Algae biomass cultivation in nitrogen rich biogas digestate

I. Krustok, J. G. Diaz, M. Odlare and E. Nehrenheim

ABSTRACT

Because microalgae are known for quick biomass growth and nutrient uptake, there has been much interest in their use in research on wastewater treatment methods. While many studies have concentrated on the algal treatment of wastewaters with low to medium ammonium concentrations, there are several liquid waste streams with high ammonium concentrations that microalgae could potentially treat. The aim of this paper was to test ammonium tolerance of the indigenous algae community of Lake Mälaren and to use this mixed consortia of algae to remove nutrients from biogas digestate. Algae from Lake Mälaren were cultivated in Jaworski's Medium containing a range of ammonium concentrations and the resulting algal growth was determined. The algae were able to grow at NH_4-N concentrations of up to 200 mg L^{-1} after which there was significant inhibition. To test the effectiveness of the lake water algae on the treatment of biogas digestate, different pre-cultivation set-ups and biogas digestate concentrations were tested. It was determined that mixing pre-cultivated suspension algae with 25% of biogas digestate by volume, resulting in an ammonium concentration of around 300 mg L^{-1}, produced the highest algal growth. The algae were effective in removing 72.8 ± 2.2% of NH_4-N and 41.4 ± 41.4% of PO_4-P.

Key words | algae community, algae cultivation, biomass production, nitrogen rich water treatment, photobioreactor, wastewater

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ABBREVIATIONS

JM	Jaworski's Medium
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
PAR	Photosynthetically active radiation
PO_4-P	Phosphate phosphorus

INTRODUCTION

It has been difficult for wastewater treatment processes to live up to increasingly stringent treatment standards and energy and resource efficiency demands. Effective biological treatment of wastewater, ideally with nutrient recovery, is needed for many waste producing industries. Cultivation of microalgae in photobioreactors is a technology whose potential in this area is being actively investigated (Muñoz & Guieysse 2006).

Microalgae are among the fastest growing photosynthetic organisms. They have carbon fixation rates an order of magnitude higher than those of land grown plants, and can be continuously harvested with cycles ranging between one and ten days, a feature that is useful for biofuel production (Chisti 2007) or composting (Han *et al.* 2014). They have been shown to assimilate significant amounts of nutrients due to their high requirements for nitrogen and phosphorus for proteins (45–60% of microalgae dry weight), nucleic acids and phospholipids synthesis. While many authors have shown that algae can be successfully grown in domestic wastewater (Sivakumar *et al.* 2012; Rawat *et al.* 2013), cultivation in side stream wastewaters with high nitrogen concentrations, such as biogas digestate from the anaerobic digestion process, has received less attention.

Enhancing growth performance in high ammonia/ammonium conditions would enable simultaneous biomass growth, recovery of nutrients and wastewater treatment using a variety of wastewaters, such as biogas digestate, swine waste and landfill leachate. This could help

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wastewater treatment plants achieve higher standards of treatment and energy use.

High concentrations of nitrogen (in the range of 500–1,500 mg-N/L), mostly in the form of ammonia/ammonium, can inhibit growth of algae. This is because free ammonia is toxic to several microalgae strains (Crofts 1966), complicating the use of such substrates. Because the speciation of ammonia/ammonium is related to pH, the inhibition is weaker at lower pH values. At pH values of 9 and above, however, it can have a substantial effect on algal growth (Azov & Goldman 1982). Despite this, researchers such as Yuan *et al.* (2011) have argued that the inhibition effect can be controlled by increasing the hydraulic retention time and thus increasing the biomass concentration in the reactor, or by diluting the high ammonia containing wastewaters with another source of wastewater. Another option is to look for algal strains that are less susceptible to nitrogen inhibition, such as *Chlorella vulgaris* (Cai *et al.* 2013).

This study focused on growing algae in biogas digestate, which is a nitrogen rich wastewater removed from the anaerobic digestion process by centrifugation. Specific aims were to study: (1) the tolerance to high ammonium concentrations of the algae and determine the conditions that negatively affect algae growth; (2) the growth of the algae in ammonium rich biogas digestate; and (3) the uptake, i.e., treatment potential, of nitrogen, phosphorus and heavy metals of the cultures.

