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Bioremediation of Aluminium from the Waste Water of a Conventional Water Treatment Plant Using the Freshwater Macroalga *Oedogonium*

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Abstract: Conventional water treatment processes use aluminium sulphate (alum) as a coagulant in the production of potable water. While alum is an inexpensive and reliable means of treating water, the process generates waste water containing dissolved Al. This waste water is primarily dealt with via on-site retention. In this study we investigate the cultivation of the freshwater macroalga *Oedogonium* as a means to sequester dissolved Al from waste water from a conventional water treatment plant. Furthermore, we examine the use of CO_2 to manipulate the pH of cultivation as a means of enhancing the sequestration of Al by either increasing the productivity of *Oedogonium* or increasing the bioavailability of Al in the waste water. The relative bioavailability of Al under conditions of CO_2 and no-CO_2 provision was contrasted by comparing Al uptake by Diffusive Gradients in Thin Films (DGTs). *Oedogonium* was able to grow rapidly in the waste water (12 g dry weight m^{-2} day^{-1}) while consistently sequestering Al. The *Oedogonium*-treated waste water had a sufficiently low Al concentration that it could be used in unrestricted irrigation in the surrounding region. When CO_2 was added to the waste water containing concentrations of Al up to 8 mg L^{-1}, there was a slight increase (~10%) in the rate of sequestration of Al by *Oedogonium* relative to waste water not receiving CO_2. This was due to two concurrent processes. The provision of CO_2 increased the productivity of *Oedogonium* by 15% and the bioavailability of Al by up to 200%, as measured by the DGTs. Despite this strong effect of CO_2 on Al bioavailability, the increase in Al sequestration by *Oedogonium* when CO_2 was provided was modest (~10%). Al was sequestered by *Oedogonium* to concentrations below permissible limits for discharge without the need for the addition CO_2. The cultivation of *Oedogonium* in waste water from conventional treatments plants can simultaneously treat waste water for re-use and provide a biomass source for value-added applications.

Keywords: conventional water treatment; bioremediation; macroalgae; aluminium; alum; coagulation; flocculent

1. Introduction

Conventional treatment of surface water is the dominant approach to producing potable water from surface water storages and supplies around the world and consists of five core steps; coagulation, flocculation, clarification, filtration and disinfection [1]. The primary stages of conventional water treatment (coagulation and flocculation) are reliant on the use of metal-salts, particularly aluminium

sulphate (otherwise known as alum), which is used as a chemical coagulant. Alum is added to surface water and dissolves to release a number of Al(III) chemical species that will then form flocs with negatively charged dissolved and colloidal impurities in the feed water [2]. These flocs are then settled and removed from water through the subsequent clarification and filtration processes. While alum does have some drawbacks, in particular the difficulty in predicting the requisite alum dose in supplies with variable water quality, it is a relatively affordable and reliable means of enhancing coagulation in the water treatment process [2]. Alum has therefore been used for centuries in water treatment and remains the dominant means of coagulation in developing and developed nations alike [3,4].

The precipitated flocs that are produced through alum addition are referred to as water treatment residuals (WTR). These WTR are continuously removed from water treatment basins during conventional water treatment. The average water treatment plant servicing a population of 100,000 people generates approximately 100,000 L of WTR each day and these are removed from the treatment systems as sludge to be stored in settlement ponds prior to further treatment or disposal [5]. While WTR are themselves relatively inert, they do have poor compaction traits and as a result, WTR sludge will typically comprise only 0.5–2.0% solids [5]. Consequently, up to 99.5% of WTR sludge is reject water which can itself contain high concentrations of dissolved Al, and this component of the waste stream from conventional water treatment may pose a management challenge where the scope for onsite detention is limited [5].

Al is a non-essential trace element with no known biological functions and can be toxic to aquatic organisms at relatively low concentrations [6]. This is particularly true in acidified waters which encourage greater solubility of Al, leading to a higher risk of toxicity to aquatic life [6]. Al is rapidly accumulated by macroalgae from water and poses a potential ecological threat in natural water bodies. However, recent research has also shown that the ability of macroalgae to sequester Al can be harnessed in intensive bioremediation systems to treat waste water contaminated with Al [7,8]. For example, the freshwater green macroalga *Oedogonium* is tolerant of high concentrations of Al and has been cultivated at scale at an Australian coal-fired power station to sequester Al from waste water at concentrations of up to 0.15 mg L^{-1} [8]. In this previous study, *Oedogonium* sequestered Al at a rate that reduced dissolved Al concentrations to <10 μg L^{-1} within 3 days, resulting in treated water with Al concentrations below regulatory limits for discharge [8]. The bioremediation of waste water with *Oedogonium* therefore offers an efficient means of sequestering Al and delivering an improved quality of water for discharge to the environment.

