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Combined remediation and lipid production using *Chlorella sorokiniana* grown on wastewater and exhaust gases

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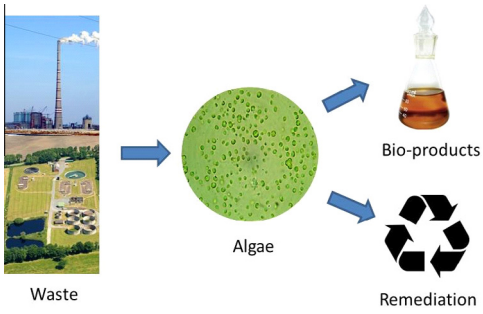
HIGHLIGHTS

- *Chlorella sorokiniana* can grow phototrophically using wastewater and exhaust gas.
- Biomass yields from waste streams were comparable to commercial media.
- Lipid production was highest in the final effluent augmented with 12% CO_2.
- CO_2 addition improved the rate of nitrogen removal in both wastewater types.
- The cultures removed 20–30% of CO, 30–45% of CO_2 and 95–100% of NO_x.

GRAPHICAL ABSTRACT



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ABSTRACT

Substitution of conventional feedstock with waste based alternatives is one route towards both remediation and reducing costs associated with production of algal biomass. This work explores whether exhaust gases and wastewater can replace conventional feedstock in the production of biomass from *Chlorella sorokiniana*. Exhaust gases were used to augment production in final effluent, anaerobic digester centrate or in standard medium. Cultures were grown in 1 L bottles under illumination of 80 μmol m^{-2} - s^{-1}. The results showed an average μ_{max} ranging between 0.04 and 0.07 h^{-1}, whilst the final biomass yield in different media ranged between 220 and 330 mg L^{-1}. Lipid yield was increased over time to 31 mg L^{-1}. CO_2 addition resulted in complete nitrogen removal between 48 and 96 h in both final effluent and centrate. The results also indicated that levels of carbon monoxide, carbon dioxide and nitrogen oxides in the exhaust gases can be reduced by between 20% and 95%.

1. Introduction

Large scale production of algal bio-products currently faces a number of cost related bottlenecks (Campbell et al., 2011; Gallagher, 2011; Lee, 2011). Prominent concerns include feedstock requirements and cultivation strategies, as well as the necessity for energy-intensive growth and harvesting methods (Greenwell et al., 2010). One promising approach to achieve cost and energy reductions during production is to integrate algal facilities within existing industrial or waste treatment activity. For example, co-location of an algal process with power plants or wastewater treatment facilities would allow for the utilisation of feedstock and waste streams. This type of industrial symbiosis can result in greatly reduced economic and environmental cost, whilst also performing valuable remediation services.

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Nomenclature

BBM	Bold's basal medium	FE	Final effluent
CO	Carbon monoxide	NO*x*	Nitrogen oxides
CO_2	Carbon dioxide		

Numerous studies have been undertaken which show that traditional feedstock for algal cultivation can be replaced with a variety of waste derived alternatives (Muñoz and Guieysse, 2006). One particularly suitable source of feedstock is the wastewater industry, which already utilises mixed algal consortia within conventional treatment processes (Oswald, 1988). This is because the nutrients required for algal growth (such as compounds of nitrogen, phosphorous, trace metals and vitamins) can often be sourced directly from secondary or tertiary wastewater (Greenwell et al., 2010). Unsurprisingly, the composition of the wastewater has a critical impact on algal cultivation. Nitrogen content is of particular importance, as both a key macronutrient and a trigger for lipid accumulation in algal cells, making it an attractive benchmark when selecting waste feedstock. Some of the highest levels of nitrogen can be found in the anaerobic digester centrate (or centrifuged supernatant), often in the form of ammonia and ammonium ions, making it a particularly favourable feedstock (Pittman et al., 2011; Wang et al., 2010). Another prominent source of nitrogen can be found in the final effluent discharged from the wastewater treatment process, albeit at lower concentrations.

2The effects of augmenting algal cultures with dissolved carbon dioxide to improve overall yield are well understood (Nielsen and Jensen, 1958; Park et al., 2011). One interesting avenue is to utilise waste carbon dioxide from industrial processes to increase algal growth and thereby reduce the costs associated with cultivation. The composition of most industrial exhaust (or flue) gases varies dependent on source, but usually contains between 5% and 15% carbon dioxide, alongside oxides of nitrogen (and sulphur in the case of coal fired generators), un-burnt hydrocarbons and soot particulates. Research has shown that some species of algae can tolerate flue gas and its contaminants, without the need for any pretreatment (Doucha et al., 2005; Yoshihara et al., 1996). Reports from the literature indicate up to 95% removal efficiency of carbon dioxide from the gas input stream (Doucha et al., 2005; Vunjak-Novakovic et al., 2005). Furthermore, it can be seen that with the potential of algae to produce lipids and biomass suitable for fuels (Demirbas and Fatih Demirbas, 2011; Um and Kim, 2009; Yang et al., 2011), a virtuous cycle of carbon release and capture can be implemented.