MATERIALS AND METHODS

Biogas digestate and lake water origin and properties

Biogas digestate was sampled from the mesophilic anaerobic digestion of sewage sludge in the Västerås wastewater treatment plant after the sludge dewatering process. The plant uses a conventional treatment process with screening, and biological and chemical steps. Iron sulphate is used to precipitate the phosphorus. Chemical characteristics of the biogas digestate sample are shown in Table 1.

The lake water used as a source for algae was sampled from the highly eutrophicated Lake Mälaren in mid Sweden. The lake is known for its seasonal algae blooms in spring and late summer when diatoms, green algae and cyanobacteria are abundant. Previous studies have shown that wastewater-treating photobioreactors using algae from Lake Mälaren contain mainly *Scenedesmus, Desmodesmus* and *Chlorella* species, with *Scenedesmus obliquus* being dominant (Krustok *et al.* 2015a). For the present experiment, sampling was performed during winter, and thus the water was sampled from the top 10 cm layer of lake water after drilling through the frozen surface. All water samples were taken with sterilised equipment according to the SS/ISO 5667-3:2004 standard and immediately transported and used in the experimental setup. Chemical characteristics of the lake water used are shown in Table 1.

Table 1 | Chemical parameters of the biogas digestate and lake water used in the experiments. Average values and standard deviations of the parameters between experiments are displayed for the lake water. Abbreviations: Chl a – Chlorophyll a, NH_4-N – Ammonium nitrogen, NO_3-N – Nitrate nitrogen, PO_4-P – Phosphate phosphorus

Parameter	Units	Biogas digestate	Lake water
Chl a	mg L^{-1}	0.06	0.05 ± 0.01
pH		8.2	7.2 ± 0.1
NH_4-N	mg L^{-1}	542	0.5 ± 0.4
NO_3-N	mg L^{-1}	3.9	1.0 ± 0.9
PO_4-P	mg L^{-1}	10.8	0.02 ± 0.02
Cr	μg L^{-1}	1.8	2.6 ± 0.6
Co	μg L^{-1}	7	1.3 ± 1.1
Ni	μg L^{-1}	13.8	14.6 ± 1.7
Cu	μg L^{-1}	14.4	15.0 ± 12.1
Zn	μg L^{-1}	117	170.0 ± 110.6
As	μg L^{-1}	14.8	1.0 ± 0.3
Cd	μg L^{-1}	0.13	0.08 ± 0.03
Pb	μg L^{-1}	3.4	1.4 ± 1.3

Experimental setup

Tolerance of the Lake Mälaren algae to high ammonia concentrations

The tolerance of the algae from the lake to high concentrations of ammonium was tested in triplicate initially. Based on the results obtained by Odlare *et al.* (2011), 10 ml of Jaworski's Medium (JM) was added to 100 ml of lake water, which also served as a source of algae. To simulate high concentrations of NH_4+, additional NH_4Cl was added to the mixtures in order to achieve ammonium nitrogen (NH_4-N) concentrations of 50, 100, 200, 500, 900, 1,900, 2,900, 3,600 and 5,000 mg L^{-1}.

Determining the biogas digestate to lake water ratio

The algae community cultivated in biogas digestate was slowly adapted to the high ammonium concentration by

pre-cultivating the algae in a low concentration of biogas digestate first. After the algae had grown, a higher concentration of biogas digestate was added. To determine how much biogas digestate to add to the lake water to achieve maximum growth, 500 ml flasks were incubated in duplicate at 22 ± 0.5 °C with 16 hours of light and 8 hours of dark per 24 hours for 14 days at a photosynthetically active radiation (PAR) level of around 100 μmol/m^{2}s (Tang *et al.* 2011). The flasks contained 5, 10 or 15% by volume of the biogas digestate (centrate). The remainder was made up with either lake water or distilled water, which was used as a control. The best performing mixture was then mixed with 25, 30, 35 and 40% of biogas digestate by volume. The algae were incubated in 100 ml flasks with 60 ml of working volume in duplicate in the same conditions as used in the pre-cultivation.

