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ORIGINAL RESEARCH

Open pond cultures of indigenous algae grown on non-arable land in an arid desert using wastewater

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Abstract The algae production on non-arable land in the al-Wusta region of the Sultanate of Oman was evaluated. Pre-cleaned production water (pPW) from the oil production was enriched with nutrients and used as growth medium. The indigenous isolate *Scenedesmus* sp. MKB was grown in open ponds under semi-continuous conditions. A productivity of 16.1 g/m^2/day in 2013 and 15.4 g/m^2/day in 2014 was reached during four different experiments during March and April of 2013 and 2014. Thereby, no influences of light intensity (maximum daily values in between 2400 and 3050 µmol photons/m^2/s) or temperature (maximum daily temperatures were between 29.9 and 41.9 °C) on productivity were observed (correlation coefficient below ±0.5). Weed algae, mainly Cyanobacteria and diatoms were detected microscopically in all cultures by the first to seventh day of growth. The increase in weed algae had a negative influence on the maximum photosynthetic efficiency (*Y*). *Y* decreased after a weed algae concentration of ca. 2 % (cell/cell) was reached.

Keywords *Scenedesmus* · Open pond · Arid environment · Produced water · Weed algae · PAM

Introduction

The possible use of non-arable land (Wagener 1983) would increase the overall land area available for biomass production. A high annual temperature regime, low cloud coverage, and a maximum light exposure, which are typical for semi-arid regions, together with an already existing infrastructure are said to be favorable factors to increase the probability of economical viable microalgal production processes (Grobbelaar 2009). Algae capable of growing in brackish and non-potable water can be used as production strain, and such a choice would result in a reduced water footprint and therefore might result in an increase in potable or agriculturally usable water (Guieysse et al. 2013). However, the use of brackish water might lead to an increase in salt concentration due to evaporation which may cause possible salt stress (Winckelmann et al. 2014).

One of the major issues with the growth of microalgae is the possibility of a sudden and inevitable culture collapse (Hamilton et al. 2014), which can be caused by different means beginning with grazers (Becker 1994; Tillmann 2004), contaminations and pathogens (Gachon et al. 2010), and climatological factors (Grobbelaar

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2010). Another reason for the loss of revenue are weed algae overgrowing the algae to be produced. Weed algae are indigenous algae more suitable for the conditions at the production site (Becker 1994). Depending on the final use of the algae biomass, even small amounts of foreign algae can render an algae culture unsuitable for further processing (Gilroy et al. 2000; Gantar and Svirčev 2008).

The use of indigenous species as production strains is said to reduce the risk of culture loss. Their origin at the same climate as the production site will reduce the risk of culture crash due to climatological and other abiotic factors, and it is assumed that they will be able to outcompete or at least suppress other indigenous algae (Mutanda et al. 2011; Odlare et al. 2011; Rawat et al. 2013).

The aim of this study was to test if indigenous algae grown in industrial waste water can be grown on non-arable land in an arid region.

The crude oil production in the al-Wusta region of the Sultanate Oman results in 800,000 m^3 production water per day (Breuer and Al-Asmi 2010). A fraction of approximately 100,000 m^3 is treated in a reed bed water treatment plant in Nimr. Uses for the resulting hydrocarbon-free brackish water (pPW) to increase revenue are sought for and the location of Nimr being a semi-arid region led to the consideration to establish an algae production facility.

In February 2014, an open pond culture of *Cyanobacterium aponinum* grown in Nimr was invaded by green algae. The algae used for this study, *Scenedesmus* Sp. (SC) strain MKB, was isolated from the mixed culture.

To evaluate the stability of the production process, SC was grown in 2013 and 2014. The productivity and the overall efficiency of photon capture (Grobbelaar 2009) were chosen as an indicator for the efficiency of the production process. The introduction of weed algae might lead to unwanted competition for nutrients and sunlight. It was monitored microscopically and using a multi-channel pulse-amplitude-modulation chlorophyll fluorometer [PAM; (Kolbowski and Schreiber 1995)].

Methods

Analytical methods

Temperature (°C), wind velocity (m/S), and light intensity (W/m^2) were measured by an adjacent weather station (data kindly provided by Bauer Nimr LLc). Light (µmol photons/m^2/s) was measured using a Mini Quantum Sensor MQS-B (Heinz Walz GmbH, Germany). A conversion factor from W/m^2 to µmol photons/m^2/s of 2.9 was calculated. The mass transfer coefficient for oxygen [$k_La(O_2)$] of the open ponds was determined using the dynamic method (Doran 1995). The $k_La(O_2)$ can be used to quantify the oxygen transfer from the liquid phase to the surrounding air. It is also used to calculate the $k_La(CO_2)$ using the equations from Talbot et al. (1990). Flow velocity inside an open pond was measured using a current meter (C2 with a 30 mm blade (gradient 0.1) and counter Z30 OTT Hydromet GmbH, Germany).

The biomass was measured by means of optical density at 660 nm (Lovibond MaxiDirect photometer, Tintometer GmbH, Germany). Culture composition was determined microscopically (using a Zeiss Axiostar Plus light microscope, Carl Zeiss GmbH, Germany) and a Thoma Neu chamber with a depth of 0.1 mm (Paul Marienfeld GmbH & Co. KG, Germany). For the determination of bio dry weight (dw), part of a culture was washed and filtered. The filter was dried at 105 °C until the weight was constant. Increase of salinity was measured by means of conductivity (Water quality meter U-52, Horiba Ltd., Japan). The photosynthetic yield (*Y*) and all photosynthetic parameters were measured using a phyto-PAM with phyto-EDF (Heinz Walz GmbH, Germany) as described previously (Winckelmann et al. 2014). Rapid light curves (Ralph and Gademann 2005), the illumination of a sample with increasing light intensities, were measured to estimate the photosynthetic performance. Thereby, light intensities from 0 to 197 µmol photons/m^2/s were used in nine steps lasting 1 min each.