One aspect that can potentially enhance the rate of metal sequestration in algal bioremediation systems is the use of CO_2 as a supplemental C source. The addition of CO_2 increases the availability of dissolved inorganic C for photosynthesis, leading to greater productivity of algae [9]. In a bioremediation context, the enhanced productivity of algal biomass should provide additional substrate for metal sequestration. Additionally, when CO_2 is added to waste water containing metals, the resulting reduction in pH can increase the bioavailability of dissolved metals, leading to more rapid bioaccumulation by algae [7]. While previous research has shown *Oedogonium* to be tolerant of relatively high concentrations of Al, there are likely to be limits to which CO_2 can be used for these dual purposes. The intensive dissolution of CO_2 could increase metal availability to the point at which it becomes toxic, which will make it necessary to identify the tolerance of *Oedogonium* to Al in cultures with, and without, CO_2 dissolution [7]. It is known, for example, that the toxicity of Al is strongly reliant on the pH of water [6]. Therefore, the use of CO_2 as a means to increase growth of algae in waste water bioremediation ponds will need to balance the requirements of algae for supplemental C with the potential to cause toxicity due to increased metal availability at low pH. It will also need to balance the cost of CO_2 addition which can be a significant proportion of operational expenditure for large-scale algal cultivation [10]. Furthermore, it is currently unclear whether this technique can be used to enhance the sequestration of Al species that are found in WTR retention ponds. Diffusive Gradients in Thin Films (DGTs) are passive sampling devices that can be deployed in waste water to quantify the relative bioavailability of metals under different physico-chemical conditions. DGTs contain a

metal-binding Chelex resin which only absorbs bioavailable metal cations [11]. By deploying DGTs in cultures containing *Oedogonium*, it is possible to quantify the bioavailability of metals independently of any biological interactions that may occur in macroalgal bioaccumulation. This technique has been successfully used in conjunction with algal bioremediation to characterise the bioavailability of metals under different culture conditions [7].

While *Oedogonium* can be cultivated in waste water to sequester Al, there is limited data to predict growth rates and treatment in waste water with the range of Al concentrations experienced in waste water from alum disposal. Similarly, while CO_2 can enhance the bioremediation of Al from waste water with relatively low initial Al concentrations [7], it is not known whether CO_2 can be used to enhance Al sequestration at higher concentrations that may be encountered in waste water containing WTR sludge treated with alum. There is consequently a need to determine the potential interactions between the addition of CO_2 to waste water and the ability of macroalgae to sequester Al across a range of physico-chemical conditions.

This study investigates the cultivation of *Oedogonium* in waste water from a WTR detention basin in north Queensland, Australia. Specifically, the study will address the following questions. First, can *Oedogonium* be cultivated in waste water from WTR disposal to sequester dissolved Al? Second, can supplemental CO_2 be used to increase biomass productivity of macroalgae and the sequestration of Al for improved waste water treatment across a range of initial concentrations? Together these studies will provide the empirical data needed to assess the efficacy of algal-based bioremediation at scale in conjunction with conventional water treatment plants.

2. Methods

2.1. Study Site and Water Collection

These experiments were conducted on waste water collected from the Giru Drinking Water Treatment Plant (GDWTP) in Giru, north Queensland, Australia (19°30′17.99″ S, 147°5′38.71″ E). GDWTP draws intake water from the nearby Haughton River and uses conventional treatment to produce potable water for Cungulla and Giru Township. The GDTWP produces 0.15 ML of WTR each day which is pumped into four settling lagoons for clarification. The water is not discharged from the settling lagoons due to the residual dissolved Al in the water. Rather, the site relies on evaporation to maintain water levels below the capacity of the settling ponds. The facility has an aspirational target of treating the aqueous phase of the WTRs to a total Al concentration of <0.1 mg L^{-1}, which would enable reuse of the water in local irrigation practices (J. Tickle, Operations Manager, personal communication). Waste water for the first experiment was collected directly from the coagulation tank sludge bed by site staff in January, 2016 and water for the second experiment was collected in the same way in April, 2016. In both instances the water was pumped into 1000 L plastic containers and shipped to James Cook University (JCU) in Townsville, Australia (19°19′47.49″ S, 146°45′40.43″ E) for experimental studies. Experimental studies were conducted in outdoor research facilities at the Centre for Macroalgal Resources and Biotechnology at JCU.

2.2. Productivity of Oedogonium in Giru Waste Water and Sequestration of Al

Oedogonium was cultivated in waste water from GDWTP and its productivity was compared with that of *Oedogonium* cultivated in dechlorinated water. The cultivation experiment was conducted in 20 L buckets for 8 weeks. Waste water was inoculated with *Oedogonium* from stock cultures at a density of 0.25 g fresh weight (FW) L^{-1}. An algal growth media (Manutec® MAF f/2, Cavan, Australia) was added to all cultures at 0.1 g L^{-1} and there were 5 replicate cultures of *Oedogonium* in the waste water and dechlorinated water. Water samples were taken from each bucket at the beginning of the experiment to determine initial concentrations of dissolved Al. The water samples were filtered through a 0.45 µm filter, acidified to pH < 2 with Ultrapure HNO_3 and stored at 4 °C until analysis (see "Analytical Methods" for a detailed description of metals analysis). A water sample was also

taken from each bucket after 7 day of algal culture to quantify the final dissolved Al concentration in the waste water.

