Comparing Harvest Productivity of the Filamentous Alga *Oedogonium* with Microalgae

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Abstract

As the planet faces depletion of its natural resources, alternative and sustainable energy sources are becoming increasingly sought after. Research on the growth of algae has revealed their potential for carbon capture to reduce greenhouse gas emissions and for conversion into a fuel source for bioenergy applications. Filamentous algae have attracted recent attention as an optimal species due to their ease of harvest and dominance over other species. To determine the most suitable species for future biomass applications, a 1000-L open raceway pond was inoculated with the freshwater filamentous alga, *Oedogonium*, with the addition of CO_2. An additional two 1000-L raceway ponds with established cultures of microalgae already receiving CO_2 were used as a comparison to the growth of *Oedogonium*. The pond culture was harvested weekly to determine culture density/growth (mg VSS/L) and harvest productivity (g VSS/m^2-day). After 3 weeks, *Oedogonium* harvest productivity exceeded both microalgal ponds at 13.7 ($\pm$ 0.3) g VSS/m^2-day compared to 9.3 ($\pm$ 0.6) and 9.5 ($\pm$ 0.5) g VSS/m^2-day for the microalgae. Thus, *Oedogonium* could serve as a suitable species for biomass production due to its higher productivity rates when compared with microalgal growth.

Keywords: algae, *Oedogonium*, bioenergy, filamentous, carbon dioxide

Introduction

With a large dependence on non-renewable fossil fuels as a source of energy, more sustainable options are being researched to meet society's energy demands without diminishing finite resources. The growth of various species of algae could potentially provide an alternative energy source, in addition to many other environmental benefits. Algae can be used as a feedstock to produce methane gas, a form of bioenergy, through the process of anaerobic digestion where bacteria break down the organic components of algal biomass and release methane as a by-product (Wilkie, 2008). Arable land is not required for the growth of algae, reducing direct competition with land use for agricultural purposes (Roberts et al., 2013). Algae remove nutrients and toxins from wastewater streams, helping to keep water sources free of contaminants (Cole et al., 2016; Edmundson and Wilkie, 2011; Lincoln et al., 1996; Wilkie and Mulbry, 2002). As part

of the process of photosynthesis, algae capture carbon dioxide (CO_2), a greenhouse gas, from the atmosphere and convert it into their biomass (Lawton et al., 2014).

In many countries, the combustion of coal at power plants generates the majority of their energy in the form of electricity. This process releases large amounts of CO_2 into the atmosphere, contributing to an increase in the earth's warming potential (Singh et al., 2003). To offset the negative impacts of emissions from coal power plants, new ways to capture this carbon are being studied, including the use of algae. Generating bioenergy from algal biomass results in carbon capture that is equivalent to the carbon release once the fuel is burned, creating an overall carbon neutral process (Schamphelaire and Verstraete, 2009). The uptake of carbon directly from the atmosphere by algae is inefficient, but CO_2 dissolution in water allows better access to the carbon. Depending upon the pH of the water, carbon equilibrates between three different forms: CO_2 (carbon dioxide), HCO_3^- (bicarbonate), or CO_3^{2-} (carbonate). HCO_3^- is the form of carbon most readily available for algal uptake and is at its highest concentration at a pH of approximately 8 (Choo et al., 2008). Roberts et al. (2013) and Cole et al. (2014) determined that CO_2 addition by diffusion in water resulted in an increase in algal biomass productivity. The algal biomass can then be used for multiple applications.

Among the different species of algae being cultivated, *Oedogonium*, a freshwater filamentous alga, is gaining attention. Typically, cultivation of microalgae has been the most popular choice in recent years for its use in various cosmetic products and in human and animal nutrition (Spolaore et al., 2006). However, filamentous algae are now being researched due to their dominance over other species in most environmental conditions (Lawton et al., 2013). A study based on creating polycultures of the filamentous algae *Oedogonium*, *Cladophora*, and *Spirogyra* and subjecting them to different cultivation conditions to determine a target species for future biomass production demonstrated equal proportions of *Oedogonium*, *Cladophora*, and *Spirogyra* initially. However, after three weeks, the concentration of *Oedogonium* reached over 80% in all treatments (Lawton et al., 2013). This revealed the potential for *Oedogonium* as a superior species for biomass applications in terms of its high productivity and dominance over other filamentous species. Additionally, *Oedogonium* was shown to be a promising species compared to three strains of filamentous algae where increases in lipid and starch concentrations were revealed under nitrogen-starved conditions, properties important for biofuel production (Zhang et al., 2016). Filamentous algae are also becoming favored in production due to the relative ease of