Isolation and characterization

Scenedesmus Sp. MKB was isolated from *Cyanobacterium aponinum* cultures and characterized as described earlier (Winckelmann et al. 2014). It was identified as described earlier (Winckelmann et al. 2014) using

primer pair ITS4 and ITS5 (Connell 2000) (Results: GenBank KM873329) and EukA and EukB without GC-clamp (Medlin et al. 1988) (results: GenBank KM873328).

Biomass growth for inoculation

The initial inoculum was cultured in Wuxal-media (Winckelmann et al. 2014) until a biomass of above 0.5 g dry weight/L was reached. The cultures were transferred to 1.5 L bottle reactors which were situated outside; the place selected was in full sunlight until midday and covered in shade afterward. The cultures were aerated with a rate of 30 L/h. For the first 4 days of outdoor cultivation, unsterilized bottled water with 0.05 % (v/v) Wuxal (Wuxal® Universaldünger liquid plant fertilizer, Wilhelm Haug GmbH & Co. KG, Germany) was used as growth media. The cultures were divided each time so the biomass reached a dry weight of above 0.5 g dry weight/L and diluted with unfiltered (2013) or filtered (2014) pPW. The experiment performed in April 2014 was inoculated with sedimented culture from the experiment conducted in March 2013.

Production site and composition of produced water

Algae ponds were constructed on site of the Bauer Nimr water treatment plant(Abed et al. 2014). The growth media used was made up of pPW (440–550 mg/L SO_4, N–NH_3 0.3–0.6 mg/L, N–NO_3 0.2–1.0 mg/L, P–PO_4 <0.2 mg/L, salinity 7–12 ppt; analysis kindly provided by Bauer Nimr LLc; values measured before the addition of fertilizer) and 0.5 % (v/v) Wuxal.

Pond construction

Open ponds (4 m long, 80 cm wide, and 38 cm deep) were constructed on a leveled area. Building bricks (39 cm long, 19 cm wide, and 19 cm deep) were used for the outer walls and 2 mm thick high density polyethylene (HDPE) sheets were used as liner. A centrifugal pump (2 in. diameter; Pedrollo, HFm 5AM) was used for mixing.

Biomass production in open ponds

Growth experiments were conducted with a volume of 600 L. The growth medium used was pPW, which was left standing for at least 2 days in a one cubic meter container, only the top 70 % was used as feed water and 0.05 % (v/v) Wuxal was added directly into the pond. The ponds were filled to a depth of 20 cm and inoculated with 18 L inoculum (biomass content of at least 1 g dry weight/L). The cultures were mixed 24 h per day if not stated otherwise.

Harvesting

Part of the culture was exchanged on a regular basis. Therefore, the culture was removed until the depth was 10 cm. Fresh pPW containing 0.05 % v/v fertilizer was added until a depth of 20 cm was reached, and on days without removal the evaporated water was replaced with fresh pPW.

Results

Environmental conditions

The light intensity during all experiments reached medium values over the course of the sunlight hours of a day, which were in between 975 and 1740 µmol photons/m^2/s and maximum values in between 2400 and 3050 µmol photons/m^2/s. The duration of sunlight was between 12 and 14 h per day. The maximum temperature was between 29.9 and 41.9 °C (Fig. 1). The monthly mean temperatures did not differ significantly between 2013 and 2014. The monthly mean and maximum light intensities were 770 and 3030 in March 2013 and 810 and 3010 in April 2013. These values were higher than the recorded values of 2014 which were 730