The algae was harvested from each bucket every 7 day by pouring the water and algae through fabric bags. The bags were placed in a domestic washing machine on the spin cycle for 7 min to remove excess moisture, and the algal FW was recorded to the nearest 0.01 g. A sub-sample of the FW biomass was then returned to new GDWTP waste water or dechlorinated water at the same initial stocking density (0.25 g FW L^{-1}) and left for another 7 day. This process was repeated for a total of 8 weeks. In order to measure the growth through the cultivation period, the surplus FW biomass (i.e., the final minus initial biomass) from each weekly harvest was first converted into a dry weight (DW) using a dehydrator (60 °C, 24 h). The DW was then used to calculate the DW productivity, which is expressed as g DW m^{-2} day^{-1}. A sub-sample of the dried biomass was retained for analysis of the concentration of Al.

The growth of *Oedogonium* in the waste water and the dechlorinated control treatment was contrasted using a 2 way Analysis of Variance (ANOVA) including the random factor "time" (weeks 1–8) and the fixed factor "water source" (dechlorinated vs. waste water water). The assumptions of normality and homogeneity of variance were checked prior to analysis with residual histograms and scatterplots of residuals vs. estimates, respectively [12]. In addition, a mass balance model was developed to quantify the proportion of dissolved Al initially in the waste water that was removed from the water in the harvested algae. Briefly, the initial mass of dissolved Al (mg) in the waste water was calculated as the product of the concentration of Al in the water (mg L^{-1}) at the beginning of each harvest cycle and the volume of waste water in each bucket (L). The final mass of dissolved Al (mg) in the waste water was calculated as the product of the concentration of Al in water (mg L^{-1}) at the end of each harvest cycle and the volume of waste water in each bucket, allowing for evaporation. The final mass of dissolved Al was subtracted from the initial mass of dissolved Al to quantify the amount of Al removed during the harvest cycle. The amount of Al sequestered by *Oedogonium* (mg) was calculated as the product of the concentration of Al in the harvested biomass (mg kg^{-1}) and the mass of *Oedogonium* cultivated in each week (kg DW).

2.3. The Effect of CO_2 on Oedogonium Productivity and Al Sequestration from Giru Waste Water

A second experiment was conducted to investigate the interaction between the concentration of Al in the waste water, and the addition of supplemental CO_2, the latter to manipulate culture pH and provide a source of inorganic C to support photosynthesis. The experiment was conducted in 20 L buckets in an outdoor cultivation facility. The experiment used waste water collected from GDWTP in April 2016 with an initial Al concentrations of 0.17 mg L^{-1} Al. The waste water was spiked with an Al stock solution to obtain initial Al concentrations of 0.17 mg L^{-1} (raw, un-spiked waste water) to a maximum of 8 mg L^{-1} total Al. These concentrations of Al were crossed with a CO_2 addition treatment. For treatments receiving CO_2, the gas was added through an air stone connected to a digital solenoid timer that activated CO_2 flow from a connected cylinder of CO_2 (BOC gases). The solenoid was set to deliver a pulse of CO_2 at a rate of 4 L min^{-1} for 4 s every 20 min between 09:00 and 16:00 each day. This addition of CO_2 maintained a stable day-time pH of approximately 8.0 in the cultures receiving CO_2 (see results Section 3.2). The treatments not receiving CO_2 had daily pH fluctuating between approximately 8.0 and 10.0 during day-light hours. There were 4 replicate buckets for each combination of Al concentration and CO_2 addition.

The Al concentrations were created for the experiment from a diluted $AlCl_3 \cdot 6H_2O$ stock solution (Sigma, St. Louis, MO, USA) which was made according to the methods previously described in Golding et al. (2015). Briefly, a 1 g L^{-1} Al stock solution was created by adding the appropriate amount of $AlCl_3 \cdot 6H_2O$ to a 0.1 M NaOH solution [13]. This caused a white precipitate of aluminium hydroxide to form which then dissolved when diluted in the test waters. An appropriate amount of this stock solution was then added to each bucket to achieve the desired initial Al concentrations in each treatment. The experimental cultures were stocked with *Oedogonium* taken from long-term stock

cultures maintained at the research facility. Each bucket was stocked with biomass at an initial density of 0.25 g fresh weight (FW) L^{-1} and provided with f/2 algal nutrient mix at 0.1 g L^{-1}. The buckets were continually aerated with compressed air to keep the filaments in suspension and left for 7 d. After 7 day they were harvested as described earlier. A sub-sample of the biomass was returned to re-stock the cultures in new water at 0.25 g L^{-1} and the remaining biomass was dried for 24 h in a dehydrator at 60 °C and the dry weights recorded (nearest 0.01 g). This process was repeated for 4 weeks.

Initial water samples were collected from each bucket each week to determine the starting concentrations of dissolved Al in the 0.1–8 mg L^{-1} total Al treatments. The water samples were collected with a syringe and filtered (0.45 µm) before being acidified to pH < 2 with ultrapure HNO_3. The dried biomass samples were retained for metals analysis as described above for the first experiment. In addition, the flux of bioavailable Al was determined in each treatment with the use of Diffusive Gradients in Thin-films (DGTs) which were deployed for one of the 7 day periods in the bucket cultures. DGTs contain a metal-binding resin that specifically accumulates the bioavailability fraction of Al in waste water. These devices can be used to quantify the bioavailability of Al to aquatic life under different physico-chemical conditions. The DGTs were placed in the cultures at the start of the experiment and recovered during the harvest in week 1. The DGT resin was removed from the gel unit and eluted in 1 ml of ultrapure HNO_3 for 24 h. After the elution, the samples were diluted with 14 mL of deionised water and analysed for total Al concentrations.

