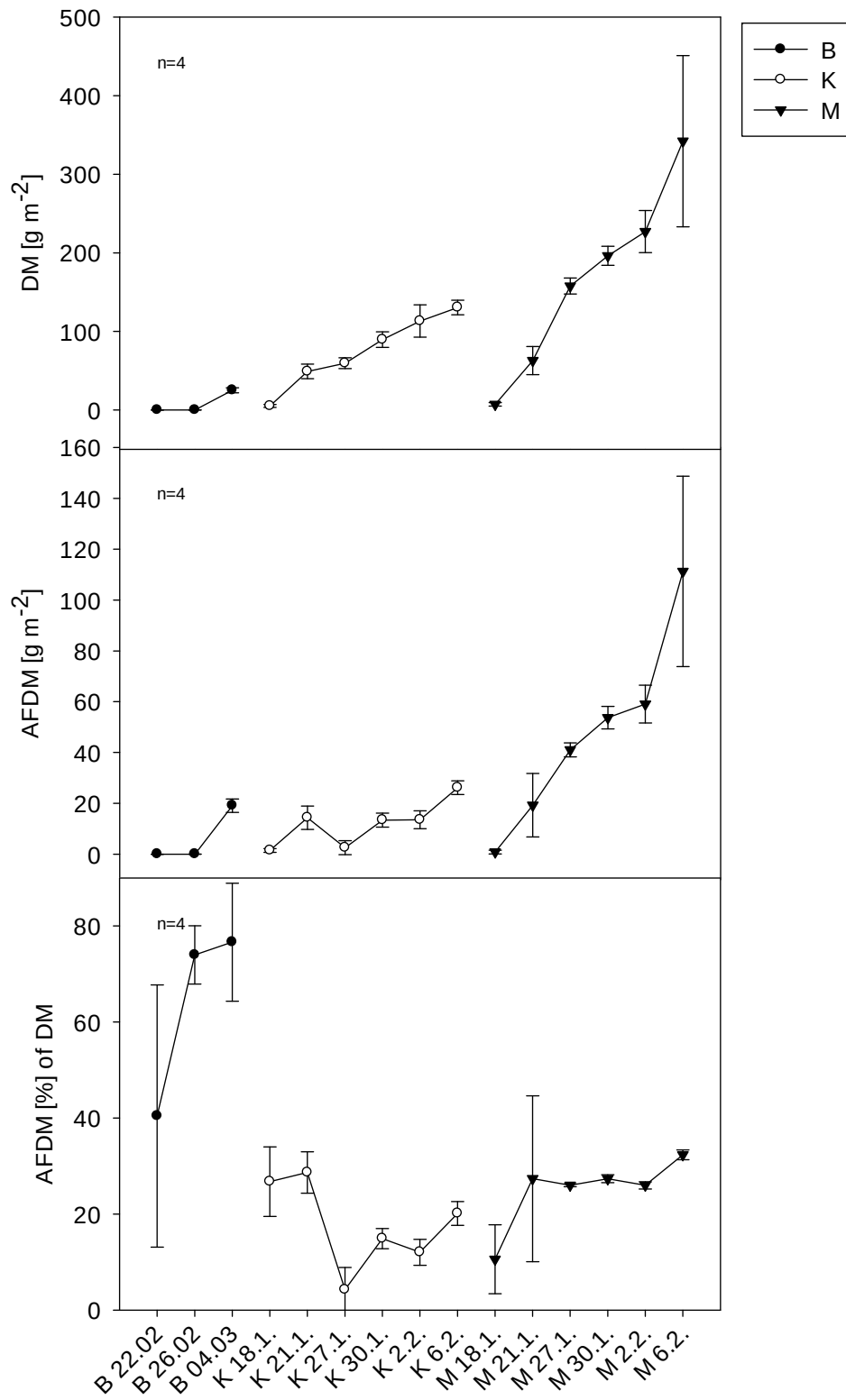


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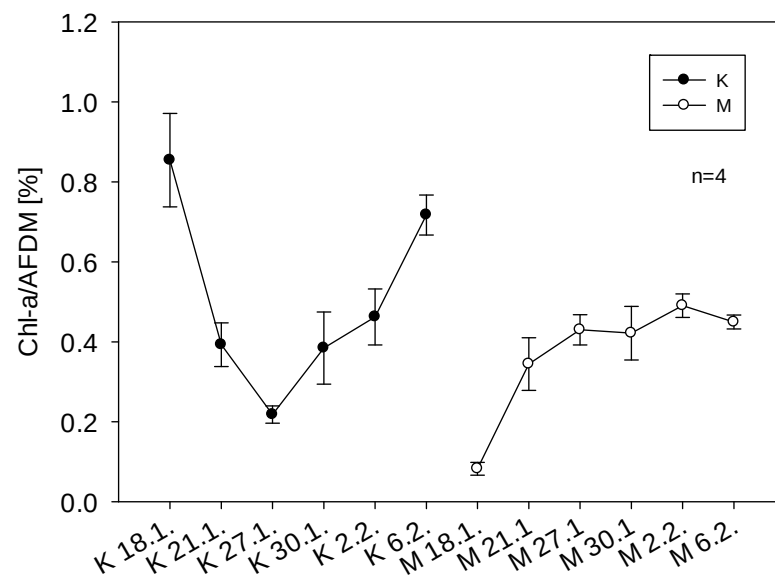


Figure 7 Ratio of Chl-a to AFDM in flumes in % for M and K, mean and standard deviation.