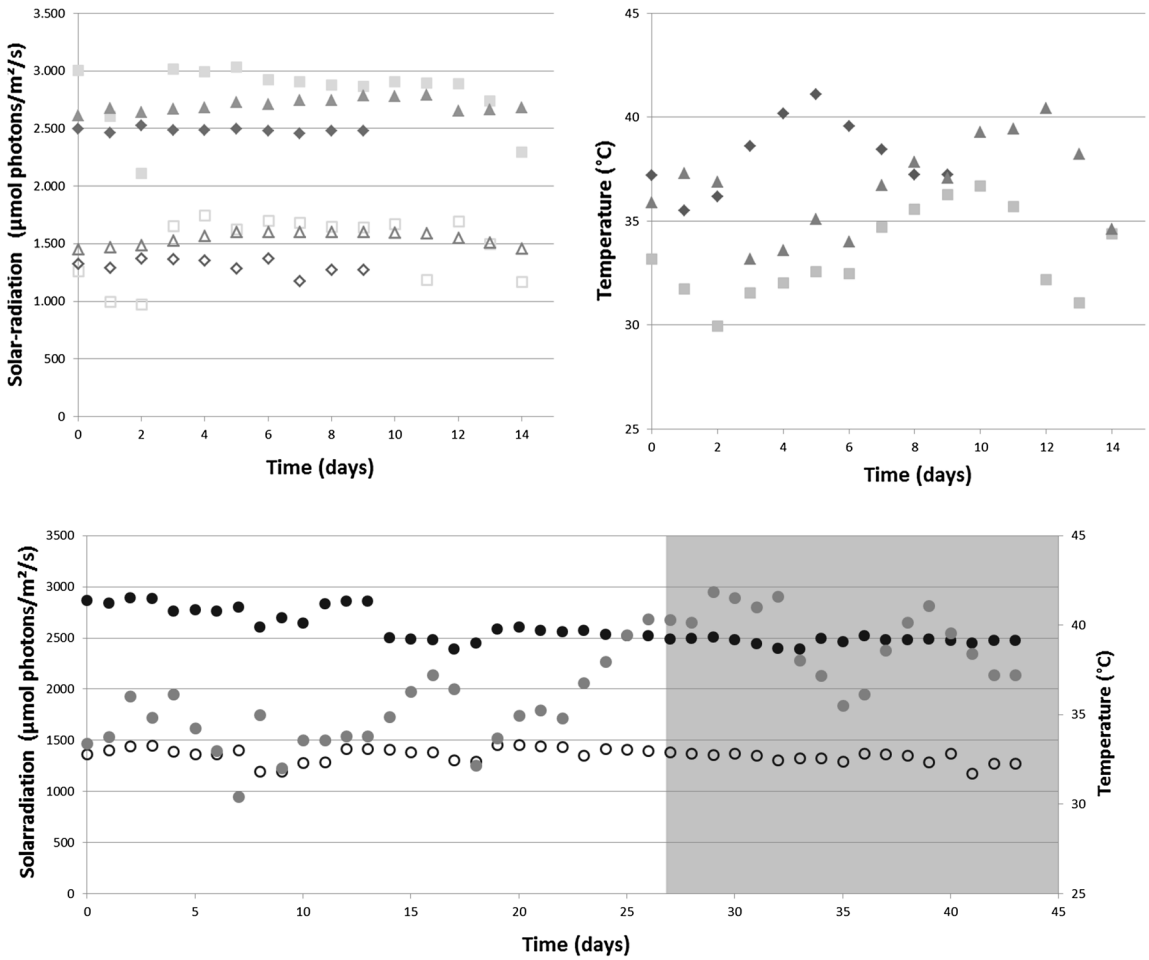

Fig. 1 Progression of light intensity and temperature. *Top row* shows three experiments (*square* March 2013; *triangle* April 2013; *diamond* April 2014) with durations of 14 days or less; *left* light intensity (*solid symbols* maximum intensity, *open symbols* medium intensity); *right* maximum temperature. *Lower row* shows the maximum light intensity (*black solid circle*), medium light intensity (*black open circle*), and maximum temperature (*gray-closed circle*) of a growth experiment lasting 43 days (March–April 2014). The area shaded in *gray* indicates a decreased water circulation

and 2890 in March and 710 and 2430 in April (all measurements in µmol photons/m^2/s). During both periods, days with dust or cloud coverage were observed (9 days in 2013 and 5 days in 2014). Heavy winds and dust led to the termination of the experiment in March 2013.

Open pond characteristics

To be able to assess CO_2 supply, oxygen degassing, and biomass sedimentation, the open pond characteristics were determined. The ponds showed a $k_La(O_2)$ value of 0.04/s (SD ±0.01), a $k_La(CO_2)$ of 0.033/s, and current velocities 5 cm above the ground were between 0.2 and 2.3 m/s (Fig. 2).

Culture composition

The cultures were examined microscopically to observe the influence of weed algae or grazers on the culture's stability. The culture's health was examined using the chlorophyll fluorescence as the parameter. We found that all cultures were mixed cultures and bacteria were found. During all experiments, three different groups of photosynthetic active organisms were found (Fig. 3) in different abundance (Fig. 4). In single cases during pump outage, the appearance of small dark brown flakes, with small air bubbles attached to it, on the surface

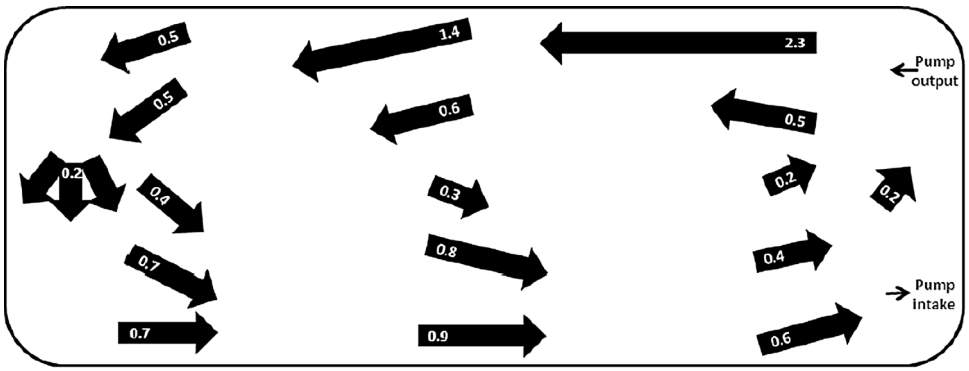

Fig. 2 Flow velocities and directions in *open* pond. *Unit* m/s. Measurement depth was 5 cm above the *bottom*. *Pond length* 4 m, *pond width* 0.8 m, *water level* 20 cm. Figure is not up to *scale*

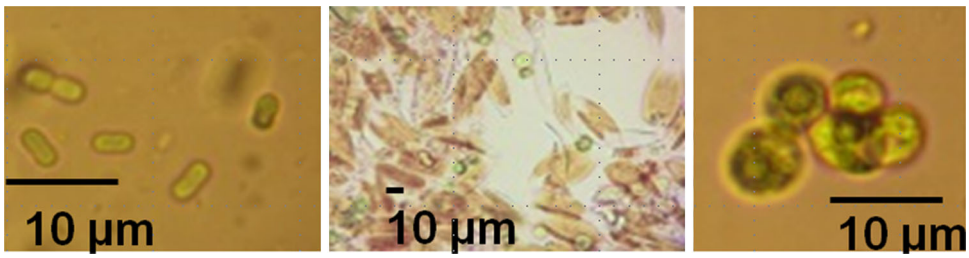

Fig. 3 Photosynthetically active organisms found in all growth experiments; from *left* to *right*: single-celled cyanobacteria, most likely *Cyanobacterium aponinum*; Diatoms; *Scenedesmus* spec