One of the most important aspects for scaling up an algal production process is the selection of a suitable algal strain. One candidate organism is the thermo-tolerant, fast growing chlorophyte alga *Chlorella sorokiniana* (Li et al., 2013). This is a small (2–4.5 μm diameter), robust single cell alga that is capable of mixotrophic growth on various carbon sources, making it ideal for cultivation on waste feedstock. Previous findings report that optimal growth can be obtained at temperatures between 35 and 40 °C (de-Bashan et al., 2008); with phototrophic doubling times as low as 4–6 h (Janssen et al., 1999). Growth under mixotrophic conditions has been observed to be even faster, with a preference for sugars such as glucose (Wan et al., 2012) or simpler carbon sources such as acetate. The species has also been shown to be robust enough for scale up in bubble columns (Béchet et al., 2012) and tubular reactors (Lee et al., 1996). Some work has also demonstrated that *C. sorokiniana* is able to grow on wastewaters under conditions that would be unfavourable for other algal species (de-Bashan et al., 2008). It has also been reported to be capable of producing a variety of lipids, polysaccharides and other cellular products which could be of interest for bioenergy or higher value commodities (Lu et al., 2012).

Within this context, the aim of this work was to compare the growth characteristics and yield of *C. sorokiniana* on both wastewater and commercial medium, whilst assessing the influence of exhaust gas on the process. This allowed for a quantitative evaluation of the benefits of coupling biomass production to remediation.

2. Methods

2.1. Experimental set-up

C. sorokiniana UTEX1230 was obtained from the Culture Collection of Algae, University of Texas at Austen (http://web.biosci.utexas.edu/utex/) and was maintained on Bold's basal medium (BBM) obtained from chemical suppliers (Sigma–Aldrich). The experimental vessels were made by converting 1 L Duran bottles into photobioreactors. Mixing air was kept consistent throughout the experiments, and introduced into all of the bottles at 0.2 vvm (volume of air per volume of liquid sparged per minute). This was achieved by using an air compressor (Hailea) and a ceramic diffuser. Fig. 1 shows the experimental set-up in greater detail. The experiments were undertaken at 30 °C in batch, under 80 μmol m^{-2} s^{-1} of artificial light (low light conditions (de-Bashan et al., 2008)), provided by two 8 W Gro-lux lights (Sylvania). Each experimental condition was undertaken in triplicate, with growth monitored by measuring the optical density at 750 nm, and converting it to a biomass dry weight, using a calibration curve. Care was taken to prevent false readings by subtracting empty media-only values from those containing algae.

The maximum specific growth rate (μ_{max}) was calculated according to Eq. (1). Where N_1 and N_0 corresponds to the algal density at times t_1 and t_0, respectively.

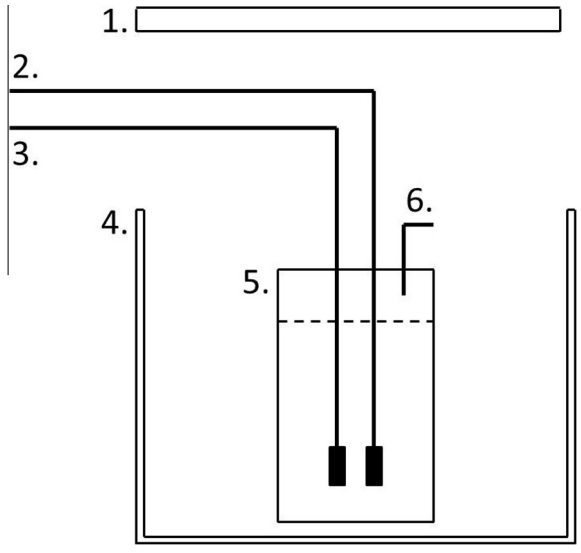

Fig. 1. Experimental apparatus. (1) Light source. (2) Mixing airline. (3) Exhaust gas line from compressor. (4) Growth chamber. (5) Culture vessel. (6) Gas and sampling outlet.

$$\mu_{max} = \frac{\ln(N_1) - \ln(N_0)}{t_1 - t_0} \quad (1)$$

Biomass and lipid productivity were calculated on a batch basis, by dividing the product yield by the total number of days within the experiment.

2.2. Flue gas composition and analysis

All cultures were mixed with atmospheric air, and half of the cultures were supplemented with exhaust gas, which was continuously bubbled at a rate of 20 cm^3 min^{-1} (Vunjak-Novakovic et al., 2005). The exhaust gas for these experiments was produced by a single cylinder diesel engine specially designed for combustion and fuels research (Ricardo Hydra with Ford Duratorque head). The gas was stored under 10 bar of pressure in a modified air compressor (Einhell). The engine was operated on a fossil diesel fuel, with zero fatty acid methyl ester (FAME) content, at a variable load condition to produce a constant exhaust gas composition of 12% CO_2. Exhaust gas sampling took place downstream of the engine using an automotive gas analyser system (Horiba MEXA9100 HEGR). The composition of the exhaust gas was determined by the following methods: NO_x concentrations were determined by chemiluminescence; CO and CO_2 concentrations by non-dispersive infrared detection, and O_2 concentrations with paramagnetic analysis (Hellier and Ladommatos, 2011). Table 1 shows the dry composition of the exhaust gas supplied to the cultures.

2.3. Media composition

Wastewater was sourced from a UK municipal treatment works dealing with domestic waste streams. The tested wastewater included final effluent generated after secondary treatment (due for discharge) and the centrate produced from the separation of solids from an anaerobic digester. BBM was used as a benchmark in the experiments, and diluted 1:50, according to manufacturer's instructions (Sigma). The wastewater samples were autoclaved at 121 °C for 15 min, and diluted 1:10 with deionised water for the purposes of the experiment. Table 2 contains further details regarding the chemical composition of the media.

