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Citation for the original published paper (version of record):

Krustok, I., Odlare, M., M.A., S., Truu, J., Truu, M. et al. (2015)

Characterization of algal and microbial community growth in a wastewater treating batch photo-bioreactor inoculated with lake water.

Algal Research

<http://dx.doi.org/10.1016/j.algal.2015.02.005>

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**Characterization of algal and microbial community growth in a
wastewater treating batch photo-bioreactor inoculated with lake water**

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Characterization of algal and microbial community growth in a wastewater treating batch photo-bioreactor inoculated with lake water

Microalgae grown in photo-bioreactors can be a valuable source of biomass, especially when combined with wastewater treatment. While most published research has studied pure cultures, the consortia of algae and bacteria from wastewater have more complex community dynamics which affect both the biomass production and pollutant removal. In this paper we investigate dynamics of algal and bacterial growth in wastewater treating batch photo-bioreactors. The photo-bioreactors were inoculated with water from a nearby lake. Lake water was obtained in August, November and December in order to add native algae species and study the effects of the season. The photo-bioreactors inoculated with lake water obtained in August and November produced more biomass and grew faster than those that only contained the algae from wastewater. The results indicated a rapid decline in bacterial abundance before algae began to multiply in reactors supplemented with lake water in November and December. The reactors were also successful in removing nitrogen and phosphorous from wastewater.

Keywords: algae cultivation; biomass production; community analysis; photo-bioreactors; wastewater treatment

Abbreviations

DOC – Dissolved Organic Carbon

DW – Dry Weight

LW – Lake Water

PE – Purification efficiency

qPCR – Quantitative polymerase chain reaction

TOC – Total Organic Carbon

TP – Total phosphorous

WW – Wastewater

WWTP – Wastewater treatment plant

Research highlights

- Adding lake water to the photo-bioreactors had an effect on the algal growth dependant on the season of sampling.
- The most dominant algae in the photo-bioreactors studied were *Scenedesmus*, *Desmodesmus* and *Chlorella*.
- In experiments performed with lake water sampled in November and December, there was a decrease in bacterial populations.
- The reactors were effective in removing nitrogen from the wastewater.

1. INTRODUCTION

Recent studies have shown that cultivation of algae in wastewater may be an effective way to recover nutrients and cultivate biomass (Odlare et al., 2011; Su et al. 2011; Termini et al. 2011). Wastewater is also readily accessible in most urban environments making its use as a growth medium possible in a wide variety of locations.

The use of wastewater as a medium introduces a whole consortium of microorganisms into the process making it more robust in terms of metabolic pathways present (Muñoz and Guieysse, 2006). In addition, natural inoculants such as lake water may serve as a source for indigenous algae strains. This removes the need to find a perfect pure culture to use in the process and let the medium do the selection (Olguín, 2012).

Compared to conventional monoculture photo-bioreactors, it is important to study the algal and microbial communities in these systems in order to understand and control the process (Su et al., 2011; Lakaniemi et al., 2012; Olguín, 2012). Lakaniemi et al. (2012) concluded that understanding of the interactions between microorganisms in photo-bioreactors is needed to increase the biomass production. The potential of the bacterial and algal consortium in similar biotechnological applications has also been described by

Subashchandrabose et al. (2011), who concluded that understanding the community relationships is crucial when algae biomass is produced in the course of different wastewater treatments and the degradation of pollutants and production of metabolites (proteins, fatty acids, steroids, carotenoids, phycocolloids, lectins, mycosporine-like amino acids, halogenated compounds, and polyketides) is highly desirable.

The aim of the present study was to investigate algal growth and nutrient removal in a photo-bioreactor using water containing indigenous algae from an inland lake in central Sweden (Lake Mälaren) and inflow wastewater to a wastewater treatment plant (WWTP) treating the water of a medium sized town in central Sweden (Västerås) as a growth medium in three different seasons. Samples were taken in August, when the algal biomass growth in the lake was high, in November, when there is no visible algal biomass growth in the lake and in December, when the lake surface had frozen. Specific objectives were to:

- (1) Investigate the dynamics of the microbial and algal growth in a wastewater treating batch photo-bioreactor after introduction of indigenous algae from a nearby inland lake taken at different seasons.