The Al concentration in the water samples, in DGT extracts and in the DW biomass samples for all experiments were analysed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Advanced Analytical Centre (JCU).

3. Results and Discussion

3.1. Productivity of Oedogonium and Al Sequestration in Giru Waste Water

There was no significant difference in the mean (time-averaged) productivity of *Oedogonium* in the waste water from GDWTP and the dechlorinated water (two-tailed *t*-test: $p = 0.537$). The productivity of *Oedogonium* in the Giru waste water over the 8 weeks averaged 12.1 ± 0.4 g DW m^{-2} day^{-1}, compared with 11.6 ± 0.6 g DW m^{-2} day^{-1} in the dechlorinated control water (Figure 1). The week-to-week productivity of *Oedogonium* varied very little over the course of the experiment in the Giru waste water, from a minimum of 10.3 g DW m^{-2} day^{-1} (week 2) to a maximum of 13.6 g DW m^{-2} day^{-1} (week 6) (Figure 1). The productivity of *Oedogonium* in the dechlorinated control treatment was initially slightly lower, and ranged from a minimum of 9.0 g DW m^{-2} day^{-1} (week 1) to a maximum of 14.0 g DW m^{-2} day^{-1} (week 6) (Figure 1).

The initial concentration of dissolved Al in the Giru waste water was 0.260 mg L^{-1} and this decreased to 0.068 mg L^{-1} after each 7-day cultivation period (Table 1). A mass balance model was constructed to confirm that the reduction in dissolved Al could be attributed to the bioaccumulation of Al within the *Oedogonium* biomass. There was a mean removal of 2.68 mg of Al each week from each replicate bucket (Table 1). The *Oedogonium* cultivated in the waste water had a mean Al content of 467 mg kg^{-1} and an average of 5.4 g DW of biomass was harvested from each replicate each week (Table 1). Therefore, an average of 2.52 mg of Al was removed from the buckets in the harvested *Oedogonium* biomass each week. This represents 89.5% of the Al that was removed on a weekly basis from each culture (Table 1). The remaining 10.5% of Al that is unaccounted for is likely to be a combination of Al that precipitated in the buckets (and was therefore excluded from the water sample by the 0.45 µm syringe filter), Al that was bound by microorganisms that were in the buckets, and analytical and measurement error. Regardless, the mass balance calculations show that the majority of Al removed from the cultures was accounted for in the harvested *Oedogonium* biomass.

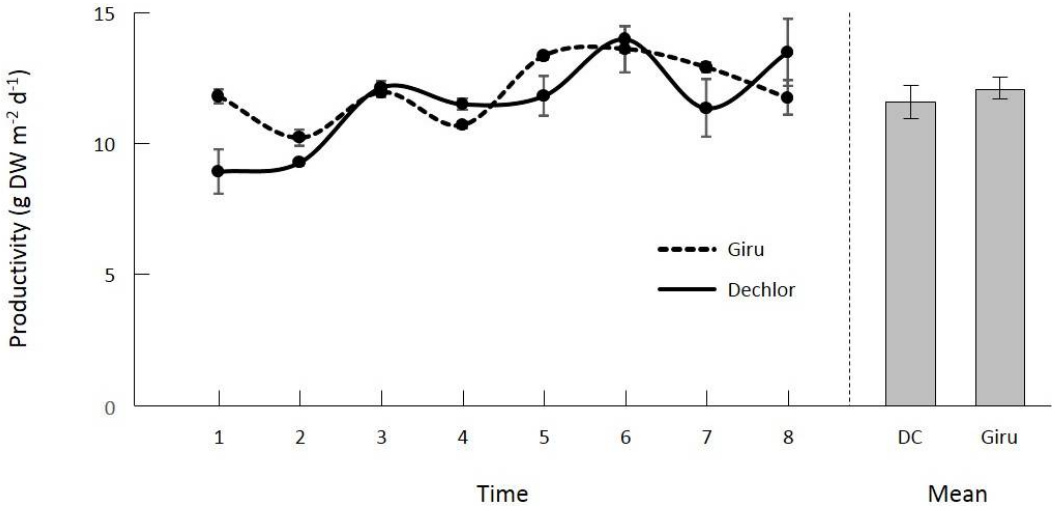

Figure 1. Mean productivity (g DW m^{-2} day^{-1}) of *Oedogonium* in dechlorinated water and Giru waste water over the course of the initial 8 week experiment. Data show mean productivities ± standard error (n = 5) for each time point. The experiment wide mean productivity is also shown for each treatment by the columns ("DC" = dechlorinated water). The error terms for the columns are based on the error associated with the mean productivity in each of the 8 weeks.