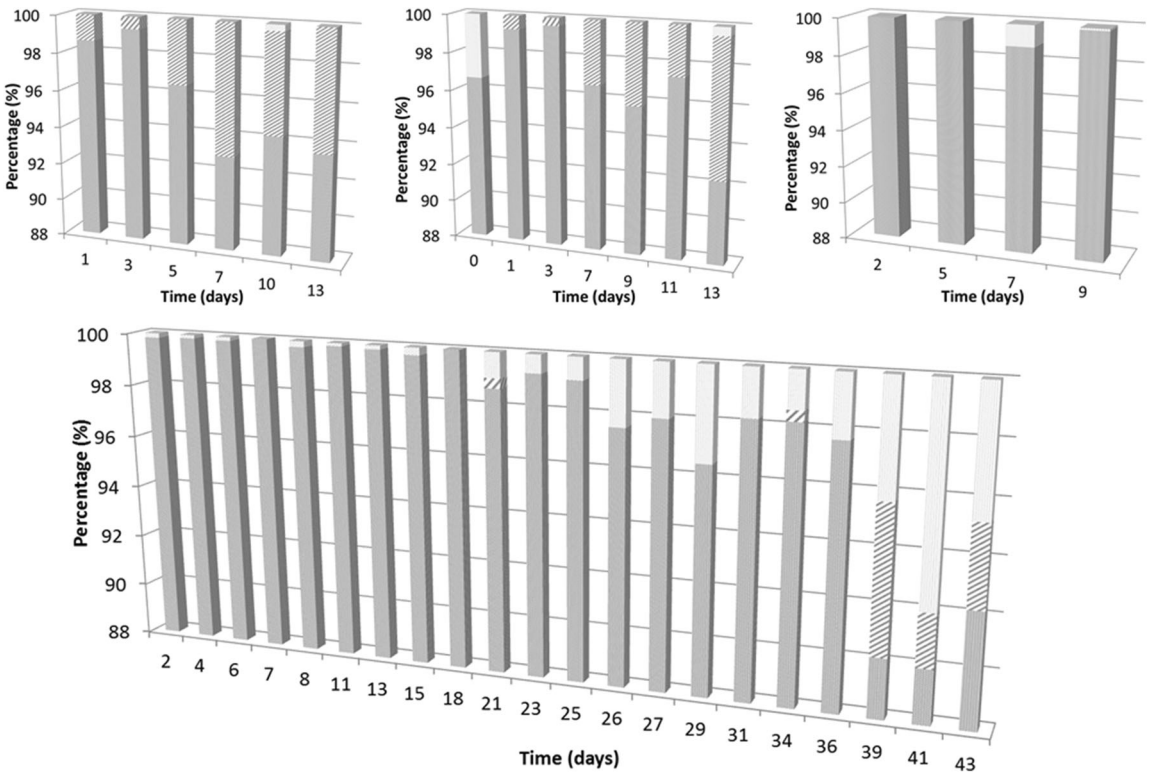

Fig. 4 Distribution of different photosynthetic active groups over time. First *row left* experiment March 2013; *middle* experiment April 2013; *right* experiment April 2014; *lower row* March–April 2014. *Graphs* show the microscopically determined share of *Scenedesmus* (*gray*, *bottom*), Diatoms (*stripes*, *middle*), and cyanobacteria (*white*, *top*)

of the culture was observed. The flakes and swabs from biofilms formed in pond regions with insufficient water movement were microscopically observed and showed a mixture of organisms and inorganic particles dominated by diatoms (Fig. 5). The amount of biofilm formed was not evaluated.

The maximum photosynthetic yield of all cultures increased above 0.7 during the first 2 days of cultivation, and it decreased right after a harvest was performed but recovered within hours. The non-photochemical quenching (NPQ) increased in the beginning above two. The overall photosynthetic characteristics in the morning, before dilution, did not differ significantly in between experiments (Table 1).

The experiment conducted in March 2013 showed signs of contamination from the early beginning. It was contaminated with diatoms and the contamination concentration breached the two percent threshold on day 5 and on the same day decreased *Y* below 0.7 and the maximum NPQ reached only 1.5 (Fig. 6). After the restart of the experiment in April 2014, the culture showed a high contamination with cyanobacteria on the day of inoculation; however, over the course of the cultivation nearly no cyanobacteria were detected until day 13. Although diatoms were detected on day 1 and after day 5, *Y* decreased below 0.7 and the maximum NPQ did only reach a maximum of around 1.5 after day 7.

The experiment conducted in April 2014 showed no sign of contamination in the beginning but a small amount of cyanobacteria was observed on day 7 and decreased until day 9. No change in *Y* or NPQ was observed.

The experiments conducted in March and April 2014 were contaminated with cyanobacteria and diatoms from the early beginning. While the diatoms were nearly undetectable using a microscope, the concentration of cyanobacteria breached the two percent threshold on day 26. The pump failed and had to be replaced with a smaller one, which only had 20 percent of performance rate on day 27 (part in Figs. 1, 3, 6, 7 lower row, which is underlayed gray). On day 36, the *Y* dropped permanently under 0.7 and the diatom concentration became detectable on day 39. The maximum NPQ was between 1.5 and 2 for most part of this experiment but dropped to values below 1 after day 36.

