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Biorecovery of nutrient waste as protein in freshwater macroalgae

Andrew J. Cole *, Rocky de Nys, Nicholas A. Paul

MACRO — the Centre for Macroalgal Resources and Biotechnology, James Cook University, Queensland 4811, Australia

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ABSTRACT

Over 150 million tonnes of protein are consumed annually by domesticated animals and this demand is expected to double by 2050. Freshwater macroalgae are a group of organisms that could contribute significantly to these future protein requirements as they can be cultured on-site, utilising the nutrient waste water from animal production. The aims of this study were to investigate the relationship between nitrogen supply, biomass productivity and the quantity and quality of protein in the freshwater macroalga, *Oedogonium*, cultured in situ in the waste water from an intensive freshwater fish farm. The dry weight (DW) productivity of *Oedogonium* ranged between 23.9 and 35.7 $g \cdot m^{-2} \cdot day^{-1}$, whilst on an ash free basis the rate of productivity ranged between 17.1 and 23.6 $g \cdot m^{-2} \cdot day$. These productivities are the highest documented for freshwater macroalgae. The protein content (sum of amino acids) of this biomass increased linearly with increasing nitrogen content of the biomass from a minimum of 3.96 $g \cdot 100\ g^{-1}$ DW when the internal nitrogen content was 0.86%, to a maximum of 18.07 $g \cdot 100\ g^{-1}$ DW when the nitrogen content was 4.16%. The quality of the protein in *Oedogonium* was high, with the essential amino acids accounting for 43.1–43.8% of the total amino acids. Methionine accounted for between 1.6 and 1.9%, and lysine 6.8 and 7.3% of this protein, with the proportion of each slightly increasing as the internal nitrogen content of the biomass decreased. The quantity and quality of protein in the *Oedogonium* biomass in this study are equivalent to, or higher than, many terrestrial crops that are currently used as a protein source in animal feeds. As such, integrating the production of *Oedogonium* into the waste management of intensive animal production will provide a mechanism to recover nutrients which, firstly, delivers a novel source of protein for the agricultural sector and, secondly, contributes to the environmental sustainability of intensive animal production through bioremediation.

1. Introduction

Approximately 70% of all harvested agricultural crops, which account for more than 150 million tonnes of protein, are consumed annually by domesticated animals [10,59]. By 2050 the world's population is predicted to increase by 2–4 billion people and the global demand for protein to feed to animals is predicted to double [13,28,57]. It is unlikely that the cultivation of traditional protein sources such as legumes, cereals and grains can meet this demand [23,25,33,39,56]. These crops are restricted to arable, fertile land and there is a very limited scope to increase the production of these crops without clearing large areas of rainforest [19,25,56,57] and using large quantities of synthetic fertilisers, which are themselves a finite resource with environmental consequences for poor application practices [16,38,39,54]. Overcoming these challenges and meeting the protein requirements of the future will require the adoption of unconventional sources of protein [2,10,62]. Furthermore, as the majority of these novel sources of protein will ultimately be fed to animals, they need not be tasty or attractive to humans. Rather, they need only be nutritionally complete and supply the protein required to facilitate the rapid growth of animals.

Freshwater macroalgae are a group of organisms that could contribute significantly to these requirements and provide protein at an industrial scale [14,34,44,45,47]. Two major advantages of freshwater macroalgae are that they do not compete directly with terrestrial crops for arable land, and they do not require fertiliser inputs as they can be cultivated using high-nutrient waste water [14,32,43,44,63]. Moreover, freshwater macroalgae have high rates of biomass production, often exceeding 15 $g \cdot DW \cdot m^{-2} \cdot day^{-1}$, and high rates of nutrient uptake and uptake efficiency [14,43]. At these high productivities between 50 and 85% of the supplied nitrogen is incorporated into the algal biomass [14,44,63]. This translates to two key features — the ability to efficiently recover dissolved nutrients at high rates and the conversion of these nutrients into protein. Moreover, freshwater macroalgae have relatively simple structures, characterised by limited cell differentiation which means that all cells are capable of photosynthesis and nutrient assimilation, such that the entire cultured biomass can be treated as a homogenous protein source.