- (2) Investigate how nutrient concentrations change in the algae cultivation process to assess the water purification efficiency in the photo-bioreactors.

2. MATERIALS AND METHODS

2.1 Experimental setup

2.1.1 Determining wastewater and lake water ratio

The 70% wastewater and 30% lake water mixture was determined based on a flask experiment. Ten 250 ml flasks were set up in a climate chamber with automatic light and temperature regulation using a protocol of 12 h of light and 12 h of dark per 24 h at

23±0.5°C. Lake- and wastewater ratios of 30/70, 50/50 and 70/30 were tested. Stirring was not added however the flasks were manually shaken every day. They were compared to pure lake- and wastewater. The experiment indicated that a 70/30 ratio of lake- and wastewater had the highest increase (1.6x) of optical density (at 630 nm) over 14 days of growth compared to the other samples.

2.1.2 Determining the effect of lake water addition

Three similar 16 day laboratory experiments were conducted with lake water sampled in November, December and August. Four photo-bioreactors (height 18 cm, diam. 10 cm) with a total volume of 1L of water mixture were set up and the following variants were treated simultaneously in each experiment: 1) wastewater (WW), 2) 70/30 mixture of wastewater and lake water (WW+LW), and 3) 70/30 mixture of wastewater and distilled water (WW+W). A sterilized wastewater reactor was set up as a control to detect cross contamination. For the control reactor, wastewater was sterilized by autoclaving at 121°C for 20 minutes. All measurements on the sterilized wastewater were performed after the sterilization process.

The reactors were glass cylinders with stainless steel tops and bottoms. During the experiments, the reactors were closed and filter paper was used to cover openings on the top in order to allow gas exchange. The light source was above the reactors and mirrors were used to reflect light in order to increase lighting efficiency. The experimental light and temperature conditions were manipulated in order to simulate conditions that stimulate the maximum growth rate of algae during the summer. The reactors were lit by 4 fluorescent tubes (Aura Long Life 51W/830) with 16 h of light and 8 h of dark per 24 h at around 100 µmol/m²s, measured from the inside wall of the reactor. The temperature in the reactors was set to 23±0.5°C (Tang et al., 2011) and the mixture was stirred with magnetic stirrer bars at around 350 rpm (Tang et al., 2011) throughout the experiment.

Air was pumped into the reactors through a 0.22 µm Millipore filter (3L/min) to optimize the gas exchange and to prevent excessive pH increases in the reactors. 100 mL water samples were taken from each reactor at 4 day intervals to determine nutrient concentrations, chlorophyll a concentration and pH. The pH of the samples was measured using a 744 pH meter (Metrohm AG, Herisau, Switzerland).

2.2 Wastewater and lake water origin and properties

Inflow wastewater obtained from the WWTP in Västerås (central Sweden) was used in this study. The plant uses a conventional treatment process, treating sewage from the equivalent of a 118 000 population, yielding 12 000 tonnes of dewatered (25% dry matter) sludge per year. In the current water treatment process, influent raw wastewater is screened, pre-precipitated with iron sulphate, and biologically treated by an activated sludge process with pre-denitrification supported with glycol as the external carbon and energy source. Wastewater for the experimental system was collected from the WWTP inflow (from the top layer of the centre of the mixed basin).

Lake water for algae inoculation was taken from Lake Mälaren, which has an area of 1096 km², a mean depth of 12.8 m and a maximum depth of 64 m. It is the third largest lake in Sweden, with a water volume of 14 km³ (Kvarnäs, 2001). Mälaren lake water was collected from a yacht harbour next to the WWTP from the upper layer (0.5 m) of the lake.

All lake and WWTP samples were taken with sterilised equipment according to the SS/ISO 5667-3:2004 standard and were immediately transported and used in the experimental setup. For initial nutrient analysis 50 mL of the sampled waters were filtered through a Whatman GF/C filter (1.2 µm) and preserved with 0.5 mL of concentrated sulphuric acid (98%, Thermo Fisher Scientific, Waltham, MA, USA). The samples were

stored at -20°C until further analysis. Initial chemical compositions of the waters and mixtures used in the experiments are in Table 1.