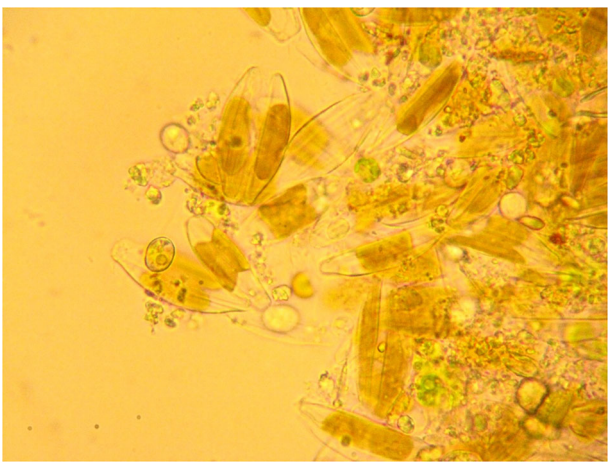

Fig. 5 Exemplary picture of community found in biofilm developed in regions with insufficient culture movement

Table 1 Overall photosynthetic performance as determined using photosynthesis-irradiance curves

	March 2013	April 2013	April 2014	March–April 2013—till day 27	March–April 2013—after day 27
Alpha (SD)	0.27 (0.02)	0.26 (0.02)	0.32 (0.07)	0.30 (0.01)	0.28 (0.03)
I_K (SD)	14 (3)	12 (5)	16 (2)	14 (2.6)	17 (5)
P_{max} (SD)	51 (12)	47 (18)	52 (13)	45 (10)	61 (19)

All data were measured in the morning between 9 and 10 a.m. before dilution of the culture. Data are not normalized against biomass

Alpha maximum light use coefficient for photosystem II, I_K transition point between light limited and light saturated photosynthesis, P_{max} maximum photosynthetic rates

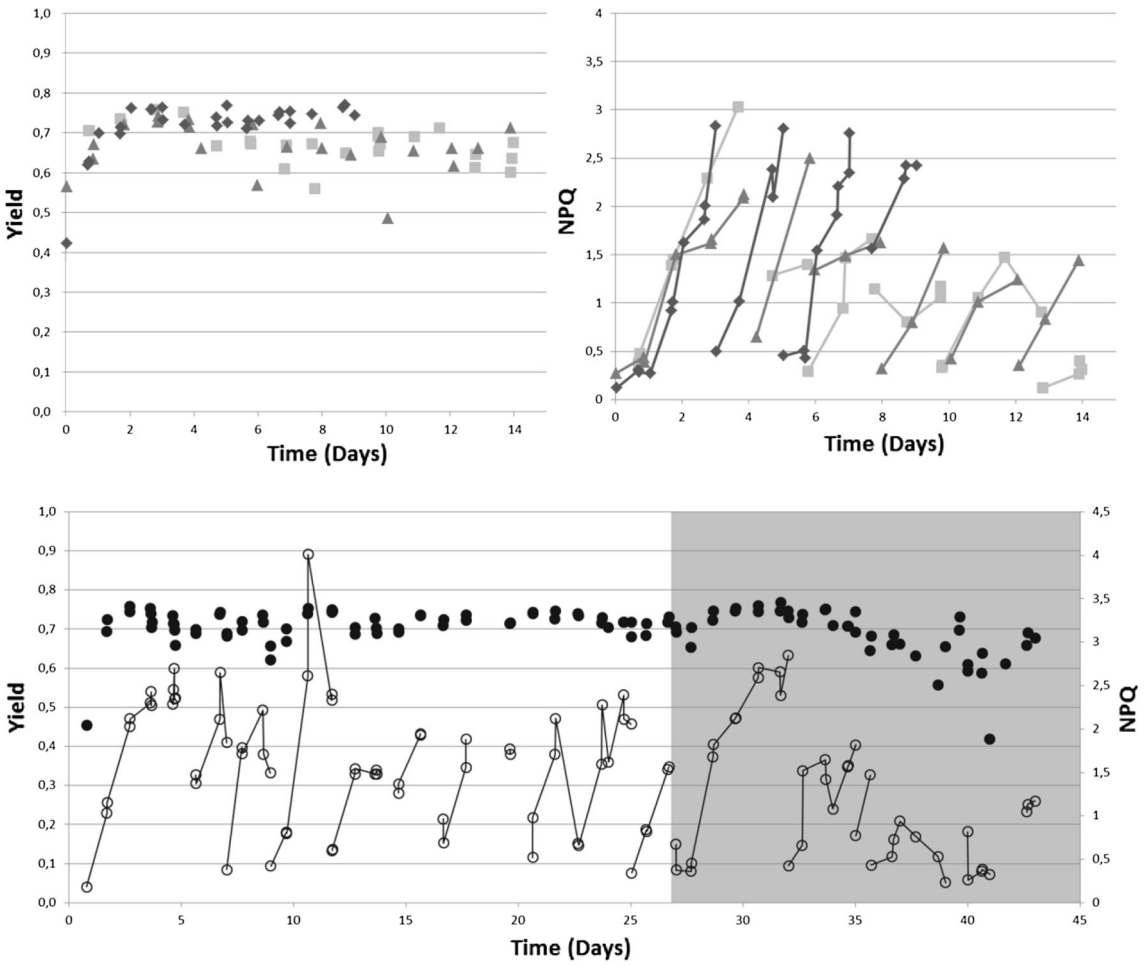

Fig. 6 Development of maximum quantum yield and non-photochemical quenching over time; samples were *dark* adapted for 5 min. Light of a wavelength of 645 nm was used for excitation. *Unlinked data points* in NPQ indicate dilution of the culture. *Top row* shows three experiments (*square* March 2013; *triangle* April 2013; *diamond* April 2014) with durations of 14 days or less; *Left* maximum quantum yield; *right* NPQ. *Lower row* shows the maximum quantum yield (*black solid circle*) and NPQ (*black open circle*) of a growth experiment lasting 43 days (March–April 2014). The area shaded in *gray* indicates a decreased water circulation

Productivity and media parameter

The overall biomass productivity and the pH and conductivity of the culture media were measured to determine the influence of a high pH and the salinity to be reached on the productivity.