harvesting long thin filaments compared to the small (3-30 µm diameter) cells of microalgae (Molina Grima et al., 2003; Wilkie et al., 2011). Harvesting filamentous algae could lead to a reduction in the costs of algal production (Zhang et al., 2016).

The objective of the current study was to assess the harvest productivity and culture density of *Oedogonium* with the addition of CO_2 and compare it to the growth of different polycultures of established microalgae already receiving CO_2. This will determine the most suitable species of algae for carbon capture and future biomass production.

Materials and Methods

Algal Inoculation and Culture Condition

A 1000-L open raceway pond (Pond 2) in Gainesville, Florida was inoculated with a local culture of the filamentous algae, *Oedogonium*. The pond included a rotating paddle wheel to promote continuous mixing of the algae to enhance sunlight penetration. An additional two 1000-L raceway ponds (Ponds 1 & 3) with local polycultures of microalgae (*Scenedesmus*, *Dictyosphaerium*, *Ankistrodesmus*, and others) that were already established and receiving CO_2 were used as a comparison to the growth of the freshwater alga, *Oedogonium*. Each pond was supplied with CO_2 through diffusion to maintain a set pH of 8.0 and fertilized with Miracle-Gro (N-P-K: 30-10-10) at a rate of 41.6 g/week. The CO_2 addition was only new to the *Oedogonium* pond. Temperature and pH readings were monitored weekly over the course of the four-week study from March 13, 2018 to April 10, 2018. Daily average pond temperatures ranged from 18.1 °C to 18.3 °C.

Harvesting and Sample Collection

Each week, pond depths were measured. If the pond levels were below 25cm, water was added to adjust depths to 25cm to make up for evaporative losses. Pre-harvest samples (1 liter) were collected after the sides and bottoms of each pond were brushed to release any settled algae and provide a representative sample. A large plastic scoop was used to sample a section of each pond and the contents were placed in a container. Once the pre-harvest samples were collected, the ponds were harvested to half-volume (12.5cm) using a suction pump and refilled with dechlorinated tap water to restore to full volume (25cm). After full volume was reached, post-harvest samples were collected by the same method as for the pre-harvest samples.

Each pre-harvest and post-harvest sample was blended and filtered in triplicate. Total and volatile suspended solids were measured according to Standard Methods (APHA, 2012). To determine total suspended solids (mg TSS/L), dry weights were recorded by placing the samples in an oven at 105°C for one hour. The samples were placed in a muffle furnace at 550°C for two hours to determine volatile suspended solids (mg VSS/L). All VSS and TSS data were normalized to correspond with a pond depth of 25cm in case of rain events where depths would increase and culture density would become diluted. Weekly culture growth was calculated by the equation: $G = VSS_{pre} - VSS_{post}$, where VSS_{pre} represents ashed pre-harvest culture density averages from the current week and VSS_{post} represents ashed post-harvest culture density averages from the previous week. Harvest productivity (g VSS/m^2-day) was calculated by using the equation: $P = (G*0.25m)/T$, where G represents culture growth, $0.25m$ represents pond depth and T represents the number of days.

Microscopic Analysis of Cultures

Photomicrographs of the algae were taken at 100x, 250x, and 400x using brightfield microscopy. The images were analyzed on a weekly basis to determine the species makeup and changes in morphotype.

Results

Culture Density/Growth

Changes in culture density were determined from the algae harvest data on a weekly basis to analyze culture growth. An increase in culture density was revealed across all ponds (Table 1). Initially, the established microalgal ponds had higher increases in culture density than the *Oedogonium* pond. During week 3 of the experiment, *Oedogonium* had the highest increase in culture density, 382.0 (± 8.0) mg VSS/L compared to the microalgae with 265.3 (± 18.0) and 260.0 (± 13.9) mg VSS/L for Ponds 1 and 3, respectively.