Table 1. Chemical parameters of the wastewater (WW), sterilized wastewater (WW Ster), wastewater/lake water mixture (WW+LW), wastewater/water mixture (WW+W) and lake water (LW). Average values and standard deviations of the parameters between the three different experiments are displayed. Abbreviations: TOC – total organic carbon, DOC – dissolved organic carbon, TP - total phosphorous, COD – chemical oxygen demand, nd - not determined.

Parameter	WW	WW Ster	WW+LW	WW+W	LW
pH	7.5±0.3	8.3±0.8	7.7±0.4	7.3±0.6	7.3±0.4
Chl a (mg L ⁻¹)	0.03±0.02	0.04±0.04	0.02±0.01	0.01±0.01	0.04±0.04
TOC (mg L ⁻¹)	112.3±50.2	154.3±74.5	105.8±66.8	126.7±91.0	30.2±24.3
DOC (mg L ⁻¹)	24.9±11.0	45.3±21.8	17.8±11.3	15.1±10.9	nd
COD (mg L ⁻¹)*	508±200	622±273	559±250	670±322	nd
NH ₄ -N (mg L ⁻¹)	35.2±9.6	27.8±8.5	27.5±9.0	24.6±4.6	0.7±0.7
NO ₃ -N (mg L ⁻¹)	2.6±4.0	3.3±5.8	1.9±2.4	2.0±2.3	0.1±0.1
TP (mg L ⁻¹)	1.12±0.68	0.61±0.55	1.36±1.15	0.90±0.97	0.01±0.01

*calculated based on the equation presented in Dubber and Gray (2010).

2.3 Chlorophyll a and algal community analysis

Chlorophyll a concentration was measured at room temperature to assess algae biomass growth (Bellinger and Sigee, 2010). 25 mL of water sample was filtered through a Whatman GF/C filter (1.2 µm). Chlorophyll a was extracted with acetone (99%, Thermo Fisher Scientific, Waltham, MA, USA). Absorbance was measured at 665 nm (chlorophyll a) and 750 nm (turbidity) with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech, Sweden), and the chlorophyll a concentration (mg L⁻¹) was calculated with the following equation (1):

$$C = (A_{665} - A_{750}) * V/V_s * 11.3/L / 1000 \quad (1)$$

where C is chlorophyll concentration (mg L⁻¹), V is the volume of the solvent (mL), V_s is the volume of the sample (L), L is the light path (cm), A is absorbance and 11.3 is the

specific extraction coefficient for acetone.

Dry weight (DW) was assessed by filtering 25 mL of sample through a Durapore 0.45 µm filter (Merck Millipore). The filters were dried and weighed before and after filtering and DW was calculated as the difference between the filter with and without the sample. At each sampling time, 5 mL of sample was taken for microscopic analysis of the algal community. 250 µl of Lugol's iodine was added to the samples which were then stored at 4°C prior to microscopic analysis.

The algae community was studied using an Alphaphot-2 YS2 microscope (Nikon Instruments Inc., Tokyo). The samples were concentrated 5x by centrifugation. 50 µl of sample was placed under a cover glass and studied under 60x lens. Images were taken using a Sony NEX 5N camera with an APS-C size sensor (1.5x crop factor).

2.4 Quantitative PCR and data analysis

25 mL of water was filtered through a 0.22 µm Millipore filter and stored at -20°C for microbial community analysis.

DNA was extracted from the samples using a MoBio PowerWater DNA extraction kit (Mobio Laboratories Inc., Carlsbad, CA, USA). The extraction was performed according to the manufacturer's protocol. The quality and concentration of the extracted DNA was measured at 260 and 280 nm using an Infinite 200 PRO spectrophotometer (Tecan Group Ltd, Männedorf, Switzerland) and NanoQuant plate (Tecan Group Ltd, Männedorf, Switzerland).

