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Adding value to the treatment of municipal wastewater through the intensive production of freshwater macroalgae

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ABSTRACT

Municipal wastewater treatment plants discharge large quantities of treated water that, in many regions, is not productively used and is instead released directly into the environment. In this study we examine the use of freshwater macroalgae as an in-line tertiary treatment process for existing municipal treatment plants. We examine the suitability of using the treated discharge water from a 29,000 $m^3.day^{-1}$ municipal wastewater treatment plant as the sole source of water and nutrients for the intensive cultivation of the freshwater macroalga *Oedogonium intermedium*. A monoculture of algae was initially cultivated for a 3 month period in which water quality and biomass productivity were quantified and the composition of the biomass characterized. These cultures were then maintained for a further 9 months to determine the average monthly biomass productivity, and seasonal variation, over a 12 month period. The cultivation of *Oedogonium* significantly improved the quality of the discharged water with a 36% reduction in total nitrogen and a 65% reduction in total phosphorous. The average monthly biomass productivity of *Oedogonium* ranged between a minimum of 8.9 g $DW\cdot m^{-2}\cdot day^{-1}$ in June (austral winter – dry season) and a maximum of 15.8 g $DW\cdot m^{-2}\cdot day^{-1}$ in January (austral summer – wet season) with an average annual rate of 12.5 g $DW\cdot m^{-2}\cdot day^{-1}$. The biomass produced was of a high quality with a total protein content of 23 $g\cdot 100\ g^{-1}$ and a total lipid content of 10 $g\cdot 100\ g^{-1}$. Both the protein (10 $g\cdot 100\ g^{-1}$ of essential amino acids) and lipid (4.5 $g\cdot 100\ g^{-1}$ of polyunsaturated fatty acids) provide product opportunities for animal nutrition. This study demonstrates that the production of algae integrated with the operation of conventional wastewater treatment can complement and add value to existing processes by recovering residual nutrients and metals and, at the same time, create a high-quality biomass resource for product development.

1. Introduction

The intensive production of micro- and macro-algae, both as a tool to recover waste nutrients and as a source of sustainable biomass, has developed significant momentum in the last decade and is now viewed as a viable technology for application at commercial scales [13,46,53,59,61]. Algae are particularly attractive as part of a transformative solution where the nutrients in wastewater produced by industry can be recovered and converted into biomass, increasing the environmental sustainability of industry by treating water while simultaneously helping to meet the ever increasing demand for food and energy [13,22,31,36,51,66]. In order to implement these "closed loop" systems [40], and produce biomass at a scale required for it to be commoditized, there is a need to integrate the land-based production of algae with existing water and nutrient sources [11,24,50,63]. Specifically, a large and consistent supply of water is fundamental as every hectare of algal production will require approximately 5000 m^3 of water to initially fill the shallow culture ponds and ongoing daily water exchanges are required to deliver nutrients to the cultures, facilitate harvesting of the biomass and replace water lost to evaporation [12,67]. In general, for freshwater macroalgae, a water dilution rate of between 50 and 100% per day is sufficient to maintain cultures in a highly productive state when using wastewater with relatively low concentrations of nutrients (<5 $mg\cdot L^{-1}$ N) [12,13].

There are a range of industries that could be explored for the purpose of integration, however, the largest and most consistent source of wastewater that contains nutrients is from municipal wastewater treatment plants (WWTP). Worldwide, at least 181 km^3 of municipal wastewater is treated annually and <13% of this treated effluent is reused, with the majority discharged into the environment [10,57]. Modern sewage treatment plants are very efficient at removing nutrient and biological contamination from municipal wastewater [10,23,56]. Despite this, their discharge water contains a relatively high residual concentration of nitrogen (>3 $mg\cdot L^{-1}$) and phosphorous (>0.5 $mg\cdot L^{-1}$) compared to the receiving environments [10]. When these concentrations are multiplied by the amount of water discharged daily, which for larger plants can exceed 100,000 $m^3\cdot day^{-1}$, it represents a large export of nutrients to the

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environment [10]. Moreover, due to their low concentrations, these residual nutrients are expensive to treat on a per unit basis compared to the higher concentrations found in bulk sewage [56]. As such, an innovative approach to recover the residual nutrients is to utilize them for the targeted production of high-value species of algae.

There are many qualities of existing discharge water from sewage treatment plants that make it an attractive water source for the intensive production of algae. It is a stable source of nutrients with minimal fluctuations throughout the year and, if passed through membranes or clarified, its turbidity is low permitting high penetration of light. In addition, it is relatively clean of microbial contaminants that could affect the algal cultures [25,35]. Consequently, the algal biomass produced in this treated water should be of a high and consistent quality, potentially making it suitable for higher value applications). Furthermore, the production of this algal biomass will benefit the treatment plant itself, as the quality of the discharge water will be further improved prior to discharge into the environment. This could notionally extend the life of existing infrastructure as the treatment capacity of the plant could be increased thereby complying with environmental discharge limits and postponing the need for capital expenditure to upgrade the plant.

The aim of this study is therefore to demonstrate that freshwater macroalgae can be continuously cultured in the treated discharge water of a municipal treatment plant to, firstly, add value to the existing process of wastewater treatment by recovering nutrients and improving the quality of the discharged water that is released into the environment, and secondly, to generate a homogenous biomass resource that is suitable for a range of product applications. Specifically, we quantify the amount of nitrogen, phosphorous and metals recovered by the algae over a 3 month culture period and characterize the biochemical composition of the produced biomass to identify areas where value can be realized. We subsequently extend the cultivation to cover a 12 month period and quantify the average monthly biomass production in this continuous culture system. These data provide a basis to determine the scale of algal operations and volume of products that could be supported by treatment plants.