Table 1. Weekly algal culture growth

	Microalgae Pond 1	*Oedogonium* Pond 2	Microalgae Pond 3
	(mg VSS/L)	(mg VSS/L)	(mg VSS/L)
Week 1	64.8 ± 3.9	14.4 ± 3.9	66.2 ± 12.0
Week 2	202.6 ± 8.3	130.0 ± 7.2	213.3 ± 2.3
Week 3	265.3 ± 18.0	382.0 ± 8.0	260.0 ± 13.9
Week 4	141.1 ± 17.4	248.2 ± 12.6	175.4 ± 6.2

Data are means ± standard deviation (n=3).

Harvest Productivity

Over the course of the four-week experiment, *Oedogonium* in Pond 2 surpassed the harvest productivity of microalgae in both Ponds 1 and 3 (Figure 1). Initially, *Oedogonium* had a lower productivity at 0.6 (± 0.1) g VSS/m^2-day during week 1 and 4.6 (± 0.3) g VSS/m^2-day during week 2, but increased to 13.7 (± 0.3) g VSS/m^2-day by week 3 and 9.1 (± 0.3) g VSS/m^2-day by the final week. Microalgae in Ponds 1 and 3 had harvest productivities of 2.3 (± 0.1) and 2.3 (± 0.4) g VSS/m^2-day during week 1, 7.4 (± 0.3) and 7.7 (± 0.1) g VSS/m^2-day during week 2, 9.3 (± 0.6) and 9.5 (± 0.5) g VSS/m^2-day during week 3, and 5.2 (± 0.6) and 6.4 (± 0.2) g VSS/m^2-day by the final week, respectively.
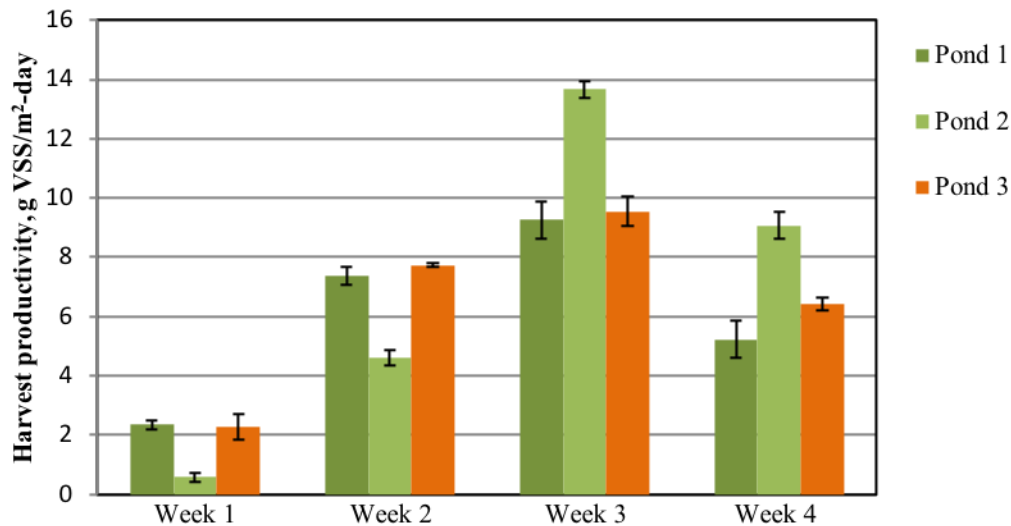

Figure 1. Harvest productivity of *Oedogonium* (Pond 2) and microalgae (Ponds 1 and 3). Data are means ± standard deviation (n=3).

Microscopic Analysis

During the first week, *Oedogonium* filaments were a dull green color with cell components aggregated towards the center, away from the cell walls (Figure 2). After three weeks, cell components dispersed, filling the entire capacity of the cell wall (Figure 3). The filaments appeared more robust with a brighter green color. *Dictyosphaerium* was still the dominant microalgae over *Scenedesmus* in Pond 1 after 3 weeks (Figures 4 and 5). Microalgae in Pond 3 transitioned from a balanced culture of *Scenedesmus* and *Ankistrodesmus* in week 3 to a culture dominated primarily by *Ankistrodesmus* during the final week (Figures 6 and 7).