The development of the bacterial community was estimated from 16S rRNA gene copy numbers. The L-V6 (5'-GAACGCGARGAACCTTACC-3') and R-V6 (5'-ACAACACGAGCTGACGAC-3') primers were used to amplify the bacterial 16S rRNA gene 111bp fragment from the V6 hypervariable region (Gloor et al. 2010). Quantitative PCR (qPCR) was performed with a Rotor-Gene Q (Qiagen, CA, USA). The qPCR

program was as follows: 2 min at 95°C, 45 cycles of 15s at 95°C, 30s at 54°C and 30s at 72°C. Melting curve analysis was performed at 65–90 °C. The standard curve was constructed using the standard plasmid as described by Nölvak et al. (2013). 50 copies of standard plasmid were diluted in 10 µl of reaction mixture for the standard curve preparation. Quantitative PCR data were analysed as described by Nölvak et al. (2012). Target gene copy numbers were calculated and presented as copies per mL of water sample (copies/mL).

2.5 Nutrient analysis

NH₄-N and NO₃-N concentrations were measured using FOSS FIASTAR 5000 Fluid Injection Analysis. Measurements were made as specified in the standard FOSS protocol. Total phosphorous (TP) concentration was measured from filtered and unfiltered samples using HACH LANGE cuvette tests LCK349 and LCK350. TOC and DOC were measured using HACH LANGE cuvette tests LCK380 and LCK381. 25 mL of each sample was filtered through a Whatman GF/C filter (1.2 µm) for analysis of nutrients in the water phase and acidified by adding 0.5 mL of concentrated sulphuric acid (98%, Thermo Fisher Scientific, Waltham, MA, USA). The samples were stored at -20°C prior to measurement according to the manufacturer's protocol.

3. RESULTS AND DISCUSSION

3.1 Algal growth dynamics

As was expected the algal growth was highest in the reactors with lake water sampled during the summer season (Fig. 1a). In November, after the algal growth season the maximum chlorophyll a concentration was around half of what it was during the summer and growth was considerably slower compared to the summer season, in all reactors (Fig. 1b). In experiments conducted after the lake had frozen, algal growth was lower still with

maximum chlorophyll a value being under 2 mg L⁻¹ compared to 7.4 mg L⁻¹ reported in the experiments conducted in August.

The addition of lake water to the wastewater had a noticeable effect on the algal growth in both experiments conducted in August and November (Fig. 1a-b). While in August, wastewater diluted with distilled water reached a comparable maximum chlorophyll a concentration, the growth rate in the WW+LW reactor was 2-3 days faster than in the WW+W reactor. There was no significant difference between WW+LW and WW+W reactors in the experiment conducted in December (Fig. 1c), probably due to the low lake water temperature and the algae being dormant.

Due to cross-contamination the sterilized wastewater reactor showed some algal growth in the experiments conducted in November and December. However, in the experiment conducted in August, there was no change in the chlorophyll a concentration in the reactor with sterilized wastewater.

The general dynamics of algae growth were similar to those reported in literature (Chiu et al., 2008; Odlare et al., 2011; Pegallapati and Nirmalakhandan, 2013), although in our case there was a longer lag phase especially in the experiments conducted outside the algal growth season. It took around 8 days for the algae to start to grow in these experiments (Fig. 1b-c). In August it only took 4 days for the algae to start their active growth (Fig. 1a). One way to reduce the lag time would be to supply a higher concentration of CO₂ to the reactors than is found in ambient air. Chiu et al. (2008) and Pegallapati and Nirmalakhandan (2013) reported a shorter lag phase and faster growth when 2-5% CO₂ was mixed into the air supply. Odlare et al. (2011) observed a similarly longer lag phase when they used low nutrient concentrations. Because the WWTP aims to reduce phosphorus level before wastewater enters the plant, phosphorous may be a limiting factor for algal growth.

233 There was a 2.61, 3.89 and 3.93 time increase in the DW of the WW, WW+LW and
234 WW+W reactors, respectively in the August experiment. From the initial DW values of
235 204, 144 and 112 mg L⁻¹ the values increased to 532, 560 and 440 mg L⁻¹ in the WW,
236 WW+LW and WW+W reactors, respectively. However, WW+W had the highest
237 percentage of chlorophyll a in the DW at 1.7%, followed by WW+LW at 1.2% and WW
238 at 1%.