2.4. Ion chromatography of wastewater and commercial media

Ion chromatography (IC) was performed to analyse the remediation potential of *C. sorokiniana* in terms of reducing nitrate, phosphate and sulphate levels. The runs were undertaken on a KS-1100 IC instrument (Dionex), using an AS23 4 × 250 mm carbonate eluent anion-exchange column (Dionex). Anion mode analysis was carried out according to the manufacturer's recommendations, using a mobile phase of 4.5 mM Na_2CO_3. The flow rate was set at 1 mL min^{-1}, with a total run time of 30 min and temperature held at 30 °C. Cation analysis was undertaken using an IonPac CS16–5 μm (5 × 250 mm) column with 30 mM methanesulfonic acid as the eluent. The flow rate was set at 1 mL min^{-1}, with a total run time of 25 min and temperature held at 40 °C. Detection of ion peaks in both conditions was undertaken by suppressed conductivity measurements at 25 mA. The spectra were analysed using a set of standards and software provided by Dionex.

2.5. Conductivity and pH

Conductivity and pH were measured to better ascertain some of the key changes in characteristics within the wastewater. Conductivity was measured using an S230 conductivity meter (Mettler Toledo). The pH change in the media was monitored during the course of the experiment with a pH probe (Mettler Toledo).

2.6. Total dry weight and lipid analysis

The biomass produced during batch growth was concentrated by centrifugation (10 min at 4370*g*), washed and lyophilised prior to weighing. Lipid accumulation was assessed by fluorescence spectroscopy using the fluorescent dye, Nile Red (Cooksey et al., 1987). Staining was performed by adding Nile Red to culture samples to a final concentration of 2 μg/mL. Fluorescence was measured using a Perkin–Elmer LS-55 Luminescence Spectrometer with the excitation wavelength set at 510 nm and the emission scanned between 530 and 750 nm. Comparison to a Triolein standard (Sigma) was used for estimation of total lipid levels.

2.7. Data analysis

Data was analysed and plotted on Microsoft Excel 2010. Triplicate results display error bars with 1 standard deviation. Significant differences between each treatment condition (+CO_2 and −CO_2) were analysed at 96 h by one-way ANOVA with a statistical significance of $P \leqslant 0.05$.

Table 1
Mean dry exhaust gas composition according to media type.

Media type	Mean dry exhaust gas composition				
	CO (ppm)	CO_2 (%)	O_2 (%)	THC (ppm)	NO_x (ppm)
Bold's basal medium	5766.5	11.72	4.06	207.9	555.7
Final effluent	2216.4	12.02	4.11	119.9	613.5
Centrate	5009.6	11.65	4.48	126.6	555.3

Table 2
Characteristics of growth media after dilution.

Media type	Media characteristics				
	pH	Conductivity (μS/cm)	Total (*N*) (mg L^{-1})	Total (*P*) (mg L^{-1})	TOC (mg L^{-1})
Bold's basal medium	6.32	778.5	34	47	0.35
Final effluent	7.40	161.4	8	2.6	2.1
Centrate	9.47	262	53	9.4	9.56

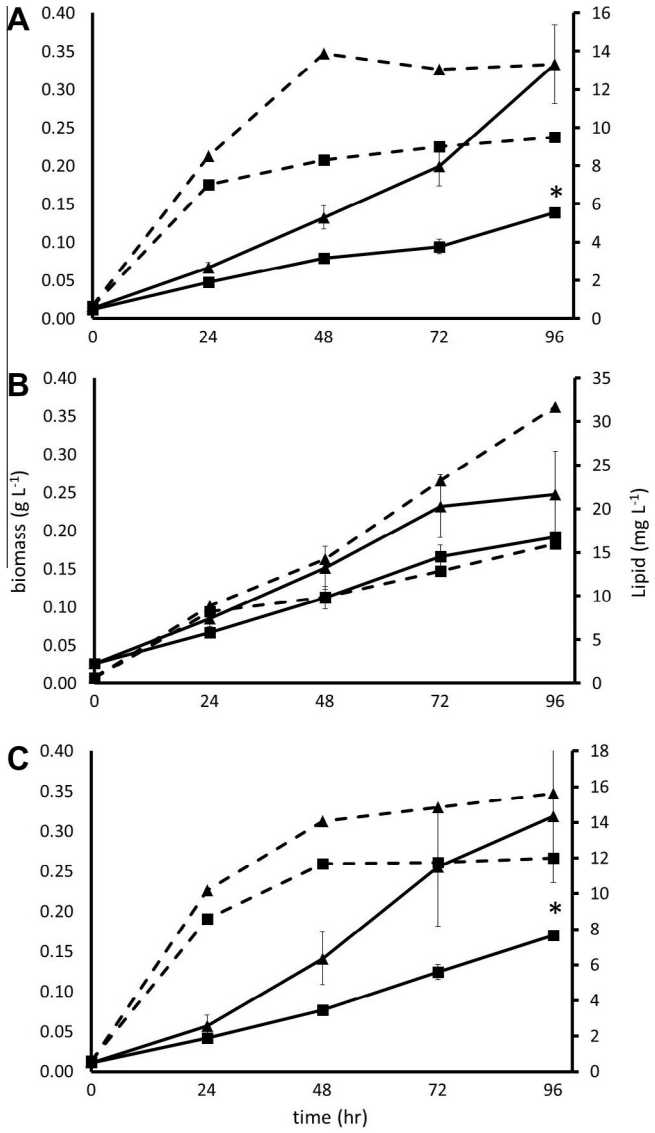

Fig. 2. Growth of *C. sorokiniana* on the different wastewaters and commercial media. (A) Bold's basal medium. (B) Final Effluent. (C) Centrate. Triangles represent media augmented with exhaust gas containing 12% carbon dioxide; Squares show media that has not received any additional carbon dioxide. Solid lines, on the left axis showing biomass concentration; Dashed lines, on the right axis showing lipid concentration. $n = 3$, error bars show 1 standard deviation from the mean. An asterisk denotes significant differences between the yield of $\pm CO_2$ conditions, $P \leqslant 0.05$.

