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

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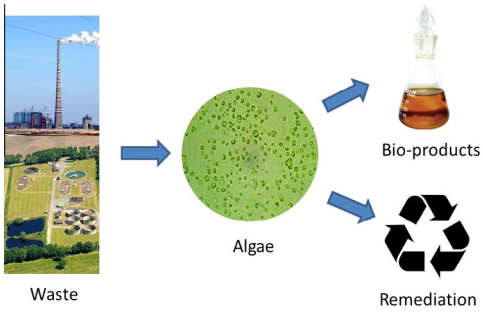
HIGHLIGHTS

- *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.

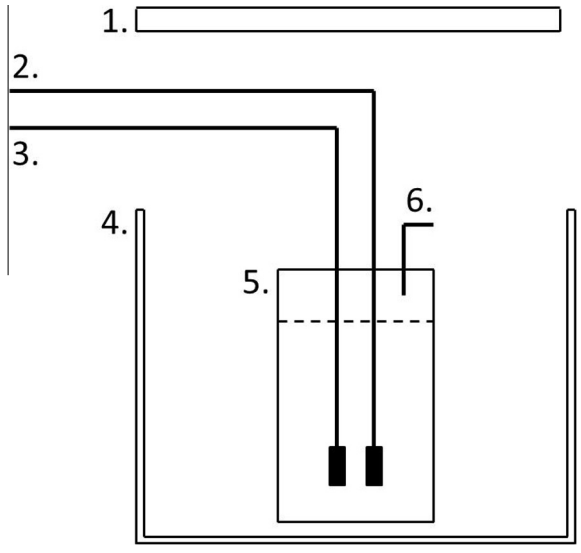

Fig. 1. Experimental apparatus. (1) Light source. (2) Mixing airline. (3) Exhaust gas line from compressor. (4) Growth chamber. (5) Culture vessel. (6) Gas and sampling outlet.