239 The average mean pH at the start of all experiments was slightly above neutral at 7.7±0.4.
240 The pH values in each reactor is given in Table 1. Because there was no pH control in the
241 experiment, pH values started to increase as the algae started to grow (Fig. 1d-f). The
242 highest pH value was measured in the WW+LW reactor during the November experiment
243 at 10.1. In other cases the pH value remained below 9.5. The increase in pH values as the
244 algae started to grow is expected due to the rapid assimilation of CO₂. Where the algal
245 growth was particularly low, for example in the WW reactor during the experiment
246 conducted in December there was a decline in pH, due to low assimilation of CO₂ (Fig.
247 1f). The variation of the pH between the experiments can be explained by a high variation
248 in the inflowing wastewater. The algal and bacterial community can also have an effect
249 on the final pH. The pH increase in most reactors was similar to data previously reported
250 by Pegallapati and Nirmalakhandan (2013).

Fig. 1. Dynamics of chlorophyll a concentrations and pH values in the August (a and d), November (b and e) and December (c and f) experiments. Abbreviations: WW – wastewater; WW+LW – 70% wastewater and 30% lake water; WW+W – 70% wastewater and 30% water.

3.2 Bacterial growth dynamics

The 16S rRNA gene concentration was used as a measure of bacterial abundance in the reactors. The dynamics of bacterial 16S rRNA gene copy numbers in all three experiments are shown in Figure 2. The lake water contained 2.7×10^6 , 1.7×10^7 and 4.2×10^6 copies of the 16S rRNA gene per mL of water in the August, November and December experiment, respectively.

In the November and December experiments, the bacterial 16S rDNA copy number dynamics showed similar patterns in all reactors (Fig. 2b-c). The average initial gene concentration was 6.4×10^8 and 5.3×10^8 copies of the 16S rRNA gene per mL of water in the November and December experiment, respectively.

The 16S rDNA copy numbers decreased until the 8th day, after which they stabilized. This is in contrast to the chlorophyll a concentration in these experiments, which was stable until day 4-8 due to the long lag phase of the algae, after which it began to increase. 16S rDNA copy number was higher at the beginning in the first experiment, but stabilized around the same value in both experiments.

In the August experiment the dynamics were significantly different (Fig. 2a). The average initial gene concentration was 1.8×10^8 copies of the 16S rRNA gene per mL, which is about 4 times lower than in the November and 3 times lower than in the December experiment. Because of the lower initial 16S rDNA copy numbers, there was no decrease as in the experiments conducted in November and December. The average 16S rDNA copy number concentration was around 1.1×10^8 throughout the experiment with only minor changes. This is similar to the 1.1×10^8 and 9.9×10^7 copies per mL where the November and December experiments averaged out, respectively.

The lower initial 16S rRNA gene copy numbers found in the experiment conducted in August may be due to the variations in the inflowing wastewater to the plant. Although general chemical parameters were similar in all 3 experiments (Table 1), there are other factors (such as pollutants) that can limit the growth of bacteria in wastewater. In general the abundance of bacteria present in wastewater treatment plant should be comparatively stable (Harms et al., 2003).

The decrease in the bacterial community seen in the November and December experiments can be due to a decrease in available nutrients as they are consumed. Another potential reason may be the selective pressure from the photo-bioreactor selecting for bacteria better suited to the arising algal community (and pH value). Choi et al. (2010) also found that algae and cyanobacteria can inhibit nitrifying bacteria growth in a bioreactor by a factor of 4 even though the community structure of the nitrifying bacteria

was unchanged by the algae and cyanobacteria. The increase in pH could also create selective pressure on the bacteria. However, the pH change was different in all experiments (Fig. 1d-f) but the 16S rDNA copy numbers at the end of the experiments were similar in all cases (Fig. 2a-c).

Fig. 2. Dynamics of 16S rRNA gene copy numbers in experiment 1 (a) and experiment 2 (b). Abbreviations: WW – wastewater; WW+LW – 70% wastewater and 30% lake water; WW+W – 70% wastewater and 30% water.