3. Results and discussion

3.1. Growth on wastewater

The growth curves in Fig. 2 show the biomass accumulation and lipid productivity of *C. sorokiniana* on the tested media; both with and without the addition of 12% CO_2 enriched exhaust gas. The results on BBM (Fig. 2A) indicate there is a marked increase ($P \leqslant 0.05$) in biomass yield under conditions of exhaust gas sparging compared to the non-enriched condition. After 96 h growth, the CO_2-supplemented cultures gave an average final biomass yield of 330 mg L^{-1} s.d. ±50, whilst the control gave a final biomass yield of 140 mg L^{-1} s.d. ±3 (productivity of 82.5 and 35.5 mg L^{-1} - day^{-1}, respectively). The μ_{max} of the CO_2 supplemented culture was found to be 0.07 h^{-1}, whilst that of the non-enriched condition was 0.06 h^{-1}. Neutral lipid concentration increased over the course of the experiment, with the CO_2 augmented condition giving a final yield of 13 mg L^{-1} against 9.5 mg L^{-1} in the non-augmented condition (productivity of 3.25 and 2.38 mg L^{-1} day^{-1}, respectively).

The results for growth on final effluent (Fig. 2 B) show that after 96 h a final biomass yield of 250 mg L^{-1} s.d. ±56 is obtained, whilst the control showed a final biomass yield of 220 mg L^{-1} s.d. ±58 (productivity of 62.5 and 55 mg L^{-1} day^{-1}, respectively). No statistical difference was found between the two biomass yields. The μ_{max} of the CO_2 supplemented culture was 0.05 h^{-1}, whilst that of the control was 0.04 h^{-1}. Lipid yield increased after 48 h, peaking at 32 mg L^{-1} in the CO_2 supplemented condition and 16 mg L^{-1} in the non CO_2 enriched condition (productivity of 8 and 4 mg L^{-1} - day^{-1}, respectively).

The results in Fig. 2C show growth on anaerobic digester centrate. Again, the results demonstrate a significant difference ($P \leqslant 0.05$) in biomass yield after 96 h under conditions of exhaust gas sparging when compared to control. The CO_2 supplemented culture showed a final biomass yield of 320 mg L^{-1} s.d. ±83, whilst

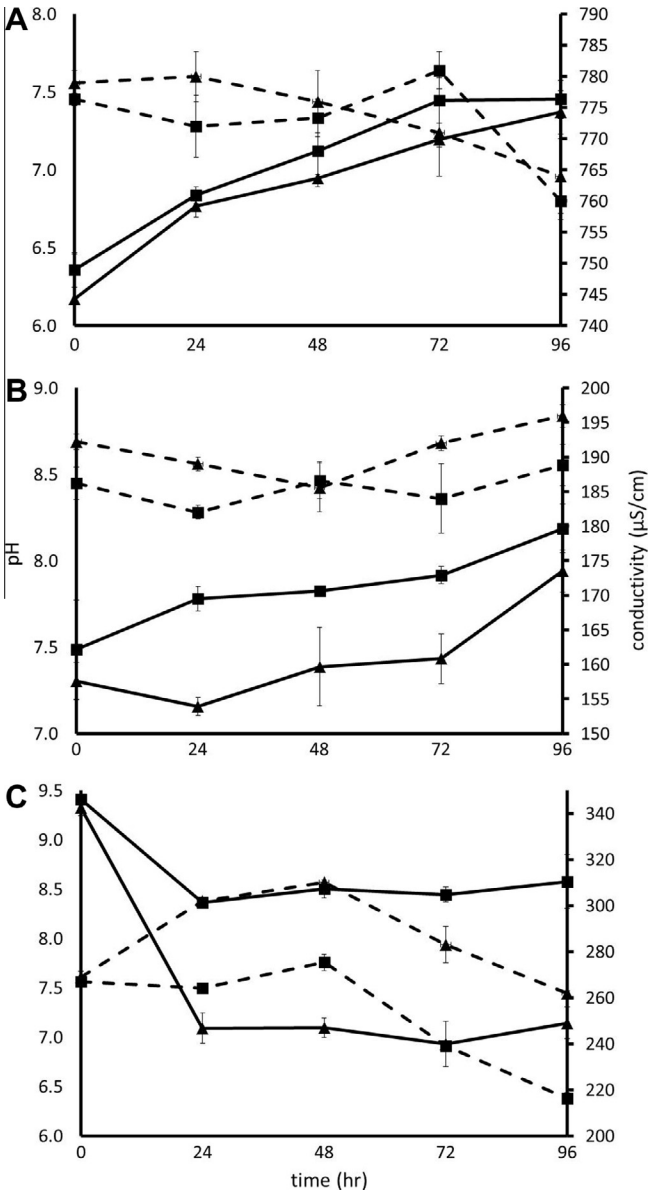

Fig. 3. Effect of algal growth on pH and conductivity within the different media types. (A) Bold's basal medium. (B) Final Effluent. (C) Centrate. Triangles indicate media augmented with 12% carbon dioxide; Squares have not received any additional carbon dioxide. Solid lines correspond to the pH represented on the left axis. Dashed lines correspond to the conductivity on the right axis. $n = 3$, error bars show 1 standard deviation from the mean.