The amount of crude protein in any organic matter is closely linked to its nitrogen content and can be calculated by using a feedstock

* Corresponding author.
E-mail address: andrew.cole3@jcu.edu.au (A.J. Cole).

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specific nitrogen-to-protein conversion factor that varies between 4.1 and 6.25 [40,65], with algae at the lower end of this spectrum [47]. Whilst there is limited data on the amount of crude protein in freshwater macroalgae, values reported in the literature range between 16 and 44% of the dry weight [14,47,63]. These values are comparable to, or higher than, many traditional protein crops that typically range between 8 and 43% [14]. However, to be an effective feed replacement, the quality of the protein must be such that amino acids are supplied in the optimal quantities and ratios, with a focus on providing essential amino acids that cannot be, or are inadequately, synthesised by animals [10,63]. Methionine and lysine are most often the limiting amino acids in non-ruminant agricultural livestock because the main protein crops fed to these animals, cereals and legumes, are naturally low sources of one or both of these essential amino acids [8,9].

The nitrogen content of freshwater macroalgae can vary significantly in response to changes in nitrogen availability both in terms of water exchange rates and nitrogen concentrations of the incoming water [14]. However, it is unclear how the amino acid profile of freshwater macroalgae changes in response to variation in the internal nitrogen concentration and if there is potential to manage the culture system to optimise both the rate of recovery of nutrients and their bioconversion into protein. The primary aim of this study was therefore to investigate the relationship between the supply of nitrogen, rate of biomass production and the quantity and quality of protein in freshwater macroalgae when it is cultured in the waste water from an intensive freshwater fish farm. To manipulate the supply of nitrogen, and potentially, the nitrogen content or protein within the biomass, three culture tanks were assembled in series where the nutrient waste water moved from one tank to the next prior to release. The productivity, rate of nitrogen uptake and the quantity and quality of the protein in the algal biomass were quantified both between the three positions in series, and to three control tanks arranged in parallel which had a single pass of nutrient waste water.

2. Methods

2.1. Study species and site

Oedogonium is a genus of unbranched filamentous green algae made up of small cylindrical cells. This genus has a worldwide distribution and is a common component of natural ecosystems where it grows either attached to the substrate or as free floating mats. *Oedogonium* is a robust and competitively dominant genus that has been identified as a key target group for the bioremediation of freshwater waste streams [15,34,35,50] and as a feedstock biomass for bioenergy applications [47,48]. Stock cultures of *Oedogonium* sp. (GenBank accession number: KF606977) as described in Lawton et al. [35], and hereafter referred to as *Oedogonium*, were sourced from stock cultures at the Marine & Aquaculture Research Facilities Unit, at James Cook University (JCU), Townsville (latitude: 19.33°S; longitude 146.76°E) and transported to the study site at the Good Fortune Bay Fisheries Ltd., a 450 T. yr^{-1} freshwater barramundi farm also located in Townsville (latitude: 19.33°S; longitude 146.76°E). Barramundi are cultured in large outdoor ponds, constantly supplied with clean bore water. Pond effluents are passively cleaned using reed beds and settlement ponds to reduce suspended solids. In this experiment, pond effluent water was pumped out of the settlement pond and screened to 150 μm using a sandfilter. This water was then used to feed fifteen cylindrical tanks (Duraplas AP 1000; 1000 L capacity, 1.19 m^2 surface area) to a depth of 70 cm that gave a total culture volume of 864 L. In these tanks *Oedogonium* was maintained in tumble culture through the use of a central aeration ring (45 cm circumference). A 750 μm mesh screen was used to prevent the filaments of *Oedogonium* from exiting the tank with the outgoing water, permitting water exchanges without loss of biomass.