Table 1. Mass balance of Al sequestration in Experiment 1. The data shows the mean initial and final concentration of Al in the waste water after each 7-day cultivation window, the amount of biomass produced, and the concentration of Al in the *Oedogonium* biomass from each week. From this data a mass balance model was derived, with approximately 90% of the Al removed from the waste water each week being accounted for in the harvested *Oedogonium* biomass.

Variable	Units	Value
Initial [Al] in waste water	mg L^{-1}	0.260 ± 0.020
Final [Al] in waste water	mg L^{-1}	0.068 ± 0.007
Mass Al lost from waste water	Mg	2.68 ± 0.11
[Al] in harvested *Oedogonium*	mg kg^{-1}	467 ± 25
Mass harvested *Oedogonium*	g DW	5.40 ± 0.13
Mass Al sequestered in *Oedogonium*	Mg	2.48 ± 0.09
Proportion of Al in *Oedogonium*	%	89.5 ± 6.5

The stable long-term productivity of *Oedogonium* in the Al-contaminated waste water, combined with the consistent uptake of Al from the waste water, demonstrates that cultivating algae is a highly effective means of treating dissolved Al. The concentrations of Al in the waste water treated with *Oedogonium* was well below the regulatory limits for irrigation water and livestock drinking water [14], as well as the 0.1 mg L^{-1} aspirational target for local discharge at GDWTP. Consequently, the treated water would be suitable for un-restricted re-use in irrigation and agriculture in the surrounding region. There are, however, cost-benefit hurdles for implementing algal bioremediation beyond efficacy. The cost of culturing any form of algae at scale, whether it be in open-pond systems or photobioreactors, is unlikely to be offset by a waste water service fee. For this reason the next step in demonstrating the economic viability of algal bioremediation of alum WTP is to determine the value of the biomass produced. For context, the concentration of Al of 467 mg kg^{-1} in the harvested biomass is below the tolerable Al content in poultry, swine, horse, cattle and sheep feeds, all of which have regulatory limits of 1000 mg kg^{-1} [15]. Furthermore, previous work on the proximate composition of *Oedogonium* across a range of different water sources demonstrate that this freshwater macroalga is an excellent and reliable source of protein, lipids, energy and minerals (see detailed review in [16]). For example, *Oedogonium* biomass could be used as a protein-rich

component of livestock feeds in the region [17,18]. It could also be used for the production of liquid biofuels [18,19] and *Oedogonium* biomass containing Al at similar concentrations is a suitable feedstock for bioenergy and biochar production through thermal conversion techniques such as pyrolysis and gasification [20,21]. A similar rationale can be found for linking biomass production of algae for liquid biofuel production with waste water utilisation or treatment [22,23], including marine algae for coastal systems [24] *Oedogonium* is also a source of carotenoids with strong anti-oxidant properties [25] which, given that other commercially important algae containing bioactives have been cultured in diverse waste waters [26,27], provides further potential for high-value application. With a mean productivity of 12.1 g DW m^{-2} day^{-1}, which is the same annualised productivity when this species is cultured at large-scale integrated with waste water at industry sites [28], annual biomass yields of 44 tonnes ha^{-1} could be expected at scale, offering a significant source of biomass for regional applications in agriculture and bioenergy production.

3.2. The Effect of CO_2 on the Productivity of Oedogonium and the Bioremediation of Al

The chemical behaviour of Al in freshwater systems is complex, with a wide range of Al species being formed under different conditions. The effects of physico-chemical parameters on the solubility of Al is typically considered from a toxicological perspective. That is, there has been great interest in understanding the conditions under which Al is most bioavailable to aquatic life and, therefore, most toxic [6]. However, in a bioremediation setting where a tolerant alga is being used to actively sequester dissolved Al, manipulating physico-chemical conditions to enhance the bioavailability of Al could increase the efficacy of the treatment process. One factor that strongly affects the bioavailability of Al in aquatic systems is the pH of the water, with the solubility of Al increasing in acidified waters [6].

The addition of CO_2 to the algal cultures maintained a constant pH, in comparison to the treatments not receiving CO_2, which fluctuated throughout the day. The treatments receiving supplemental CO_2 had a mean pH of 8.3 at 09:00 and 15:00 (Figure 2). In contrast the pH in treatments not receiving supplemental CO_2 increased from 8.6 at 09:00 to 9.5–9.8 at 15:00 (Figure 2). There was no significant interaction for pH between the Al concentration and CO_2 addition at 09:00 (two-way ANOVA "$CO_2 \times$ Al": $F_{4,30} = 2.279$, $p = 0.084$), which indicates that the effect of CO_2 was consistent across the treatments (Figure 2a). While there was an interaction for pH between Al and CO_2 at 15:00 (two-way ANOVA "$CO_2 \times$ Al": $F_{4,30} = 2.717$, $p = 0.048$), this was due to a slightly lower pH in the no-CO_2 treatments containing 5 and 10 mg L^{-1} Al, relative to the waste water with 0.1 mg L^{-1} (Figure 2b). Therefore, the addition of CO_2 affected the pH of cultures equally regardless of the concentration of Al (Figure 2b).