Studying the algal growth and pollutant removal in 1L photobioreactors

The biogas digestate to lake water ratio that yielded the highest algae growth in previous experiments was re-tested, in duplicate, in two closed laboratory-scale 1 L batch photobioreactors (height 18 cm, diam. 10 cm). The reactors were repurposed commercial fermenters consisting of glass cylinders with stainless steel tops and bottoms. The openings in the top of the reactors were closed with filter paper to avoid contamination and to allow gas exchange. The reactors were lit from above by four fluorescent light tubes with 16 hours of light and 8 hours of dark per 24 hour cycle at a PAR level of around 100 μmol/m^{2}s. Mirrors were used to reflect light onto the reactor sides in order to increase lighting efficiency. The temperature in the reactors was set to 23 ± 0.5 °C (Tang *et al.* 2011) and stirring was applied at around 350 rpm (Tang *et al.* 2011). The experimental light and temperature conditions were selected based on the prevailing conditions during the maximum growth rate of microalgae in the summer. Air was pumped into the reactors through a 0.22 μm Millipore filter (3 L min^{-1}) to optimize gas exchange through aeration and to prevent excessive pH increase in the reactors by supplying CO_2. A 60 ml sample was taken every 4 days from each reactor for chlorophyll a, nutrient and heavy metal analysis.

Algae growth analysis

In the ammonia tolerance experiments, algae growth was estimated by measuring optical density at 600 nm (Hitachi, U2000); 150 μL samples were taken from each of the 15 flasks and measured in duplicate in a 96 well microplate.

For the experiments with different mixtures containing biogas digestate, algae growth was estimated by measuring chlorophyll a concentration. Then, 25 ml of the sample was filtered through Whatman GF/C (1.2 μm) filters and chlorophyll a was extracted with acetone as described in Bellinger & Sigee (2010). The samples were centrifuged at 1,000 g at 4 °C for 5 minutes and the absorbance of the supernatant was measured at 665 nm (chlorophyll a) and 750 nm (turbidity). Chlorophyll a concentration was calculated using Equation (1).

$$C = (A_{665} - A_{750}) \times \frac{V}{V_5} \times \frac{11.3}{L}, \quad (1)$$

where C is chlorophyll concentration (mg L^{-1}), V is the volume of the solvent (ml), VS 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.

The pH of the samples was measured using a 744 pH meter (Metrohm AG, Herisau, Switzerland).

Nutrient and metal analysis

Nutrient samples were filtered through Whatman GF/C (1.2 μm) filters, acidified to pH < 2 with concentrated sulphuric acid (98%) and stored at −20 °C. The NH_4-N and phosphate phosphorus (PO_4-P) concentrations in the samples were measured using FOSS FIASTAR 500 Fluid Injection Analysis as described by the procedures supplied by the company.

Heavy metals were analyzed by ICP-MS (Agilent 7500 cx). The analysed isotopes were 53Cr, 59Co, 60Ni, 63Cu, 66Zn, 75As and 111Cd. For Pb, the sum of 206, 207 and 208 isotopes was analysed to compensate for any isotope fractionation.

Due to the unknown and visually heavy matrix of the samples, the Agilent HMI (High Matrix Introduction) system was used. The system was operated in the Ultra Robust High mode to obtain good recovery, which slightly increased the detection limit, especially for As. The analyser was calibrated after every 28 samples.