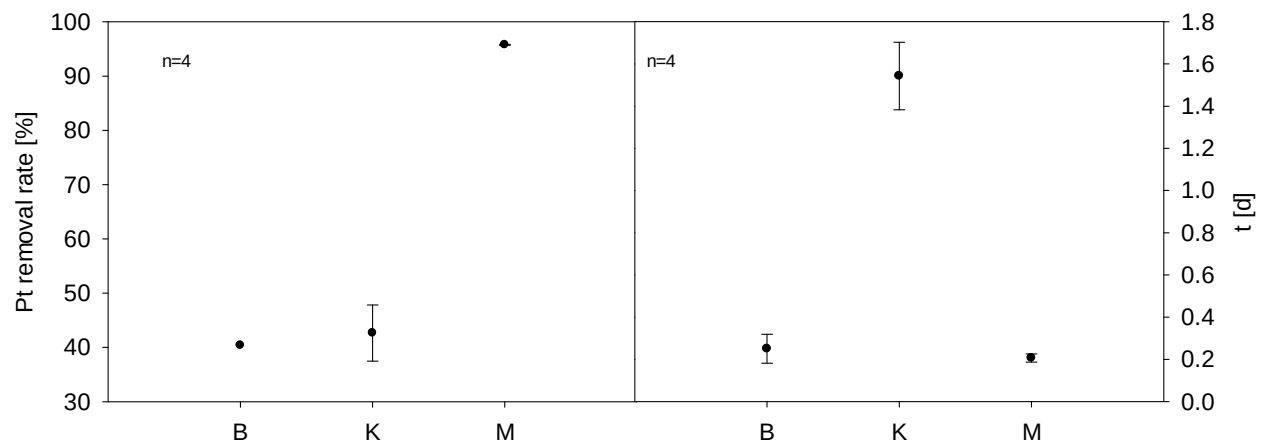


Figure 8 Pt removal rates in the flumes, mean and standard deviation (except B only single value) and Doubling times of algae in flumes, based on Chl-a analysis, mean and standard deviation.

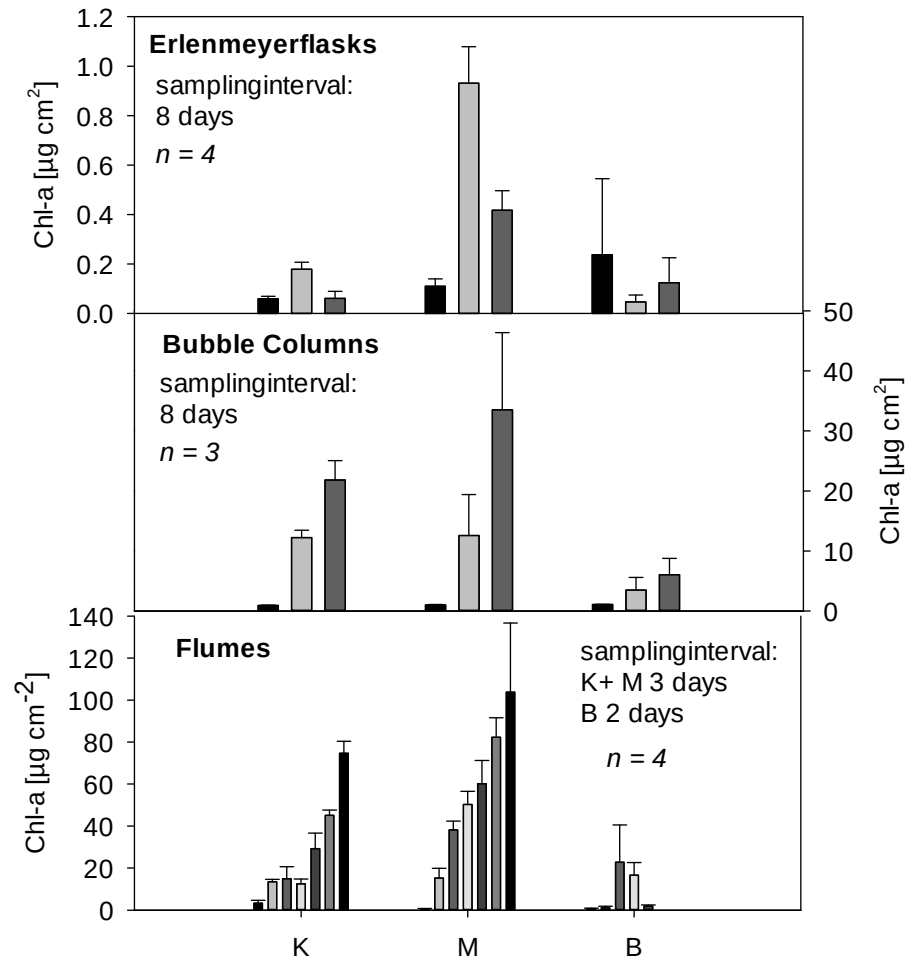


Figure 9 production comparison per irradiated area in Erlenmeyer flasks, Bubble Column Reactor and flumes, mean and standard deviation, note different Y scale.