DGTs were used to quantify the bioavailability of Al as these passive sampling devices specifically accumulate the bioavailable fraction of metals from water. DGTs provide a means of contrasting metal bioavailability under different conditions that is decoupled from any effects of the different conditions on the productivity and biology of *Oedogonium*. The DGT data clearly confirmed that the addition of CO_2 to the algal cultures significantly increased the bioavailability of Al (Figure 3). There was a significant interaction between the concentration of Al and the addition of CO_2 on the flux of Al into the DGTs during the cultivation experiment (two-way ANOVA "$CO_2 \times$ Al": $F_{4,30} = 4.05$, $p = 0.014$). The addition of CO_2 to the cultures increased the rate of flux of Al into the DGTs in cultures containing 0.17–1.59 mg L^{-1} total Al in comparison with the cultures not receiving CO_2 (Figure 3). Somewhat surprisingly, the flux of Al into the DGTs did not differ between cultures with and without CO_2 addition when the initial Al concentration was >1.59 mg L^{-1} total Al (Figure 3). It is likely that the DGT resins became saturated with Al in the treatments that combined high concentrations of Al with the addition of CO_2. This would have the effect of preventing further uptake of Al and mask any effects of CO_2 on the bioavailability of Al. A shorter deployment time of the resins in these treatments may have shown a positive effect of CO_2 on the uptake of Al into the DGT resins. Regardless, it is clear that the addition of CO_2 enhanced the bioavailability of Al, particularly in the treatments with lower concentrations of Al.

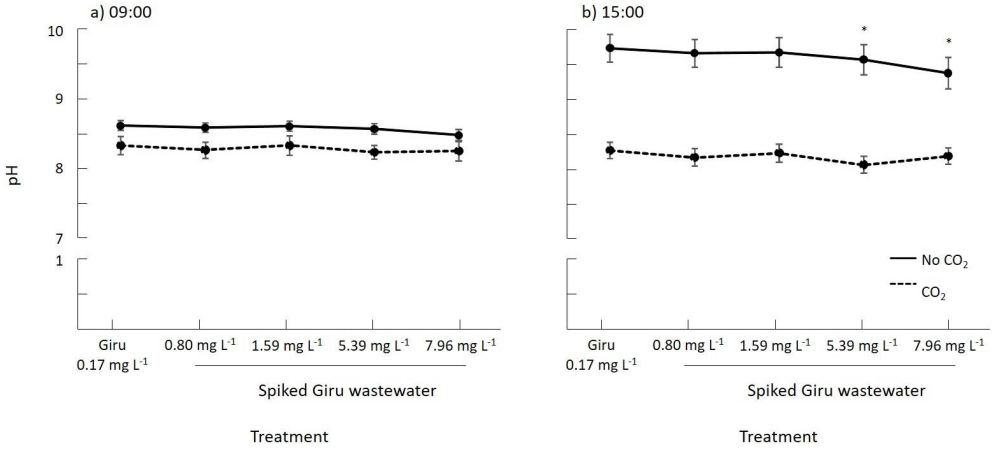

Figure 2. Mean of pH measurements in the cultivation experiments at (**a**) 09:00 and (**b**) 15:00. The pH of each culture was checked twice daily throughout the experiment and the data shown above are mean pH for each treatment over the course of the experiment. The solid line shows pH without addition of CO_2 and the dashed line shows pH with addition of CO_2. The CO_2 treatments received a 4 s pulse of CO_2 at a flow rate of 4 L min^{-1} every 20 min between 09:00 and 16:00. Asterisks above treatments indicate the results of post-hoc Tukey's test for the interaction ("CO_2 × Al"). Treatments marked with an asterisk are significantly different to the "well" treatment in that level of the interaction ($p < 0.05$). All data are mean pH ± standard error ($n = 28$).

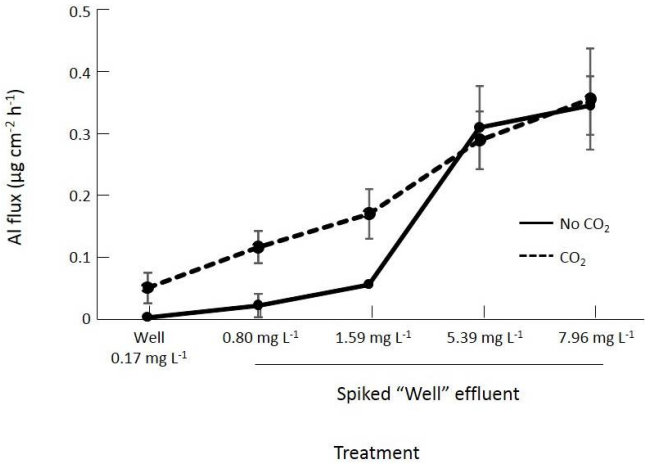

Figure 3. Flux of Al into the diffusive gradient in thin film (DGT) passive sampling devices during the outdoor cultivation experiment using waste water from Giru Drinking Water Treatment Plant (GDWTP) ("Well") spiked with a range of Al concentrations. The data show flux across a single 7 day deployment conducted in parallel with the algal cultivation. The solid line shows Al flux without the addition of CO_2 and the dashed line shows Al flux with the addition of CO_2 (see Figure 2 for pH profiles). All data are mean Al flux (µg cm^{-2} h^{-1}) ± standard error ($n = 3$).