Discussion

In terms of harvest productivity and culture growth, *Oedogonium* outperformed both microalgal ponds. Initially, *Oedogonium* had the lowest growth and a lower productivity for both week 1 and week 2. By week 3, there was a large increase in growth and productivity for the culture. This was likely due to the time required for the culture to adapt to the addition of CO_2. The physical changes of the algae indicate that the CO_2 addition promoted more robust and brighter green filaments of *Oedogonium*. These findings reveal the suitability of *Oedogonium* for future production.

Culture growth and productivity during week 4 likely decreased for all ponds because of a storm that brought 4.9 inches of rain, causing the ponds to overflow and spill some of the biomass. Solar irradiance data received from Florida Automated Weather Network (2018) revealed a significant decrease in irradiance from a high of 252.78 W/m^2 at the beginning of week 4 to a low of 21.65 W/m^2 by the end of the week (Figure 8). Because algae require sunlight for photosynthesis, their productivities likely decreased as a result of lower solar irradiance during week 4.

In a study by Cole et al. (2014), biomass productivities of *Oedogonium* in 15,000 L open outdoor tanks using dechlorinated tap water enriched with Guillards f/2 growth media reached 8.33 (± 0.51) g DW (dry weight) m^{-2}-day when adding CO_2 to maintain a pH of 7.5. Average culture temperatures during the Australian winter ranged from 18.4 ($\pm$ 2.2) to 19.1 ($\pm$ 2.24) °C. At a latitude of 19.33°S in Australia, daily average solar irradiance varies between 173.6 W/m^2 and 231.5 W/m^2 in the winter (Cole et al., 2018). By comparison, the greatest harvest productivity

Figure 2. Pond 2: *Oedogonium*, week 1.

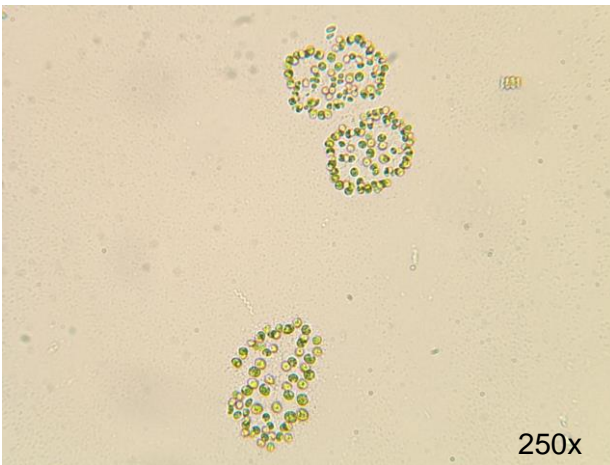**Figure 3**. Pond 2: *Oedogonium*, week 3.

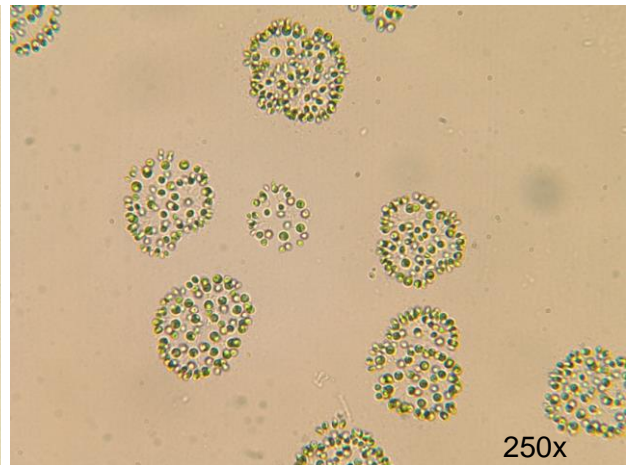**Fig. 4**. Pond 1:*Dictyosphaerium* and *Scenedesmus*, week 1.