RESULTS AND DISCUSSION

Tolerance of the Lake Mälaren algae to high ammonium concentrations

As expected, growth of the Lake Mälaren water culture was fastest with pure JM (Figure 1). However, the growth was

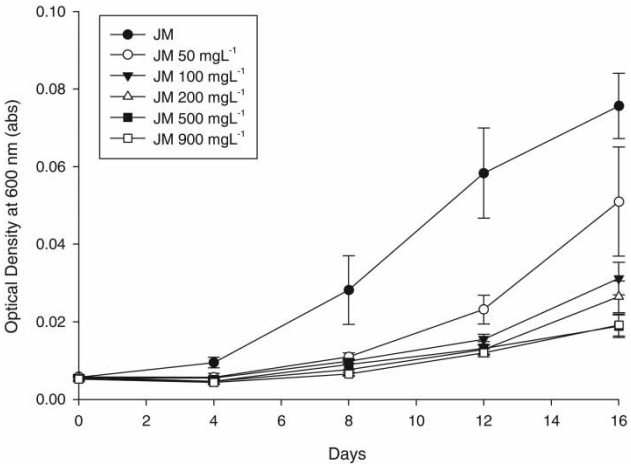

Figure 1 | Optical density in samples containing Jaworski's Medium (JM) with varying NH_4-N concentrations up to 900 mg L^{-1}. Error bars show standard deviation.

significantly slower than growth in the samples collected in the summer (Odlare *et al.* 2011). As the lake water was sampled during winter, it is possible that the algae in the water were dormant and had a lower growth rate. Previous experiments have shown that it is possible to cultivate algae from Lake Mälaren as late as November and December, but algae collected in winter require a longer adaptation period as the change of environment from lake to laboratory (4 °C to 22 °C) is more extreme (Krustok *et al.* 2015b).

In this study, growth slowed and the maximum measured OD decreased with increasing concentrations of ammonium chloride. Growth was very slow at ammonium concentrations above 200 mg L^{-1}, and there was no noticeable growth at all above 900 mg L^{-1} ammonium chloride. This is similar to the results presented by Lin *et al.* (2007). In their experiments, three different *Chlorella sp.* strains were able to grow in landfill leachate at NH_4-N concentrations up to 135 mg L^{-1}, after which growth was noticeably inhibited.

The average pH value at the start of the experiment was 7.5. During growth, the pH of the JM sample increased to 9.3 while the other samples decreased to an average pH of 5.1. It is not clear why the pH decreased so dramatically in the samples with added NH_4Cl, as the chemical itself did not affect the pH noticeably in the beginning of the experiment. However, since NH_4Cl acts as a weak acid, it is possible that at such low algae growth and high NH_4Cl concentrations, it will cause the pH to decrease.

In the JM sample, the faster removal of CO_2 as well as the formation and release of ammonia would have contributed to the increase of pH, implying that much of the ammonium had been removed. However, since there was algae growth, and the ammonium concentration at the beginning was relatively low, this effect would not have influenced the results from the other samples.

Ammonium concentration was reduced by 31.9 ± 2.0% in the samples with an initial ammonium concentration of 50 mg L^{-1} (Table 2). While the removal efficiency in these samples was low, it could be explained by the slow growth rate seen in the experiments and the absence of continuous mixing limiting the amount of nitrogen the algae had access to. Since the pH in the samples with initial ammonium concentrations of 50 mg L^{-1} remained around 7, it is likely that the ammonium was taken up by the algae. In the samples with ammonium concentration above 50 mg L^{-1}, there was very little to no ammonium uptake.

Biogas digestate and lake water ratios for optimum algal growth

Out of the tested pre-cultivation mixtures, the best growth was seen with 5% biogas digestate and 95% lake water, with an initial NH_4-N concentration of 20 mg L^{-1}. The chlorophyll a concentration increased 3-fold in this sample while it remained relatively unchanged in the samples with 10% and 15% biogas digestate. There was also no significant growth in the samples that substituted distilled water for the lake water, suggesting that the algae that grew in the lake water samples originated from the lake water.

As the 5% biogas digestate sample had the highest algae growth it was chosen as a base to determine the best ratio of pre-cultivated algae suspension to biogas digestate.

There was a significant increase in the chlorophyll a concentration in samples with 25% and 35% biogas digestate mixed with the pre-cultivated algae suspension. Chlorophyll a concentration grew 61- and 18-fold respectively in the two samples (Figure 2(a)).