3.3 Algal community analysis

There was little to no difference between the communities in the experiments performed in November and December. Because there was algae growing in the sterilized reactor, there was a possibility for cross contamination. Data from microscopic examination showed that after 16 days of growth, the two reactors with the most diverse communities were the wastewater and the wastewater + lake water reactors. Representatives from many genera of algae were found in the reactors. The most common algae were *Chlorella*, *Oocystis*, *Selenastrum*, *Scenedesmus*, *Monoraphidium*, *Sphaerocystis* and many different diatoms. In the sterilized wastewater reactor only three genera of microalgae were detected – *Chlorella*, *Oocystis* and *Scenedesmus*. Since the samples were taken in late autumn and early winter, the algae community in Lake Mälaren should be closer to the vernal community with many diatoms classes dominating, such as *Aulacoseira*, *Stephanodiscus*, *Diatoma*, *Cryptophyceae* and *Melosira* (Willén, 2001).

Since there was no cross contamination in the experiment performed in August, it should give a better overview of the algal communities in the different reactors. The genera identified with microscopic examination are brought out in Table 2 and representative images from the microscope are in Figure 3. As with the other experiments, the differences between the reactors are few and mostly associated with the dominance of certain algae. While *Scenedesmus*, *Desmodesmus* and *Chlorella* dominated in all reactors, *Coelastrum* was more dominant in the WW reactor compared to other reactors. In the WW+W there was also a high amount of *Monoraphidium* as can be seen from Figure 3d. The developed community can be considered favourable as the dominant algae are documented in other wastewater treating photo-bioreactors and are considered good for energy production from biomass (Riaño et al., 2012; Sahu et al., 2013).

Table 2. Genera identified in the wastewater (WW), sterilized wastewater (WW Ster), wastewater/lake water (WW+LW) and wastewater/water (WW+W) reactors in the experiment performed in August. X marks genera present in the reactor and XX marks a dominant genus.

Genus	WW	WW Ster	WW+LW	WW+W
Scenedesmus	XX		XX	XX
Desmodesmus	XX		XX	XX
Chlorella	XX		XX	XX
Chroococcus	X		X	
Lacunastrum			X	X
Monoraphidium	X		X	XX
Coelastrum	XX		X	
Nitzschia	X		X	X
Selenastrum				X
Oocystis	X	X	X	X

Fig. 3. Representative microscope images of the WW (wastewater) (a), WW Ster (sterilized wastewater) (b), WW+LW (wastewater/lake water) (c) and WW+W (wastewater/water) (d) reactors in the experiment performed in August. Images for each reactor were selected based on the most variety of species present.

3.4 Changes in nutrient concentrations

As the August experiment was the most successful in terms of algal growth (Fig. 1a), changes in the nutrient concentrations in the water phase were studied to assess water treatment quality of the reactors. There was a reduction in ammonium concentration and an increase in nitrate concentration during the experiment (Fig. 4). After 12 days, the concentration of $\text{NH}_4\text{-N}$ was below 0.01 mg L^{-1} in all the reactors. Similar results have been found in studies of wastewater-treating photo-bioreactors with Termini et al. (2011) and Riaño et al. (2012) reporting 90-99% and more than 99% reductions in ammonium in 1-7 day and 30 day experimental periods respectively. Although Riaño et al. (2012) had longer experimental periods, they reported ammonium reduction of 0.81-7.66 mg

NH₄-N L⁻¹d⁻¹.

Nitrate nitrogen increased to 1.6 mg L⁻¹ in WW+LW and WW+W reactors and to 1.8 mg L⁻¹ in the WW reactor. This increase is most likely due to the high concentrations of nitrifying bacteria commonly found in wastewater (Harms et al., 2003). Because the reactors are aerobic, the bacteria can quickly nitrify the ammonium before the algae start to grow. Similar results have been reported by Karya et al. (2013), who showed that 81-85% of the ammonium in a wastewater photo-bioreactor was removed by nitrification and not through uptake by algae.