All growth experiments were harvested every other day (Fig. 7) and showed a mean productivity of 16.1 g/m^2/day (SD ±1.7) in 2013 and 15.4 g/m^2/day (SD ±4.7) in 2014. The pH showed values of between 8.3 and 10.0 in the morning (Fig. 7) rising between 0.6 and 0.8 points over the day and decreasing overnight. The conductivity alternated during all experiments in various degrees between 15 and 25 mS/cm depending on the conductivity of the feed water. The dilution of the culture led to a drop in pH, and after addition of fertilizer the slope of pH-increase declined. The maximum measured pH-values of all cultures were in the afternoon after a full day of growth between 10.1 and 10.8.

During the different experiments, the circulation of the cultures stopped irregularly due to the maintenance of the generator or failure of mechanical parts (March 2013: 21 h in 14 days; April 2013: 3 h in 14 days; April 2014: 31 h in 9 days; March–April 2014: 82 h during the first 27 days and 31 h during the 16 days with reduced pump performance). Single power outages lasted as long as 3–12 h. During this time, no decrease in productivity was observed but the pH of cultures increased faster and if stopped longer than 6 h from the morning on it plateaued at values around 10.3 (SD ±0.1).

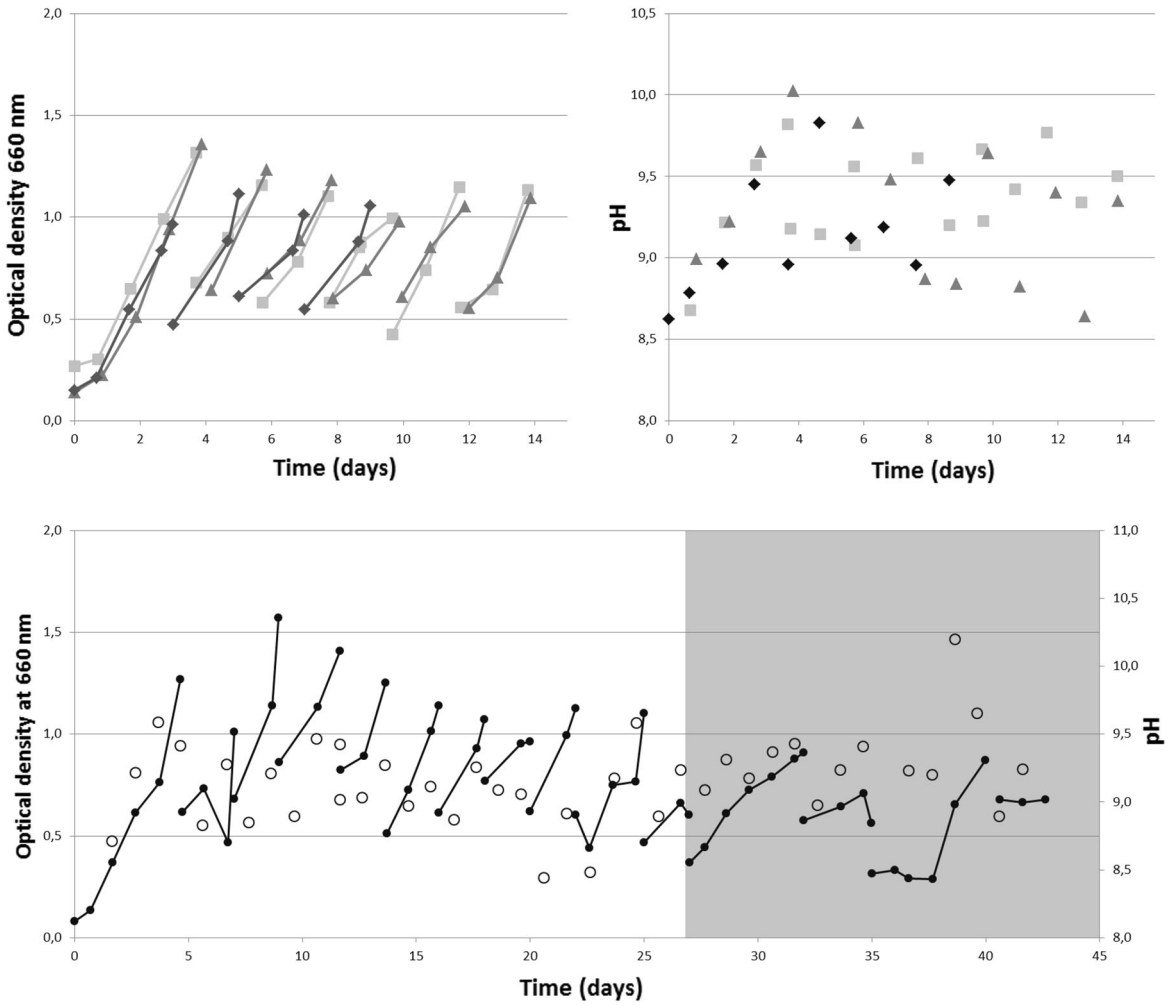

Fig. 7 Growth behavior of *Scenedesmus* Spec. MKB in 600L pre-cleaned PW while diluted by ca. 50 % every other day. Fertilizer was added after each harvest. Biomass increase is shown as determined by optical density measurement, *unlinked data points* indicate dilution. The pH shows only the mean values of 3 min measured in the morning between 8 and 10 a.m. *Top row* shows three experiments (*square* March 2013; *triangle* April 2013; *diamond* April 2014) with durations of 14 days or less; *left* biomass; *Right* pH. *Lower row* shows the biomass (*black solid circle*) and pH (*black open circle*) of a growth experiment lasting 43 days (March–April 2014). The area shaded in *gray* indicates a decreased water circulation

The productivity of the experiment conducted in March and April 2014 dropped to 6.3 g/m^2/day (SD $\pm$2.0) when the pump was replaced by a smaller one on day 27.

