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A comparative assessment on how molasses and CO_2 gas prevent carbon limitation in the large-scale culture of freshwater macroalgae

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ABSTRACT

Freshwater macroalgae are an attractive treatment option for waste streams that have very high concentrations of nutrients. However, the long water residence times required in these scenarios will result in carbon becoming a limiting nutrient that negatively impacts the rate of biomass productivity and, subsequently, the potential for nutrient uptake. This study examined how the rate of carbon supplementation influenced the biomass productivity of *Oedogonium intermedium*, during both winter and summer periods in high rate algal ponds (HRAPs) maintained under batch conditions. We also examined the novel use of molasses as a source of carbon for intensive algal cultures and compared its effect on biomass productivity to that of CO_2 gas. *Oedogonium intermedium* responded positively to carbon supplementation from both molasses and CO_2 gas, with biomass productivity increasing as the rate of carbon supplementation increased. In cultures with no carbon supplementation, the average productivity of *O. intermedium* was 2.2 (± 0.8) $g\,m^{-2}\,day^{-1}$, and a maximum of 17.3 $g\,m^{-2}\,day^{-1}$ and 20.8 $g\,m^{-2}\,day^{-1}$ during summer when carbon was supplemented through the addition of molasses and CO_2, respectively. The optimal rate of carbon supplementation was 0.06 g of carbon per liter (g (C) L^{-1}) in winter and 0.08 g (C) L^{-1} during summer. The addition of an exogenous source of carbon resulted in a decline in the culture pH and increased the availability of the dissolved inorganic carbon (DIC) pool and subsequently, carbon uptake by the algae. This study has identified a novel method to supplement carbon to algal cultures, where the waste residue from the sugar industry can be used as viable source of inorganic carbon. Importantly, we have demonstrated that *O. intermedium* can be cultivated in HRAPs without requiring a high rate of water exchange, providing that its requirements of DIC are satisfied.

1. Introduction

The large-scale cultivation of freshwater macroalgae relies on three major inputs; light, nutrients and carbon. To a large extent, the amount of sunlight an algal farm receives cannot be manipulated beyond the initial site selection and the orientation (north-south vs east-west) of the culture system. There is greater scope to manage nutrients in algal cultures by co-locating algal production with industries that produce nutrient-rich wastewater. In this setting, it is possible to manipulate the rate of water exchange to the cultures to create a flux of the key nutrients, such as nitrogen and phosphorous, that both maximizes the rate of biomass productivity and remediation outcomes [1–4]. This approach is most successful when accessing wastewater with relatively low concentrations of inorganic nitrogen ($< 5\,mg\,L^{-1}$), in particular, from aquaculture facilities [3,4], agricultural crop runoff [5] or the treated discharge water from municipal treatment plants [6]. In these situations, a nitrogen flux of approximately $1\,g\,m^{-2}\,day^{-1}$ can be generated from a water exchange rate of between 0.5 and 1 culture volumes per day (vol day^{-1}). This provides all of the nitrogen, and other macro and micro elements that are required to maintain high ($> 10\,g\,dw\,m^{-2}\cdot day^{-1}$) productivities in these cultures [2,3,6]. Conversely, the management of nitrogen becomes increasingly difficult in water with higher concentrations of nitrogen, such as from primary treated sewage [7], meat processing plants [8], liquors from anaerobic digestion [9] or manure effluents from intensive animal agriculture [2,10]. Higher nitrogen concentrations necessitate an increased residence time of the water if adequate bioremediation is to occur. The resulting low water exchange rates (0–0.1 vol day^{-1}) can rapidly exhaust the supply of dissolved inorganic carbon (DIC) in the culture system and, since the availability of carbon is a significant determinant of algal growth rates [11–13], this can have significant negative effects on the rate of biomass production and subsequent efficiency of nutrient bioremediation [3].

The preferred culture systems for large-scale operations are high rate algal ponds (HRAPs), as they are cost effective to construct and operate [14–16]. These systems use a paddlewheel to move the water

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horizontally, with minimal energy lost through turbulence. However, this low-turbulence environment has the unintended consequence of maintaining a largely unbroken water surface, which increases the air/water surface boundary layer. This restricts the potential for gas exchange and limits the passive diffusion of atmospheric carbon dioxide (CO_2) into the culture water [17].

As the scale of algal cultivation increases, ensuring that DIC is not limiting becomes increasingly important if the cultivation of algae is to be optimized and bioremediation outcomes maximized. In HRAPs with low rates of water exchange, this is made more difficult by the modifying effect that the pH of the culture water has on the availability of inorganic carbon, regardless of the DIC concentration [11,18]. The DIC forms part of the carbonate buffer system and is available as one of three species - CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) - with the proportion of each being dependent upon the pH and, to a lesser extent, on salinity and temperature [19]. In freshwater at a pH of 8 the concentration of dissolved CO_2 is minimal (< 1.5%), with ca. 95% of the DIC pool found as HCO_3^-, while at a pH of 10.5, approximately 65% of the DIC pool is found as CO_3^{2-} with the remainder as HCO_3^-. This is important as all algae can access CO_2, some can access HCO_3^-, but none are able to access CO_3^{2-} as a source of carbon for photosynthesis [19,20]. Consequently, if the pH of the culture water remains elevated, the growth of any alga will be significantly impaired.