Oedogonium cultures were successfully established and maintained in waste water containing up to 7.96 mg L^{-1} total Al with no acute toxicity in any cultures. In addition, there were only small decreases in productivity as the total concentration of Al increased. There was a significant effect of both CO_2 supplementation ("CO_2": $F_{1,150} = 44.71$, $p < 0.001$) and the Al concentration of the waste water ("Al": $F_{4,150} = 15.60$, $p < 0.001$) on *Oedogonium* productivity, but no significant interaction between the factors ("CO_2 × Al": $F_{4,150} = 0.46$, $p = 0.746$). The addition of CO_2 to the cultures increased the productivity of *Oedogonium* irrespective of the concentration of Al in the waste water (Figure 4a). The mean productivity of *Oedogonium* in waste water without CO_2 was 9.3 ± 0.2 g DW m^{-2} day^{-1},

increasing by 15% to 11.0 ± 0.3 g DW m^{-2} day^{-1} when CO_2 was added (Figure 4a). Additionally, productivity decreased as the concentration of Al in the waste water increased. There was no difference in productivity of *Oedogonium* between the waste water containing 0.17–1.59 mg L^{-1} total Al, with these treatments having a mean productivity of 10.7–11.0 g DW m^{-2} day^{-1} (Figure 4a). However, the productivity of *Oedogonium* in waste water containing 5.39 and 7.96 mg L^{-1} total Al was lower than the other treatments, with mean productivities of 9.6 and 8.4 g DW m^{-2} day^{-1}, respectively (Figure 4a).

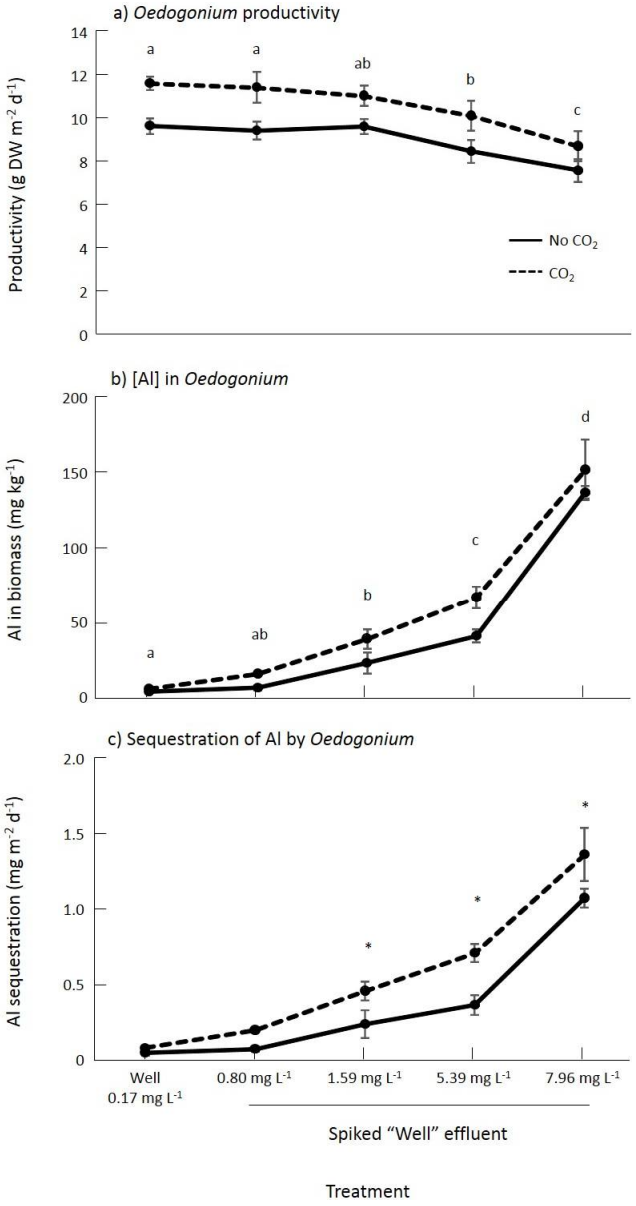

Figure 4. (**a**) Productivity of *Oedogonium*; (**b**) concentration of Al in *Oedogonium* biomass; and (**c**) rate of sequestration of Al by *Oedogonium* from Giru waste water ("Well") spiked with a range of Al concentrations. The solid lines show data for treatments without CO_2 and the dashed lines show data for treatments with CO_2 (see Figure 2 for pH profiles). The letters above treatments in panels (**a**,**b**) indicate the results of post-hoc Tukey's tests for the factor "Al". Treatments that share a common letter do not differ ($p > 0.05$). The asterisks above treatments in panel (**c**) indicate the results of post-hoc Tukey's tests for the interaction ("Al × CO_2"). Treatments marked with an asterisk differ in the CO_2 vs. no CO_2 comparison for that Al concentration. All data are means ± standard error ($n = 4$).