Discussion

Culture composition

The biomass monitored in 2013 was contaminated by weed algae, mainly diatoms, from the early beginning. This is accounted to the lack of a suitable filtration system for treatment of the pPW. The change in biomass composition from the end of the experiment in March 2013 and the beginning culture composition of the experiment performed in April 2013 showed that a significant amount of diatoms was removed from the culture via sedimentation. In 2014, diatoms were not detected (April 2014) or the earliest after 3 weeks of growth (March–April 2014), while single-celled cyanobacteria were detected in small amount during most parts of the experiments. The possible use of photosynthetic characteristics for the detection of weed algae was

examined in more detail. The maximum values of NPQ decreased when the culture matured and the maximum NPQ of the experiment in April 2014 stayed around 2.5; the NPQ of all other experiments declined. An axenic culture of *Cyanobacterium aponinum* grown under the same circumstances had a NPQ of 0.5 which increased with invading green algae and diatoms (Paper submitted for publication). During times when the NPQ did not increase as high as before, the photosynthetic yield decreased as well. The photosynthetic yield (*Y*) was proven to be influenced by numerous different factors like nutrients (Lippemeier et al. 2001), light, culture density (Schreiber and Klughammer 2013), and salt stress (Lu and Vonshak 2002) and culture composition (Kolbowski and Schreiber 1995). Since measurements were taken daily at the same time, we do not consider the difference of daylight intensity between measurement days to be a considerable influence. The culture density and the salt content of the experiment conducted April 2014 alternated in between OD 0.5 and 1.3 and 15–25 mS/cm, respectively, but no significant change in *Y* was monitored, it was therefore concluded that cell density and salinity seem to have only a small influence at best. Nutrients were added after every dilution and it is assumed that they were available in abundance. *Y* is influenced by toxic substances as well (Schmitt-Jansen and Altenburger 2008; Peña-Vázquez et al. 2010), and algae cultures are suspected to accumulate physiological products inside the medium, which might inhibit growth (Gustavs et al. 2010). It was concluded that, since the change in Y during the trial conducted in March–April 2014 occurred after 34 days, the build-up of inhibiting physiological byproducts will be neglected. Cyanobacteria and diatoms showed during laboratory studies (Winckelmann et al. 2014) a maximum *Y* of 0.45 and 0.6, respectively. The change in *Y* occurred within 2 days of the microscopically detected weed algae, it is thought the lower capability of cyanobacteria and diatoms to use light energy for fluorescence might lead to a quenching of the overall *Y*.

The use of the phyto-PAM for the detection of weed algae and overall stress in an open pond as a stand-alone method is not advised. While it would be possible to establish an online sensor, it would be influenced by a range of different factors and the growth system to be monitored has to be well understood. A well-trained operator might be capable to distinguish the influence of nutrient deprivation from the afternoon photoinhibition(Lu and Vonshak 1999), but the sensitivity is insufficient to detect weed algae in an early stage. While it was possible to detect cyanobacteria concentration of $5 \times 10\text{^}3$ cells/mL cells/mL in $2.5 \times 10\text{^}6$ cells/mL with a microscope, the phyto-PAM started to detect cyanobacteria at $5 \times 10\text{^}4$ cells/mL. Other techniques for the detection of weed algae and even non-photosynthetic organisms like grazers can be applied (Havlik et al. 2013a). Flow cytometry was found to detect weed algae with a concentration as low as 0.01 % (Fulbright et al. 2014), and even capable to monitor algae; which change shape during their growth cycle (Havlik et al. 2013b). Real-Time quantitative PCR (qPCR) could be used to monitor production systems as well; qPCR systems were able to detect known possible contaminants at levels of 0.00001 % (Fulbright et al. 2014).

Grazers were only detected in matured cultures living in the biofilm; the abundance was too low to be quantified. Only bacteria grazing was monitored. It is assumed that the high pH and temperature did minimize the growth of grazers (Bartley et al. 2013).

Productivity

The overall production of all experiments, excluding the lag phase and the time with reduced pump performance, was 15.7 g/m^2/day (SD ±3.6). The average light intensity in Oman was reported to be around 1800 µmol photons/m^2/s (Al-Rawahy et al. 2003). Values of around 1500 µmol photons/m^2/s were measured during this study. Using the calculations proposed by Grobbelaar (2009) and assuming 40 % of algal biomass to be carbon, the photosynthetic efficiency was 2.4 % and decreased to 1 % during prolonged periods of reduced mixing.

There are no data on *Scenedesmus* productivity comparable to the set-up used during this study. The productivity of a non-axenic *Scenedesmus obliquus* dominated culture was reported to range from 1.7 g/m^2/day to 16.92 g/m^2/day in Germany (Grobbelaar et al. 1990) and a *Scendesmus* sp. culture was reported to have reached 28–82 g/m^2/day (Mendoza et al. 2013). While the first study was conducted over a range of 16 months, the second was only conducted for 6 days, both used CO_2 or flue gas as additional carbon sources. Weissman et al. (1989) conducted experiments in New Mexico, under similar temperature regimes and adding CO_2 productivities of 33 g/m^2/day, reaching the photosynthetic efficiency of 6.7 %.