2.2. Experimental design

This experiment examined how both the total content of amino acids and the proportion of essential amino acids changed in response to the internal nitrogen content of *Oedogonium* cultured on-site at the largest, practical scale to accurately measure growth. To do this 1000 L tanks were connected together such that the water overflow from one tank became the inflow for the next tank, providing the sequential reduction in the availability of nitrogen to each subsequent culture without reducing the rate of water exchange (Fig. 1). A total of three tanks were linked together in this manner, with this replicated three times during each of the three week-long trials. This experimental design meant that the *Oedogonium* cultured in position one (P1, Fig. 1) had the highest availability of nitrogen; position two had the proportion of nitrogen not incorporated into the biomass of position one; and position three was expected to be the most nitrogen-limited as this biomass only received the nitrogen not assimilated by the biomass in positions one and two. Each series of three tanks had a water exchange rate of 1 tank volume per day (1 vol·day^{-1} or 864 L·day^{-1}). This renewal rate was controlled using a digital flow meter (Hoselink digital flow meters) fitted to the first tank in each series which dispensed an hourly allotment of water (36 L) into each tank in position one of each series. Three control t\anks were used in this experiment, with these tanks set up in parallel, where the waste water entered each tank and then exited the tank to waste (Fig. 1). These control tanks had a water exchange rate of 0.33 vol·day^{-1} (284 L·day^{-1}) and are hereafter referred to as control tanks. The control tanks tested an alternative operational scenario in which the same volume of waste water is split into three direct feeds and enabled a comparison in productivity, rate of nitrogen uptake and internal nitrogen content of the *Oedogonium* biomass that can be achieved relative to the sum of the tanks in any single series. Three additional stock tanks were maintained on site and received a daily water exchange of 1 volume and were stocked with 200 g (0.23 g·L^{-1}) (fresh weight: FW) of algal biomass. The biomass from these tanks was used to initially stock each tank for each of the three replicate trials. This biomass had a mean internal nitrogen content of 4.92 (±0.69) % (see the Results section) over the three separate week-long trials that were conducted in a six week experimental period between the 4th of October and the 15th of November 2013.

A 150 mL sample of incoming pond water to the tanks was collected on days one, three and six of each week long trial to determine the concentrations of ammonium-, nitrite-, nitrate–nitrogen (summed to total N) and reactive phosphorous. Water samples were analysed by

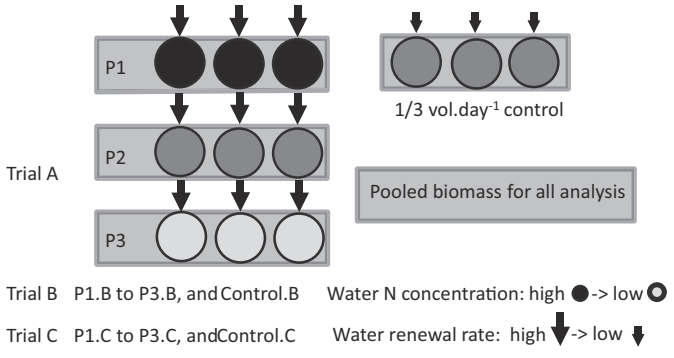

Fig. 1. Schematic of experimental set up. Positions 1, 2 and 3 (P1, P2 and P3) are connected in series, such that the inflow water flows from one position to the next. Each of the three control tanks were arranged in parallel and each received a unique water feed. The control tanks received a water exchange rate that was 1/3 that of the tanks in series. During each trial the biomass cultured in the same position (shaded rectangles) was pooled based on the proportional growth of each tank to give one replicate for each position in series and one replicate for each of the controls per trial. Three separate one week growth trials were repeated over a 6 week period. The shading of the tanks and the size of the arrows represent the relative amount of nitrogen and water exchange the different cultures received.