After 16 days, NO₃-N concentrations were <0.005, 0.16 and 0.28 mg L⁻¹ in WW+LW, WW+W and WW reactors, respectively. This means it is possible for water treated in a photo-bioreactor to meet the effluent standards set by the EU for total nitrogen (10-15 mg L⁻¹) with high algal growth. Further research in a full scale system is however needed to see if this performance scales up.

Fig. 4. NH₄-N (a) and NO₃-N (b) concentrations dynamics in the wastewater (WW), wastewater/lake water (WW+LW) and wastewater/water (WW+W) reactors in the experiment performed in August.

TP concentrations were low even at the start of the experiment (Table 1) due to the way the wastewater treatment plant treats the inflowing water. After 16 days of growth the TP

levels were below 0.05 mg L^{-1} in the water phase of all reactors. This means they were significantly lower than the EU ($1\text{-}2 \text{ mg L}^{-1}$) and Swedish ($0.2\text{-}0.5 \text{ mg L}^{-1}$) standards for water emissions.

As the algae took up CO_2 , TOC concentrations increased in all the reactors. The highest growth was in the WW+LW reactor where there was a 2.2 time increase in TOC concentration. DOC however showed a decrease, meaning the carbon present in the water phase was broken down by the bacteria present and the resulting CO_2 was taken up by the algae. In the best performing WW+LW and WW+W reactors, DOC was reduced 62.4% and 57.0%, respectively. This adds value to the process as a biological carbon capture system is highly desirable. Final COD values calculated from DOC based on Dubber and Gray (2010) were 50, 55 and 46 with a ± 14.01 prediction interval for WW, WW+LW and WW+W reactors, respectively.

CONCLUSIONS

The impact of addition of lake water to photo-bioreactor on the performance of reactors was dependent on the season when lake water was obtained. There was a benefit to algal growth when sampling was performed in August or November. In December however, when the lake was covered by ice, there was no difference between adding lake water or distilled water to the reactors. There was also a significant difference in algal growth dynamics depending on the season when sampling in the lake and wastewater is performed. When the lake water was sampled during the intensive algal growth season in the summer, the algal growth in the reactors was higher than in experiments performed with lake water sampled in November and December. The seasonal effect needs to be taken into account when moving to a full-scale system. In addition more information is needed to understand if the seasonal effect is due to the variations in the algal

communities present in the lake water or do the seasonal changes in the bacterial populations also have an effect on the final algal growth.

The most dominant algae in the photo-bioreactors studied were *Scenedesmus*, *Desmodesmus* and *Chlorella*, which are commonly seen in wastewater treating photo-bioreactors and can be potentially used for the production of biogas and biodiesel.

In the experiments performed with lake water sampled in November and December, there was a decrease in bacterial population during the first 8 days after which the bacterial abundance stabilized. In the experiment performed with lake water sampled in August, no such decrease was noted.

The reactors were effective in removing ammonia from the wastewater. This effect appeared to occur mostly through nitrification causing an increase in nitrate concentration. After 16 days of cultivation, both nitrogen and phosphorous levels in the water phase were below effluent standards in Sweden. When this level of treatment remains in a full scale systems, wastewater treatment plants could be very interested in including this process in their treatment. Due to the large investments made in anaerobic digestion in wastewater plants in Sweden, algal photo-bioreactors could be an interesting direction for both water treatment and biomass production. There was also an increase in TOC and a decrease in DOC indicating uptake of CO₂, allowing for the plants to reduce carbon emissions.

While the mixed consortia of microorganisms present in wastewater treating photo-bioreactors has advantages, it is more complex in terms of the metabolic pathways involved. There is a need to examine the microbial processes in wastewater photo-bioreactors in more depth as the microbial and algal communities seem to interact with each other, thereby influencing the nutrient dynamics. It would also be beneficial to characterize the microbiome in the reactors using high-throughput sequencing as this

would help to identify the microbial species that evolve in the system. This could open up new possibilities for control in full scale systems treating wastewater with the inclusion of native algae.

ACKNOWLEDGEMENTS

The research was conducted thanks to the support of the Knowledge Foundation, Vinnova, SVU, Lückeby Water and Mälarenergi, and by grant IUT2-16 of the Ministry of Education and Research of the Republic of Estonia (J. Truu, M. Truu, T. Ligi).

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