As for the productivity of *Oedogonium*, there was a similar effect of both CO_2 addition and the initial concentration of Al in the waste water on the concentration of Al in the harvested *Oedogonium* biomass, but no interaction between the factors ("Al × CO_2": $F_{4,30} = 0.66$, $p = 0.625$). The concentration of Al in the harvested *Oedogonium* biomass increased with the concentration of total Al in the waste water ("Al": $F_{4,30} = 107.86$, $p < 0.001$). The mean Al concentration of biomass grown in the un-spiked GDWTP waste water containing 0.17 mg L^{-1} Al was 6.04 and 4.18 mg kg^{-1} Al for CO_2 and no-CO_2 treatments, respectively, equating to a 45% increase in biomass Al concentrations (Figure 4b). This increased to a maximum of 151 and 136 mg kg^{-1} Al in CO_2 and no-CO_2 treatments containing 7.96 mg L^{-1} total Al, respectively (Figure 4b). In addition, there was an effect of the addition of CO_2 on the concentration of Al in the *Oedogonium* biomass ("Al × CO_2": $F_{1,30} = 7.74$, $p = 0.009$). The concentration of Al in *Oedogonium* grown in waste water with supplemental CO_2 was higher than the concentration of Al in *Oedogonium* grown in waste water without CO_2 (Figure 4b).

The sequestration rate of Al (mg m^{-2} day^{-1}) was calculated as the product of the productivity of *Oedogonium* (g DW m^{-2} day^{-1}) and the Al content of the harvested biomass (mg kg^{-1}). There was a significant interaction between the concentration of Al and the addition of CO_2 on the sequestration of Al from the waste water ("Al × CO_2": $F_{4,30} = 4.317$, $p = 0.007$). The Tukey's post-hoc comparisons showed that the addition of CO_2 increased the rate of sequestration of Al from the waste water when the initial Al concentrations of the waste water were greater than 0.80 mg L^{-1} (Figure 4c). The sequestration of Al reached a maximum of 1.6 mg m^{-2} day^{-1} and 1.1 mg m^{-2} day^{-1} in the waste water containing 7.96 mg L^{-1} total Al with and without CO_2 addition, respectively (Figure 4c).

Together, these data show that the use of CO_2 enhances waste water bioremediation of Al through two concurrent processes. First, CO_2 increases biomass productivity, resulting in more algae in the cultures to sequester Al. Second, the addition of CO_2 influences the speciation of Al, making it more bioavailable for sequestration by *Oedogonium* under the lower pH conditions. This results in a slightly higher concentration of Al in *Oedogonium* grown in CO_2-supplemented water. When combined, the higher biomass productivity and higher Al content of the biomass in cultures receiving CO_2 results in a more rapid sequestration of Al. It should be noted however, that the gains achieved on provisioning CO_2 are relatively modest, and will need to be balanced by the likely expense of providing CO_2 to cultures at scale, although there are opportunities to integrate with CO_2 waste streams at some sites [10,29]. A 10% increase in bioremediation efficiency and biomass production as measured in this study may not be sufficient to offset the significant capital expense associated with provisioning CO_2 to large-scale cultures [10], although cheaper alternative carbon sources are available [28]. Regardless, a positive outcome of this study is that effective bioremediation of Al can be achieved without the need to provide supplemental CO_2.

One surprising result was that the *Oedogonium* biomass grown in the un-manipulated Giru waste water in the second experiment had a relatively low concentration of Al in comparison to that grown in the first experiment. In the first experiment the concentration of Al in the Giru waste water was 0.26 mg L^{-1} and the *Oedogonium* grown in this waste water had a mean concentration of Al of 467 mg kg^{-1}. In the second experiment the concentration of Al in the waste water was 0.17 mg L^{-1}, however the *Oedogonium* grown in the waste water only had a mean concentration of Al of 4.2–6.1 mg kg^{-1}. While *Oedogonium* shows great potential to be used in the bioremediation of WTRs, there is a need to understand the site-specific factors that influence Al speciation at each facility. Specifically, while the concentration of total Al in the untreated Giru waste water changed relatively little during our experiments, it appears that the concentration of bioavailable Al did fluctuate substantially in the raw effluent. The deployment of DGTs in the waste water from conventional water treatment plants would provide a baseline dataset that examines the relative availability of Al in the WTR aqueous phase over time.

4. Conclusions

The cultivation of the freshwater macroalga *Oedogonium* in the aqueous phase of WTRs from a conventional water treatment plant was an efficient and reliable means of removing dissolved Al. While there was some variation in the uptake of Al between experiments, the waste water was consistently treated to sufficiently low concentrations that it is suitable for re-use in irrigation and for livestock drinking water. The cultivated biomass could be used as a component of animal feeds or as a substrate for the production of renewable bioenergy. The addition of CO_2 to the algal cultures resulted in modest increases in the productivity and the sequestration of Al by *Oedogonium*. The integration of macroalgal cultivation with conventional water treatment plants has the potential to enhance the sustainability of drinking water production and provide value-added linkages to regional primary industries. Few studies have considered sustainable treatment techniques for waste water streams containing WTRs from water treatment plans, including managing the waste water stream and solid residues (Lee et al., 2017). However, this will become more important as the drinking water sources become more polluted.

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