The initial ammonium concentrations in samples with 25% and 35% biogas digestate were 191 mg L^{-1} and

Table 2 | NH_4-N concentrations for samples containing Jaworski's Medium (JM) with varying NH_4-N concentrations up to 900 mg L^{-1}

Sample	Day 0	Day 16	Change
JM	<0.01	<0.01	–
JM 50	47.5 ± 1.2	32.3 ± 1.7	−31.9 ± 2.0%
JM 100	94.6 ± 0.4	90.8 ± 3.8	−4.0 ± 3.9%
JM 200	184.7 ± 5.3	176.5 ± 4.8	−4.4 ± 5.1%
JM 500	462.8 ± 2.3	489.1 ± 15.5	5.7 ± 3.5%
JM 900	896.1 ± 31.6	867.9 ± 143.5	−2.8 ± 18.4%

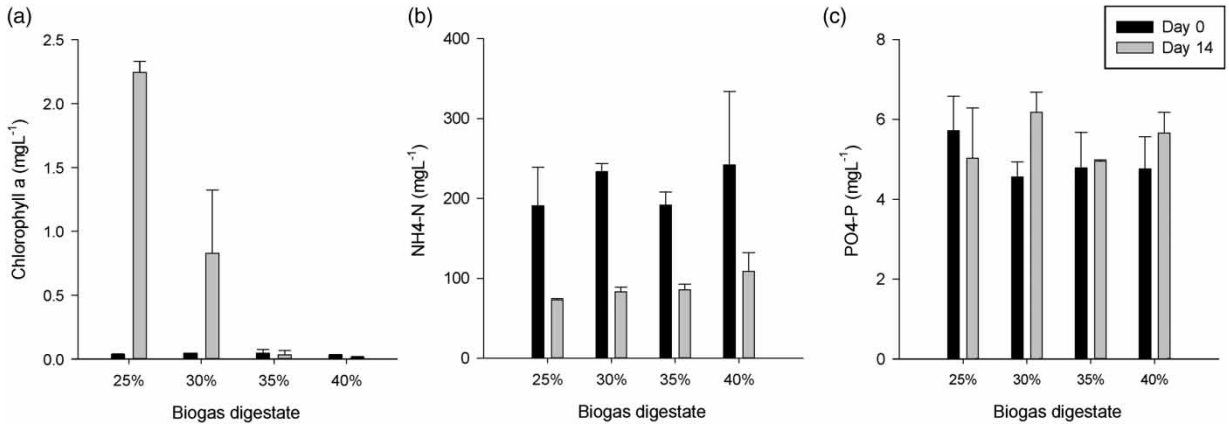

Figure 2 | Chlorophyll a (a), NH_4-N (b) and PO_4-P (c) concentrations before and after cultivation with 25%, 30%, 35% and 40% of biogas digestate mixed with pre-cultivated algae suspension and incubated for 14 days. Error bars show standard deviation.

234 mg L^{-1} respectively. These are close to the limits found in this study and previously reported by Lin *et al.* (2007). After 14 days the concentrations of ammonium had fallen to 73 and 83 mg L^{-1}, resulting in a purification efficiency of 61.8% and 64.5%, respectively (Figure 2(b)).

Since the pH decreased from 8.2–8.3 to 6.9–7.2 between the beginning and the end of the experiments, the ammonium was most likely removed biologically, either by nitrification or by assimilation by the algae. Since there was no significant correlation between the reduction of ammonium and chlorophyll growth in the samples, it is likely that most of the ammonium was nitrified and not taken up by the algae. Previous studies have also suggested nitrification as the main process behind ammonium reduction in photobioreactors (Karya *et al.* 2013).

The phosphate concentration did not change significantly during the experimental period (Figure 2(c)). While the chlorophyll a growth in samples with 25 and 30% biogas digestate indicates that at least some of the phosphorus had to be taken up by the microalgae, it is probable that more phosphate was released due to other microbiological processes.