the non CO_2 enriched group showed a final biomass yield of 170 mg L^{-1} s.d. ±20 (productivity of 80 and 42.5 mg L^{-1} day^{-1}, respectively). The μ_{max} of the CO_2 supplemented culture was 0.07 h^{-1}, whilst that of the control group was 0.05 h^{-1}. Neutral lipid yield showed a similar trend to the other experiments; with a 16 mg L^{-1} total in the CO_2 enriched condition and 12 mg L^{-1} in the non-augmented condition (productivity of 4 and 3 mg L^{-1} - day^{-1}, respectively).

The results from the *C. sorokiniana* growth experiments show that the strain can perform in a manner comparative to commercial media when grown on either wastewater final effluent or centrate under batch conditions. Growth of the strain is clearly assisted with flue gas addition, in good agreement with previous studies (Azov et al., 1982). It is also interesting that the *C. sorokiniana* seems tolerant of the various contaminants contained within the gas. The maximal growth rates and biomass yields were within a similar range over 96 h, but the BBM and anaerobic digester centrate were shown to be superior in terms of growth rate and yield compared to FE. This could be attributed to the higher nutrient levels found within these media types. It was also noted that larger productivity differences were seen between CO_2 sparged and non CO_2 sparged conditions in these richer media types. This indicates that growth can be augmented considerably when nutrient levels are sufficient.

Lipid yield was found to increase during the course of all experiments, but productivity was found to be higher in the cultures bubbled with 12% CO_2. This extra dissolved carbon has a dual effect in both augmenting growth, as well as providing excess carbon flux towards lipid production (Widjaja et al., 2009). Despite this increase, the total lipid productivity was probably underestimated slightly due to the preference of Nile Red to partition into highly hydrophobic environments. This causes it to fluoresce to a greater degree in the presence of intracellular neutral lipid droplets, as opposed to cellular membrane lipids (Greenspan et al., 1985). The highest lipid productivity was found in the final effluent condition, showing almost double the lipid yield over 96 h when compared to the other media types. This can be attributed to the lower concentration of nitrogen within the final effluent; resulting in rapid nitrogen starvation over the course of the experiment. The resulting stress response shown by *C. sorokiniana* causes lipid production and chlorosis to be triggered after 48 h, according to well understood mechanisms (Rodolfi et al., 2009).

3.2. Effect on pH and conductivity

The data in Fig. 3 illustrates the effect that algal growth can have on the pH and conductivity of the growth medium. Fig. 3A shows that in BBM the pH rises from 6.4 to 7.5 in the 12% CO_2 augmented culture, whilst the pH rises from 6.2 to 7.3 in the non-augmented culture. During this trajectory there is very little difference between the two conditions at several time points. Fig. 3B indicates that in the final effluent the pH rises from 7.5 to 8.2 in the 12% CO_2 condition, whilst the pH rises from 7.3 to 7.9 in the non 12% CO_2 condition. Fig. 3C shows that in anaerobic digester centrate the pH drops from 9.4 to 7.0 in the 12% CO_2 set-up, whilst the pH also drops from 9.4 to 8.5 in the naturally aerated set-up.

The measurement of conductivity in the BBM (Fig. 3A) shows that over the course of the experiment the conductivity remains fairly consistent, with a small drop from 779 to 764 μS/cm in the 12% CO_2 condition. A similar trend is seen in the non 12% CO_2 condition, which drops from 776 to 760 μS/cm. The results from the Final Effluent (Fig. 3B) show that the conductivity maintains an almost consistent level from 193 to 196 μS/cm in the +CO_2 cultures. A similar trend is seen with the −CO_2 cultures, which fluctuate from 186 to 189 μS/cm. The conductivity within Centrate (Fig. 3C) drops from 269 to 261 μS/cm in the +CO_2 condition, with a