Zusammenfassung

Bei der herkömmlichen Abwasserreinigung, wie sie zum Beispiel in Kläranlagen erfolgt, werden Nährstoffe und andere Verunreinigungen mit Hilfe von physikalischen, chemischen und biologischen Prozessen entfernt. Besonders die Reduktion von Phosphor und Stickstoff aus Abwässern ist aufwändig und kostspielig. Mikroalgen besitzen die Fähigkeit aus Sonnenlicht, Wasser und Kohlendioxid Energie zu gewinnen und können so Nährstoffe durch Einbau in ihre Zellen aus den Abwasser entfernen. Die dadurch aufgebaute Algebiomasse könnte industriell verwertet werden. Eine zusätzliche Reinigungsstufe des Abwassers mit Mikroalgen wäre deswegen ein umweltfreundlicher Weg, um Kosten und Nährstoffe gleichermaßen zu verringern. Ziel der Studie war ein geeignetes Abwasser zur Mikroalgenkultivierung zu finden. Dabei wurden in einer ersten Versuchsserie Abwässer einem Screening mit Microplates unterzogen um eine geeignete Verdünnung und geeignete Rein- oder Rohkultur zu finden und zu untersuchen ob Abwasser vor der Anwendung autoklaviert oder roh verwendet werden kann. Es zeigte sich, dass mit einer 1:5 Verdünnung die besten Wachstumserfolge erzielt wurden. *Chlorella emersonii* erwies sich als ideale Reinkultur. Für die Experimente in Fließrinnen wurde eine Rohkultur, die aus der Kläranlage Wieselburg entnommen wurde (Sammelprobe aus Klärbecken und Vorfluter) im Labor angezüchtet. Diese Rohkultur bestand hauptsächlich aus den Grünalgen *Coeochloris hindakii*, *Chlorolobion braunii* und *Scenedesmus sp.* Im Screeningtest zeigte sich dass nicht-autoklaviertes Abwasser am besten zur Algenkultivierung geeignet ist. Das Abwasser der Kleinkläranlage Zwettl wurde nach dem Screening für die weiteren Versuche ausgeschlossen. Die Hauptversuche wurden mit drei Abwässern (kommunale Kläranlage Wieselburg K, Brauerei Wieselburg B und Molkerei M) und drei verschiedenen Kultivierungssystemen (Erlenmeyerkolben, Bubble Column Reactor und Fließrinnen) durchgeführt. Für Erlenmeyerkolben und Bubble Column Reactor Columns wurden Reinkulturen von *Chlorella emersonii* verwendet, für Fließrinnen eine Rohkultur. Es wurden sowohl Biomassewachstum als auch Phosphoreliminierung berechnet. Hier zeigte sich dass in den Bubble Column Reactor (max. Chl-a $4.7 \pm 1.8 \text{ mg L}^{-1}$ für M) deutlich höheres Biomassewachstum als in den Erlenmeyerkolben (max. Chl-a $2.19 \pm 0.35 \text{ mg L}^{-1}$ für M) erzielt wurde. In beiden Systemen erzielte die Molkerei das höchste Wachstum. Im Vergleich Produktion pro bestrahlte Fläche der drei Kultivierungssysteme erwiesen sich Fließrinnen als das am produktivste System. In den Fließrinnen befinden sich die Algen nicht in Suspension

sonder stellen Teil eines Biofilms dar. Auch in den Fließrinnen konnte das höchste Biomassewachstum bei der Molkerei ($104 \pm 33 \mu\text{g cm}^{-2}$) beobachtet werden. Es wurde eine Phosphorreduktion von über 90% in M und ca. 40% in K und B erreicht. Die Zusammensetzung des Biofilms zeigte, dass der organische Anteil in K und M unter 30% liegt, was darauf hinweist, dass ein Großteil der Phosphorreduktion nicht über die Algen, sondern über die Biofilmmatrix erfolgt. Es konnte gezeigt werden, dass Abwässer für die Kultivierung von Mikroalgen geeignet sind (besonders Molkereiabwasser und kommunales Abwasser). Fließrinnen erwiesen sich als das produktivste System, so dass eine Synergie von Abwasserreinigung und Mikroalgenkultivierung, besonders mit Fließrinnensystemen und Algenmischkulturen und verdünntem Abwasser, eine sinnvolle Ergänzung zu herkömmlichen Abwasserreinigungssystemen darstellen kann.

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