Algal growth and pollutant removal in 1L laboratory-scale photobioreactors

As the 5 and 25% biogas digestate mixtures had the highest algae growth for pre-cultivation and cultivation, respectively, they were used in 1L photobioreactors to achieve a higher reactor volume and purification efficiency. After pre-cultivation, which lasted for 8 days, the final chlorophyll a concentration in the suspension was 0.48 ± 0.11 mg L^{-1}; 25% of the volume was then replaced with biogas digestate as in previous experiments and cultivated for another 8 days resulting in a chlorophyll a concentration of 2.1 ± 0.5 mg L^{-1} (Figure 3(a)). The growth rate seemed to be higher at the end of the

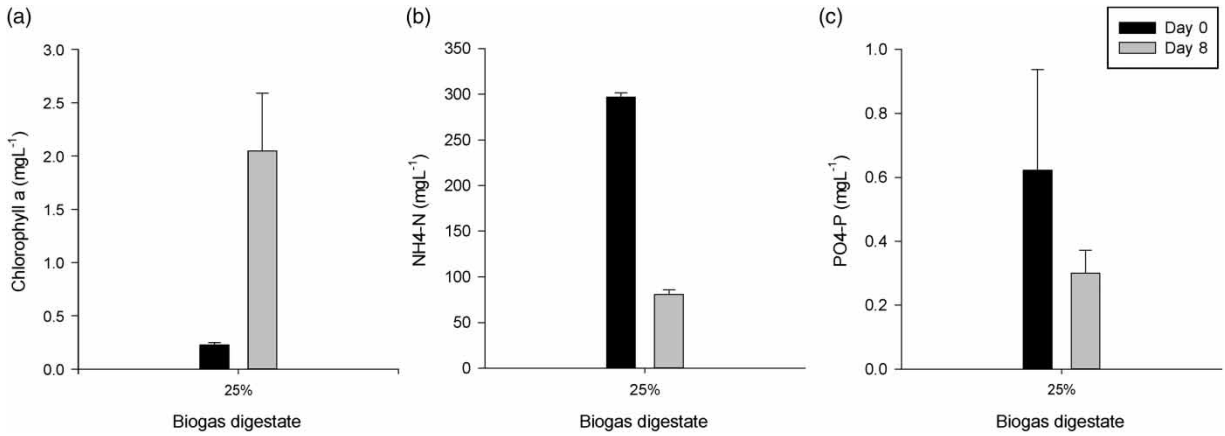

Figure 3 | Chlorophyll a (a), NH_4-N (b) and PO_4-P (c) concentrations in the cultivation step (25% biogas digestate mixed with 75% pre-cultivated algae suspension by volume). Error bars show standard deviation.

experiment, which indicates that increasing the growth period could have significantly increased the algae production. When treating municipal wastewater in a similar reactor system, comparable growth was measured in samples that were inoculated with lake water sampled in December 2012 (Krustok *et al.* 2015b). As the same research suggests, three to four times higher growth could be expected when using lake water sampled in the summer.

In the cultivation step, the algae were able to grow in 300 mg L^{-1} of NH_4-N. This is a third higher than in the previous experiments and more than twice the concentration reported by Lin *et al.* (2007) to support algal growth. This may be the result of the pre-cultivation phase where a low concentration of biogas digestate helped to select for a more stable community. In addition, while Lin *et al.* (2007) used two isolated strains of microalgae *Chlorella pyrenoidosa* and *Chlamydomonas snowiae* to treat nitrogen-rich wastewater, it can be argued that mixed consortia can be more stable in complex substrates due to the interactions between different microorganisms (Muñoz & Guieysse 2006).

NH_4-N decreased significantly in the reactors (Figure 3(b)). After pre-cultivation, the final measured concentration was 0.13 mg L^{-1} while after cultivation, 81 mg L^{-1} NH_4-N remained in the reactor, resulting in a purification efficiency of 89.9 ± 3.9 and 72.8 ± 2.2%, which is similar to the results found by Lin *et al.* (2007), although starting from a higher NH_4-N concentration.

There are several reasons for this reduction of ammonium. While ammonium may have been reduced in biological processes such as nitrification and algae uptake, the pH was between 8.1–8.6 in experiments accounting for some of the reduction in ammonium due to ammonia stripping.