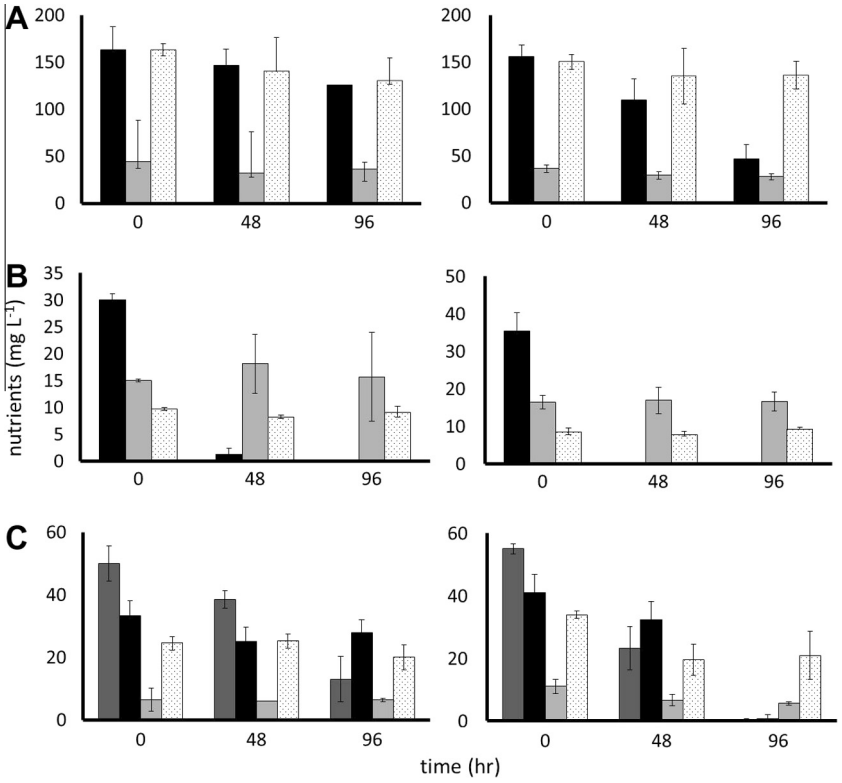

Fig. 4. Level of nutrients in wastewaters and media. (A) Growth on Bold's basal medium. (B) Growth on Final Effluent. (C) Growth on anaerobic digester centrate. Black columns represent levels of nitrates, light grey columns represent levels of sulphate; dotted columns represent phosphate and dark grey columns represent ammonia. Graphs in the left column have no addition of carbon dioxide, and graphs in the right column have the addition of 12% carbon dioxide. $n = 3$, error bars show 1 standard deviation from the mean.

peak at 310 μS/cm at 48 h. A similar but less pronounced trend is seen with the $-CO_2$ cultures, which show fluctuations from 270 to 215 μS/cm.

The pH rise found in the final effluent and BBM could be attributed to the growth of *C. sorokiniana*, and the resultant uptake of dissolved carbon species such as CO_2, from dissolved bicarbonate, leaving OH^- species under nitrate growth conditions. Likewise, the general pattern in the non CO_2 augmented cultures of higher pH levels can be explained by less dissolved carbon dioxide and fewer resultant dissociated H^+ ions. The smaller difference in pH between control and experimental bottles in the BBM is most likely due to the presence of the phosphate buffer within the medium. Interestingly, the pH is found to rapidly drop in the anaerobic digester centrate, which is due to the increased solubility of CO_2 under alkaline conditions, resulting in the production of H+ species during photosynthesis on NH_4^+. These findings show how an algal process could be used within wastewater treatment to either raise or decrease pH levels, dependent on inputs. Such pH changes could act as a 'bolt on' pre- or post-treatment step. For example, an increase in pH can be used as a sterilisation step during a wastewater treatment process (Park et al., 2011).

The higher conductivity found in the BBM reflects the greater concentration of dissolved ions in solution, whilst the dilutions of Final Effluent and centrate have overall lower levels of conductivity. These results show that the growth of *C. sorokiniana* does not appear to have a particularly marked effect on the overall conductivity of the media types, although a slight decrease is seen over time. These findings would suggest that algal growth has a low overall impact on total dissolved solid levels. However, it is interesting that the growth kinetics of *C. sorokiniana* do not seem to be overly affected by the wide range of ionic concentrations found in different media types, again suggesting suitability for use in wastewater treatment.

3.3. Nutrient uptake and removal

Fig. 4 shows how the levels of nutrients within the growth media are affected during the course of 96 h of cultivation. Fig. 4A shows the levels of nutrients in the BBM with nitrate levels around 160 mg L^{-1} at the start of cultivation. Over the course of 96 h nitrate can be reduced by 23% in the non CO_2 augmented condition and by 70% with the addition of CO_2. In both conditions the levels of phosphate and sulphate remain around 150 and 40 mg L^{-1}, respectively, with little sign of removal. Fig. 4B shows the levels of nutrients in the final effluent. The results demonstrate that the nitrate levels can be reduced by almost 100% in 48 h (from 30 to 35 mg L^{-1}) in the non-augmented condition and can be completely removed in the 12% carbon dioxide augmented condition. No ammonium ions were found in the FE, indicating a completely nitrified waste stream. In both conditions, the levels of phosphate and sulphur remain almost constant, and fluctuate around 15 and 10 mg L^{-1}, respectively. Fig. 4C depicts the levels of nutrients in the anaerobic digester centrate. The results show that the ammonium and nitrate levels were reduced by close to 100% (from a starting concentration of 55 mg L^{-1}) in the augmented condition after 96 h. In the non-augmented condition ammonium concentrations were reduced by close to 65%, whilst nitrate levels remained unchanged (starting from a concentration between 35 and 40 mg L^{-1}). In the experimental and control experiments, the levels of phosphate and sulphur fluctuate around 20–35 and 5–10 mg L^{-1}, respectively.

The results demonstrate that nitrogen can be successfully and rapidly removed by *C. sorokiniana* from waste streams, whether in the form of ammonia or nitrate. The findings also show that when the cultures are augmented with waste carbon dioxide, higher removal rates are achievable, reducing removal times to between 48 and 96 h. The findings also indicate that *C. sorokiniana* has a preference for nitrogen in ammonium form as opposed to nitrate, as demonstrated by earlier uptake within the centrate. This conforms to a metabolic preference for reduced nitrogen species that is common within many types of algae, and has been documented within this particular strain (Perez-Garcia et al., 2011).