2. Methods

2.1. Study species and location

Oedogonium 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 [12,14,38,39,47,54] and as a feedstock biomass for bioenergy and animal feed applications [13,15,46]. The species used in this study was identified as *Oedogonium intermedium*, using morphological characteristics and taxonomic keys [20,39], and hereafter referred to as *Oedogonium*. The biomass used in this study was sourced from stock cultures maintained at the Marine & Aquaculture Research Facility, at James Cook University (JCU), Townsville (Latitude: 19°19′47.37″S; Longitude 146°45′40.01″E) and was transported to the Cleveland Bay WWTP (Latitude: 19°17′19.21″S; Longitude 146°51′20.97″E).

2.2. Treatment plant capacity, processes and changes in nutrient concentrations

The Cleveland Bay WWTP receives on average 20,000 m^3 of municipal wastewater daily with a mean total nitrogen concentration of 55 $mg \cdot L^{-1}$ and a mean total phosphorous concentration of 8.3 $mg \cdot L^{-1}$ (Townsville City Council long-term operational data as reported in [47]). After initial primary treatment and sludge separation the mean total nitrogen and phosphorous concentrations in this water are reduced to 49 $mg \cdot L^{-1}$ and 7.3 $mg \cdot L^{-1}$, respectively. A 10-fold reduction in the concentration of these nutrients occurs during secondary treatment with the secondary effluent having a total nitrogen and phosphorous concentration of 4.0 $mg \cdot L^{-1}$ and 0.8 $mg \cdot L^{-1}$ respectively. Part of this treated effluent (2500 $m^3 \cdot day^{-1}$) is reused on-site for irrigation and wash down purposes, and for occupational health and safety reasons is chlorinated at a rate of 0.08 $mg \cdot L^{-1}$. This chlorinated effluent was used as the source of water and nutrients in the current study. The remaining effluent is discharged to the ocean. In the raw sewage there is a total of 1000 kg nitrogen and 160 kg of phosphorous entering the plant each day, with this concentration reduced by 92% and 90% respectively during the treatment process to result in a net export of 80 kg nitrogen and 16 kg of phosphorous per day in the treated discharge water (Fig. 1). At each stage of the treatment process, the solids are separated, dewatered and removed from the plant. During this dewatering process a low volume (800 m^3) but high nutrient effluent (centrate) is created and returned to the untreated incoming sewage stream for further treatment (Fig. 1). In total 3% of the flow but 13% of the total nitrogen and 6% of the total phosphorous from each treatment pass are re-combined with the raw sewage at the start of the treatment process (Fig. 1).

2.3. Culture conditions

Oedogonium was cultured using three large parabolic tanks (each 25 × 2 m; surface area 50m^2), filled with discharge water to a depth of 0.75 m to give a combined tank volume of ~80 m^3. This depth represents a successful trade-off between the total volume of the culture and their surface area such that a high treatment capacity is created without light limitation becoming problematic. *Oedogonium* was maintained in tumble culture in these tanks through a central 50 mm aeration line with air supplied by a side channel blower (Aerotech SD600). These cultures were maintained under constant flow, with each tank having a dilution rate of 27 $m^3 \cdot day^{-1}$ (i.e. 100% or 1 tank volume per day). This dilution rate provided a flux of 1.05 $g \cdot m^{-2} \cdot day^{-1}$ of available dissolved inorganic nitrogen (DIN = ammonia-N + nitrite-N + nitrate-N) and 0.45 $g \cdot m^{-2} \cdot day^{-1}$ phosphorous (DIP = phosphate-P). To prevent the filaments of *Oedogonium* from exiting the tanks with the outgoing water a 750 μm mesh screen covered each overflow pipe. A ring of aeration around each outlet prevented these screens from becoming clogged with the algal biomass and enabled a constant flow of water. *Oedogonium* was cultured continuously at this site for a 12 month period between the 2nd of March 2015 and the 29th of February 2016 (austral summer). During this period the water temperature and photosynthetically active radiation (PAR) were measured continuously. Water temperature in each tank was measured every hour using HOBO data loggers (UA-002-64) while PAR was measured every 5 s using a flat panel Li-190SA Quantum Sensor connected to a Li-1400 Data logger (Li-Cor, Linclone, NE, USA). The pH and electrical conductivity of the incoming effluent were measured between 2 and 3 pm, twice a week.

2.4. Nitrogen, phosphorous and metal concentrations in the treated discharge effluent

The concentrations of nitrogen, phosphorous and metals in the water were monitored during the first 3 months of cultivation (March to June 2015). During this time two water samples were taken from each of the three tanks twice a week, one sample from the incoming discharge water and another sample of equal volume from the outgoing effluent of each tank. The total nitrogen content (TN) (APHA 4500-NO_3^- F after alkaline persulfate digestion), dissolved inorganic nitrogen (DIN) (TAN [APHA-NH_3 G] + NO_2^- [APHA 4500-NO_2^- F] + NO_3^- [APHA-NO_3^- F]), total phosphorous (TP) (APHA 4500-P F after alkaline persulfate digestion) and filterable reactive phosphorous (FRP) (APHA 4500-P F) were analysed for each sample by the Australian Centre for Tropical Freshwater Research at JCU using standard methods [5]. The concentration of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr, V and Zn was measured with a Bruker 820-MS Inductively Coupled Plasma Mass Spectrometer (ICP-MS), and Ca, K, Mg and Na with a Varian Liberty series II Inductively Coupled Plasma Optical Emissions Spectrometer (ICP-OES) at the Advanced Analytical Centre at JCU, Townsville.