The PO_4-P concentrations decreased in the experiments, however, there was a large variation in the measurements, most likely due to a relatively low concentration at the start (Figure 3(c)). Due to this, the purification efficiency varied widely, being around 41.4 ± 41.4%.

Since nitrogen and phosphorus concentrations in the treatment stage were removed to unsatisfactory levels, it is possible that the duration of the treatment phase needs to be increased. While the algae biomass increased around nine-fold during the 8-day cultivation period (Figure 3(a)), there is room for improvement as, under ideal conditions, algae can double their biomass in one day (Demirbas & Demirbas 2011). There is also a need to control the pH since an increase in pH may allow for the formation of NH_3 which inhibits the algal growth (Yuan *et al.* 2011). In future experiments, pumping CO_2 instead of air into the system may provide more robust pH control.

While the growth rate and nutrient removal in these preliminary experiments was found to be low, the algae were able to grow at very high concentrations of ammonium. Similar experiments using mixed algae and bacteria consortia could help find novel biological treatment options for waste streams with high ammonium concentrations that produce suitable effluent concentrations.

Heavy metal concentrations

The Cr, Co, As, Cd and Pb showed very little change in any of the analysed samples. During the cultivation, Ni and Cu were released into the water phase while there was little change in the Zn concentration (Figure 4). Uptake of the analysed metals by the algae was not evident in any of the samples, meaning that the produced biomass did not accumulate large amounts of heavy metal. It is possible that the metals were mobilized from complexes to the water phase by microbes (Gadd 2004). This can be a positive outcome as mobilised heavy metal ions can be easily removed by ion exchange mechanisms in the filtration of clean water after biological treatment. In general, the heavy metal concentrations were very low and the differences in concentrations may have been due to measurement errors.

CONCLUSIONS

From the experiments conducted with high ammonium JM, it was determined that the algae were able to grow well in ammonium concentrations of up to 200 mg L^{-1}. At higher concentrations, there was a significant inhibition in

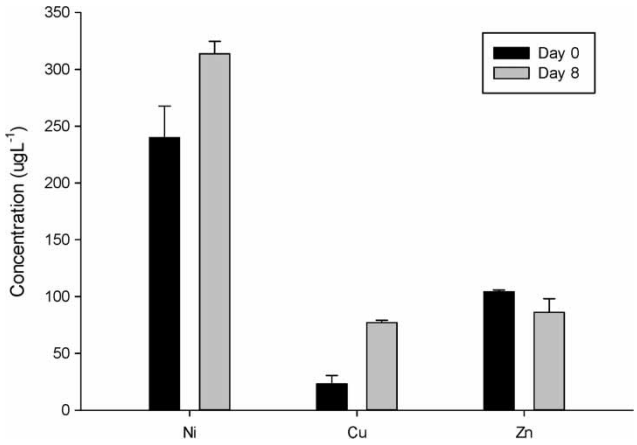

Figure 4 | Nickel, copper and zinc concentrations at the beginning and end of algae cultivation with 25% biogas digestate mixed with a pre-cultivated algae suspension. Error bars show standard deviation.

growth. This means that high ammonium waste streams such as wastewater treatment plant biogas digestate could be treated with algae, especially when the algae are pre-treated to select for more ammonium-tolerant strains.

Algae were able to grow in biogas digestate with NH_4-N concentrations up to 300 mg L^{-1} when a pre-cultivation step was used. This means that other waste streams such as swine waste and landfill leachate could also be considered as potential mediums for algae growth. Ammonium and phosphorus concentrations both decreased, but not sufficiently to meet local and EU directives for wastewater treatment effluent. The cultivation period in the experiments appeared to be too short as the algae were still growing at the end of the treatment. With longer retention times or higher growth rates, it is possible that more of the nutrients would be removed from the system.

While Cr, Co, As, Cd and Pb showed no significant difference in concentration before and after the cultivation, the concentration of free ions of Cu, Ni and Zn in the water phase increased with the algae growth in some samples. However, all the measured heavy metal levels were very low and the differences could be due to measurement error.

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