It is interesting that in all types of media there is little indication of phosphate or sulphate uptake. The sulphate findings can be attributed to a comparatively low biological requirement for the element, obscured by diminished accuracy of the IC column in resolving 'dirtier' and more complex types of media. The phosphate traces are unlikely to be caused solely by IC insensitivity and the lack of observed depletion may be the result of previously stored phosphorous carried over into the experimental media. Considerable evidence exists regarding the ability of algae to store phosphorous beyond required levels, although to our knowledge this has not been demonstrated in this particular strain (Aitchison and Butt, 1973). These findings are of particular interest for the practical application of nutrient removal utilising algae, as it would suggest that the algae should be 'starved' before applying to waste media.

3.4. Flue gas scrubbing

Fig. 5 shows the level of scrubbing that can occur by diffusing the exhaust gas through the algal growth medium. The findings from the centrate are shown herein, as these findings were the most striking of the tested media types. The results in Fig. 5A indicate that algae grown on final effluent can reduce carbon monoxide levels by 25–30% by the end of the experiment. According to

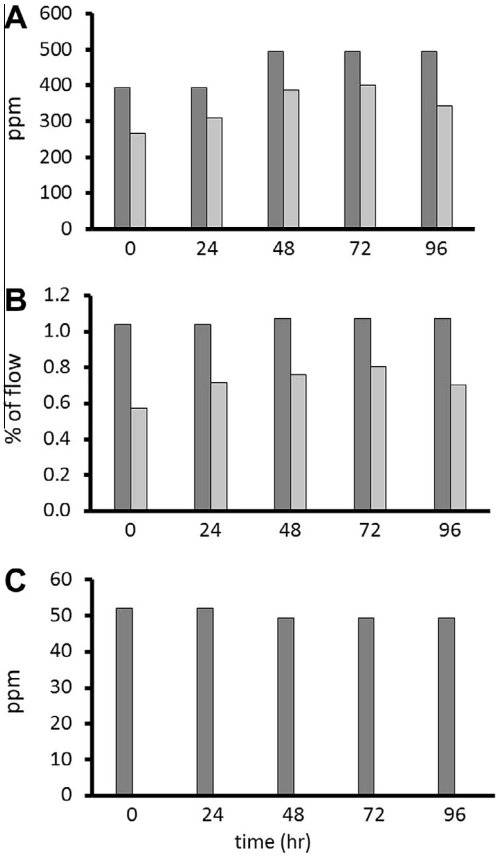

Fig. 5. Exploration of the scrubbing potential from a culture of centrate grown algae. (A) The ppm of carbon monoxide entering and leaving the reactor. (B) The percentage of carbon dioxide entering and leaving the reactor. (C) The ppm of NO_x entering and leaving the reactor. Dark grey bars represent the pollutant stream entering the reactor. Lighter grey bars represent the off-gas stream. Data is from a single culture.

data in Fig. 5B the cells are also capable of removing between 23% and 45% of the carbon dioxide entering the system over the course of the experiment. Furthermore, Fig. 5C indicates that NO_x is almost completely absent from the exiting exhaust gas during all time points, except in small quantities at 48 h. The other media types were tested and found to give similar, albeit less marked results (data not shown).

These findings show that a reasonably high level of flue gas scrubbing can be achieved within a relatively simple system, with a liquid height not too dissimilar from that of an open pond. Removal rates are in a lower range than some other findings within the literature (Doucha et al., 2005; Vunjak-Novakovic et al., 2005), although these researchers appear to have optimised gas flow rate specifically for gaseous contaminant reduction. The results presented herein are more likely to reflect realistic removal during a batch operation; especially when factors such as pH control, variable feed gas composition and flow rate are taken into account. Despite this, it is likely that the levels of gas absorption by the liquid and algal culture could be optimised further by adjusting a combination of gas flow rate, algal concentration and ion concentration within the medium. The superior removal found in the centrate as opposed to the other media types is most probably due to the higher starting pH, which gives a greater potential for neutralisation of the more acidic gases. However, given the low initial concentrations of gases such as NO_x present in the flue gas (<1000 ppm), it is not possible to conclude whether removal can be attributed in its entirety to the biological or aqueous components of the system (Svensson et al., 1987).

One consideration arising from this work is the challenge of optimising both biomass production and feedstock remediation. This is perhaps one of the greatest problems with deploying a combined production and remediation process at larger scale, due to potential conflicts between the two processes. It is most probably inevitable that one strand will take precedence over the other, dependent on the principal desired outcome.

4. Conclusions

C. sorokiniana is sufficiently robust to be grown on diluted wastewater augmented with flue gas (containing 12% CO_2). Biomass yields and lipid production were found to increase with CO_2 addition. It was also determined that nitrogen could be removed within 96 h in both wastewaters with 12% CO_2 addition. Growth in centrate media achieved CO_2 and CO reductions between 23–45% and 25–30%, respectively. NO_x was almost completely absent from the off-gas stream after scrubbing. These results indicate that waste feedstock is a good replacement for conventional media, whilst also demonstrating the potential benefits of synergistic production and remediation processes.

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References

Aitchison, P.A., Butt, V.S., 1973. The relation between the synthesis of inorganic polyphosphate and phosphate uptake by Chlorella vulgaris. J. Exp. Bot. 24, 497–510.