Previous work on the freshwater macroalga, *Oedogonium intermedium*, has demonstrated that it can maintain photosynthesis up to a pH of 10.7 and can extract DIC as either CO_2 or HCO_3^-. Despite this, the growth rate of *O. intermedium* is enhanced when the pH is lower and the proportional availability of CO_2 is higher [11]. In addition, it is expected that the amount of carbon required by *O. intermedium* to maximize the rate of biomass production will vary throughout the year in relation to changes in abiotic factors, in particular sunlight and temperature. As such, an understanding of how the rise in pH of *O. intermedium* cultures can be regulated to ensure that carbon does not become limiting and how the carbon requirements of *O. intermedium* vary seasonally will enable the targeted delivery of carbon to maximize the benefits for large-scale bioremediation applications.

The most commonly used technique to add carbon and lower the pH of the culture water is through the diffusion of CO_2 gas into wastewater containing algae [21]. However, at larger scales this technique has several limitations, including the high cost of creating and transporting pressurized CO_2, and ensuring the CO_2 gas dissolves and remains in solution [21,22]. Currently, the efficiency of adding gaseous CO_2 to open cultures is relatively low with as little as 7–35% of the carbon ultimately being sequestered by algae and incorporated into algal biomass [11,23]. This problem is often compounded at larger cultivation scales, which require a correspondingly higher rate of gas flow and/or multiple injection points to make a meaningful change to the pH and availability of DIC [22]. At these larger scales, it is unlikely to be environmentally or economically viable to use commercial grade (> 99%) CO_2 to ensure adequate concentrations of carbon are available for algal growth [24,25]. One alternative is to use wastes sources of CO_2 from industrial production, such as flue gas [22]. However, the use of flue gas is limited by the relatively low concentrations of CO_2 (4–15%), the range of other contaminants present in the flue, such as sulfur which must be stripped before use, and the requirement for algal production to be co-located with industrial production, where other essential requirements, such as a large and consistent supply of water, nutrients and sunlight, may not be available [17,22]. Therefore, there is a need to develop alternative techniques to provide carbon to algal cultures at large-scales at any location. Moreover, it is important that these solutions do not require significant infrastructure as the cost of carbon supplementation needs to be minimal to enable its viability. One potential approach is to use the natural process of microbial respiration, where the growth of bacterial populations within the algal cultures is promoted by providing an energy source, which can be metabolized to produce CO_2 *in situ*. Sugarcane molasses is a low-value, thick syrup that is a residual by-product of the sugar refining process and, which contains residual sugars (40–50%) that are not economically viable for recovery through further processing [26]. These sugars are predominantly sucrose, with lesser quantities of glucose and fructose [27]. Molasses has a calorific value of 10–12 MJ kg^{-1} and contains a range of vitamins and minerals, but virtually no nitrogen or phosphorous (Supp. A) [27,28]. In total, 3–7% of the sugarcane crushed by sugar mills ends up as molasses [27], with a market value of approximately AUD $200–300 tonnes. This provides a relatively abundant and potentially cost-effective source of carbon for the large-scale production of algae.

The aim of this study was to, therefore, examine the use of molasses as a source of carbon for the intensive culture of the freshwater macroalga, *O. intermedium*. Specifically, dose-response curves were generated showing the productivity of *Oedogonium* in cultures receiving equal amounts of carbon from two sources, molasses or CO_2, during winter and summer conditions. This data was then used to assess the carbon requirements of *O. intermedium* cultured in HRAPs that have minimal water exchange rates.

2. Methods

2.1. Study species

O. intermedium is a genus of unbranched filamentous green macroalgae with a worldwide distribution that has been identified as a key target group for the bioremediation of freshwater waste streams [4,7,29–32]. The species used in this study was identified as *O. intermedium*, using morphological characteristics and taxonomic keys [33,34]. The biomass used in this study was sourced from stock cultures maintained at the Marine & Aquaculture Research Facility at James Cook University, Townsville (latitude: 19°19′47.37″S; longitude 146°45′40.01″E).

2.2. Culture conditions

In this study, three HRAPs (20 [L] × 3 [W] × 0.4 [D] m), each having a volume of approximately 22,500 L and a surface area of 65 m^2 were used to cultivate *O. intermedium*. Each week, these HRAPs were filled with dechlorinated tap water and stocked with 5.5 kg (0.24 g L^{-1}) fresh weight (fw) of *O. intermedium* biomass that was harvested from stock cultures maintained in 10,000 L tanks. The culture water in each HRAP was enriched with MAF (Manutech Pty. Ltd.; 11.77% N, 1.46% P) growth media at a rate of 0.1 g L^{-1}. Throughout the culture period, the concentration of nitrogen and phosphorous were always maintained in excess (> 0.1 mg L^{-1} of nitrogen and > 0.01 mg L^{-1} of phosphorous) to ensure that the growth potential of the *O. intermedium* was not limited. After a five day culture period, the entire HRAP was harvested by pumping the culture water over a wedge wire sieve bend screen with 300 μm aperture (Wedgetech Pty Ltd.) to dewater the algal biomass. This biomass was then spun in a domestic washing machine (1000 rpm) and weighed prior to being dried at 60 °C to constant weight. To determine the productivity from each harvest, a fw:dry weight (dw) ratio was determined for each of the three tanks at each harvest by drying a 100 g sample of freshly harvested algae at 60 °C for 48 h. The quantity of biomass (5.5 kg fw) that was initially used to inoculate each tank was subtracted from the final harvest weight of each tank and the biomass productivity was calculated using Eq. (1) where B_f is the final weight, B_I is the initial weight, fw:dw is the fresh to dry weight ratio, A is the surface area of culture tanks and t is the number of days in culture.

$$P = \{[(B_f - B_i)/(\text{fw: dw})]/A\}/t \quad (1)$$

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