The overall photosynthetic characteristics as measured in the morning (Table 1) did not change significantly with increasing sun light, biomass density, or biomass composition, so it is assumed that the sun light is not the limiting factor during the growth experiments. However, the culture's Y usually declined over the course of the day (data not shown) indicating light inhibition or damage. An alteration of the pond design might lead to a decrease in light inhibition and thereby increasing the productivity(Vonshak et al. 2013), also the ability to cope with excess light of high intensity varies over the experiment. After the inoculation and dilution, the NPQ was below 0.5 but increased up to 3 before the next dilution. The drop in NPQ can be explained by the dilution and the decreased biomass concentration reducing the cultures self-shading (Küster et al. 2004).

It is hypothesized that the high pH plays a major role in the limitation of growth. The availability of carbon as substrate for photosynthesis is crucial. The maximum concentration of carbon dioxide in liquid with a salt content of 1 % w/v is 1.2 g/L at 30 °C (Duan and Sun 2003). With the measured $k_La(O_2)$, not only would be all oxygen produced during photosynthesis be transferred out of the media, but the high $k_La(CO_2)$ would be sufficient to supply a constant stream of carbon. On the supposition of a gradient favoring the transition of CO_2 from air to liquid and using the calculations provided by Talbot et al. (1990), a maximum of 143 g of carbon dioxide could be transferred per hour into a 600 liter culture in the ponds described above.

The uptake of HCO_3^- and CO_2 can occur simultaneously in some green algae via different pathways, leading to OH^- formation and alkalization of the medium, even if $CO_2^{(aq.)}$ is present. See Badger and Price for a review regarding the CO_2 concentrating mechanism (Badger and Price 1992). Six different green algae have shown to stop photosynthesis at a pH above 10 (Shiraiwa et al. 1993) at which most of the dissolved inorganic carbon has the form of CO_3^{2-} (Markou et al. 2014). pKa of CO_3^{2-} is 10.3 at 25 °C but decreases with increasing temperature (Rabinowitch 1945). CO_3^{2-} is unavailable for most microalgae (Markou et al. 2014). While it is known that *Scenedesmus* and some marine algae are capable of thriving at pH of 8–9, it is unknown how the excess CO_3^{2-} influences the growth (Shiraiwa et al. 1993).

Scenedesmus obliquus showed an increase of lipid content after addition of 5 g/L NaCl (Gorain et al. 2013) but were also negatively influenced by further increased NaCl level (Affenzeller et al. 2009; Salama et al. 2013). The NaCl level reported as being sufficient for repressing growth had a conductivity of 11.1 mS/cm (Salama et al. 2013). No work about salt stress and green algae isolated from brackish water was found. The fluctuating salinity during this experiment was always higher than 15 m/S/cm, but no significant correlations between conductivity and productivity were found.

Economic feasibility and suggested improvements

If algae were to be produced under similar conditions as experienced during this study and the production facility would have a pond surface of 80 % and could operate on 300 days per year, it would produce 28 tons (dw) biomass per year. It was shown that *Scenedesmus* sp. biomass, grown phototrophically outdoors in Arizonas climate, consisted of 42 % (dw) carbohydrates [7 % (dw) were starch], 15 % (dw) lipids, and 27 % (dw) proteins, which was altered after 14 days of nutrient depletion to 45 % (dw) carbohydrates (20 % (dw) were starch), 30 % (dw) lipids, and 10 % (dw) proteins (Laurens et al. 2014). Under different conditions, lipid accumulations as high as 53 % (dw) were reported (Xin et al. 2010). The lipid composition of Scenedesmus sp. has been reported to be suitable for biodiesel production (Gouveia and Oliveira 2009; Makarevi et al. 2011; Jena et al. 2012). If *Scenedesmus* sp. grown in pPW would have the same composition as shown after growth in modified BG-11 media(Laurens et al. 2014), it would be possible to harvest 9500 L of lipids (assuming a density of 800 g/L) leading to 7600 L of biodiesel per hectare per year (Chisti 2007). This would be only a fraction of the 58,700 L/ha/year predicted, but still higher than the oil yield reported for palm oil (Chisti 2007). There are multiple possibilities to increase productivity and to make the production of green algae on site more sustainable. The biomass residues not sold can be digested for biogas, which can be used as CO_2 and energy source, while the residues can be used instead of fertilizer (Chisti 2013). A change in the mixing system will change the productivity; the use of a centrifugal pump as opposed to a paddle wheel will lead to a well-mixed culture and a high turbulence which is stated to prevent algal sedimentation, nutritional gradients, and to expose the algae to variations in the quantity and quality of light (Grobbelaar 2010). But it is also less energy efficient. The floating of algae flocks at times of pump failure showed that there was a biofilm formation. The biofilm mainly consisted of diatoms and dust particles and was stronger in areas with less

turbulence. It is strongly suggested that the biofilm formation is influenced by the amount of dust, the turbulence of the culture, and the roughness of the pond liner. A change in pond design or mixing might lead to less biofilm formation and thereby reduce the risk of product contamination. It was shown that wave formation inside of a pond led to an increased productivity and a better nitrogen uptake (Chiaramonti et al. 2013). If wave formation would be obtained by intermittent reduction of the pumps delivery rate, a decrease in energy consumption might be achieved.

A final statement of economic feasibility is not possible at this stage. The energy consumption for all steps involved in upstream and downstream processing has to be included into the calculation as well as possible nutrient recycling, nutrient costs, the usability of algae residues on site, and the work hours to be invested (Collet et al. 2013).

Conclusion

It was shown that it is possible to grow indigenous algae in open ponds in an arid region using pre-cleaned waste water as growth medium. The concerns regarding salt stress due to evaporation were unwarranted. The green algae isolated on-site were suited to the conditions of high temperature and increasing salt content, and dominated the culture, while no signs of decreasing health were detectable.

High turbulence and flow regime are necessary to ensure high production rates. The design of the ponds and the means used to establish the flow regime have to be changed during scale-up to increase or at least stabilize productivity, while reducing the applied energy and thereby make economic viability possible.

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