Azov, Y., Shelef, G., Moraine, R., 1982. Carbon limitation of biomass production in high-rate oxidation ponds. Biotechnol. Bioeng. 24, 579–594.

Béchet, Q., Muñoz, R., Shilton, A., Guieysse, B., 2012. Outdoor cultivation of temperature-tolerant *Chlorella sorokiniana* in a column photobioreactor under low power-input. Biotechnol. Bioeng. 110, 118–126.

Campbell, P.K., Beer, T., Batten, D., 2011. Life cycle assessment of biodiesel production from microalgae in ponds. Bioresour. Technol. 102, 50–56.

Cooksey, K.E., Guckert, J.B., Williams, S.A., Callis, P.R., 1987. Fluorometric determination of the neutral lipid content of microalgal cells using Nile Red. J. Microbiol. Meth. 6, 333–345.

de-Bashan, L.E., Trejo, A., Huss, V.A.R., Hernandez, J.-P., Bashan, Y., de-Bashan, L.E., Trejo, A., Huss, V.A.R., Hernandez, J.-P., Bashan, Y., 2008. *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Bioresour. Technol. 99, 4980–4989.

Demirbas, A., Fatih Demirbas, M., 2011. Importance of algae oil as a source of biodiesel. Energy Convers. Manage. 52, 163–170.

Doucha, J., Straka, F., Lívanský, K., 2005. Utilization of flue gas for cultivation of microalgae, Chlorella sp in an outdoor open thin-layer photobioreactor. J. Appl. Phycol. 17, 403–412.

Gallagher, B.J., 2011. The economics of producing biodiesel from algae. Renew. Energy 36, 158–162.

Greenspan, P., Mayer, E.P., Fowler, S.D., 1985. Nile red: a selective fluorescent stain for intracellular lipid droplets. J. Cell. Biol. 100, 965–973.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2010. Placing microalgae on the biofuels priority list: a review of the technological challenges. J. R. Soc. Int. 7, 703–726.

Hellier, P., Ladommatos, N., 2011. The impact of saturated and unsaturated fuel molecules on diesel combustion and exhaust emissions. SAE J. Fuels. Lubr. 5, 106–122.

Janssen, M., Kuijpers, T.C., Veldhoen, B., Ternbach, M.B., Tramper, J., Mur, L.R., Wijffels, R.H., 1999. Specific growth rate of Chlamydomonas reinhardtii and *Chlorella sorokiniana* under medium duration light/dark cycles: 13–87 s. J. Biotechnol. 70, 323–333.

Lee, D.H., 2011. Algal biodiesel economy and competition among bio-fuels. Bioresour. Technol. 102, 43–49.

Lee, Y.-K., Ding, S.-Y., Hoe, C.-H., Low, C.-S., 1996. Mixotrophic growth of *Chlorella sorokiniana* in outdoor enclosed photobioreactor. J. Appl. Phycol. 8, 163–169.

Li, T., Zheng, Y., Yu, L., Chen, S., 2013. High productivity cultivation of a heat-resistant microalga *Chlorella sorokiniana* for biofuel production. Bioresour. Technol. 131, 60–67.

Lu, S., Wang, J., Niu, Y., Yang, J., Zhou, J., Yuan, Y., 2012. Metabolic profiling reveals growth related FAME productivity and quality of *Chlorella sorokiniana* with different inoculum sizes. Biotechnol. Bioeng. 109, 1651–1662.

Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res. 40, 2799–2815.

Nielsen, E.S., Jensen, P.K., 1958. Concentration of carbon dioxide and rate of photosynthesis in *Chlorella pyrenoidosa*. Physiol. Plant. 11, 170–180.

Oswald, W., 1988. Micro-algae and waste-water treatment. Micro-algal Biotechnol., 305–328.

Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol. 102, 35–42.

Perez-Garcia, O., Escalante, F.M.E., de-Bashan, L.E., Bashan, Y., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. Water Res. 45, 11–36.

Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresour. Technol. 102, 17–25.

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102, 100–112.

Svensson, R., Ljungström, E., Lindqvist, O., 1987. Kinetics of the reaction between nitrogen dioxide and water vapour. Atmos. Environ. 21, 1529–1539.

Um, B.-H., Kim, Y.-S., 2009. Review: a chance for Korea to advance algal-biodiesel technology. J. Ind. Eng. Chem. 15, 1–7.

Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I., Merchuk, J.C., 2005. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. Ind. Eng. Chem. Res. 44, 6154–6163.

Wan, M.-X., Wang, R.-M., Xia, J.-L., Rosenberg, J.N., Nie, Z.-Y., Kobayashi, N., Oyler, G.A., Betenbaugh, M.J., 2012. Physiological evaluation of a new *Chlorella sorokiniana* isolate for its biomass production and lipid accumulation in photoautotrophic and heterotrophic cultures. Biotechnol. Bioeng. 109, 1958–1964.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., Ruan, R.R., 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresour. Technol. 101, 2623–2628.

Widjaja, A., Chien, C.-C., Ju, Y.-H., 2009. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. J. Taiwan Inst. Chem. Eng. 40, 13–20.

Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour. Technol. 102, 159–165.

Yoshihara, K.-I., Nagase, H., Eguchi, K., Hirata, K., Miyamoto, K., 1996. Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivated in a long tubular photobioreactor. J. Ferment. Bioeng. 82, 351–354.