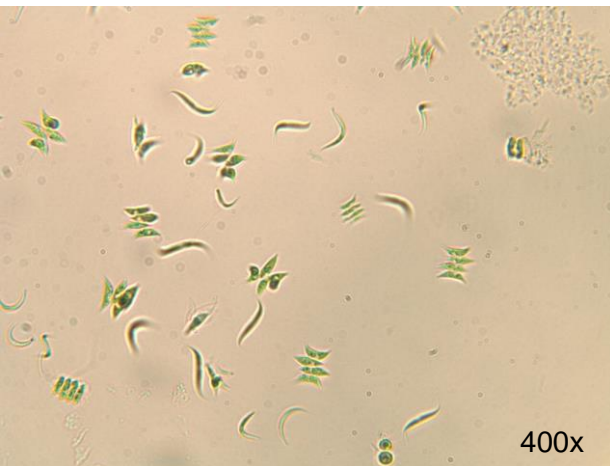**Figure 5**. Pond 1:*Dictyosphaerium*, week 3.

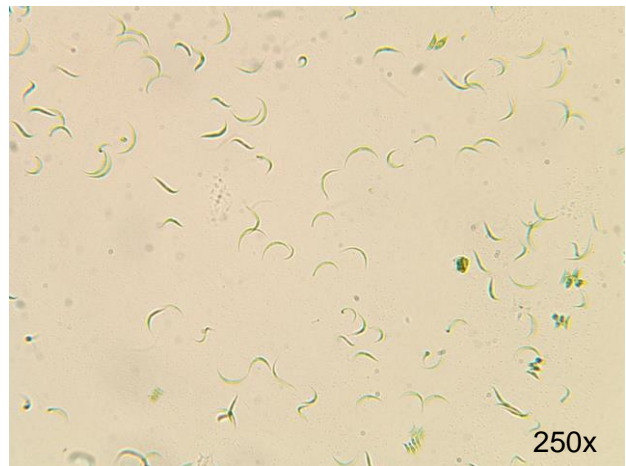**Fig. 6**. Pond 3:*Scenedesmus* and *Ankistrodesmus*, week 3.

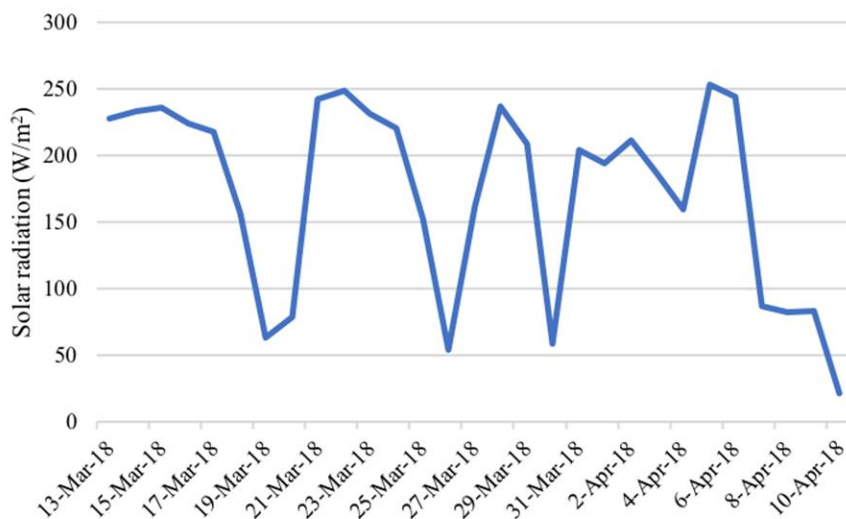**Fig. 7**. Pond 3:Dominance of *Ankistrodesmus*, week 4.

Figure 8. Solar irradiance (W/m^2) in Alachua County from
Florida Automated Weather Network (2018).

was slightly higher in this study at 13.7 ($\pm$ 0.3) g VSS/m^2-day, but both studies reveal high productivities of *Oedogonium* with CO_2 supplementation. In the current study, daily average culture temperatures ranged from 18.1 °C to 18.3 °C between ponds, which are similar to the temperatures in the Cole et al. (2014) study. Solar irradiance was also similar in the current study. Optimal temperature and solar irradiance conditions for the growth of *Oedogonium* were identified to be between 20 and 35 °C and greater than 173.6 W/m^2 (Cole et al., 2018). Thus, harvest productivity of *Oedogonium* might be even higher during summer months when temperatures are higher.

Conclusion

Overall, *Oedogonium* appears to be a suitable species to use for biomass production purposes. Not only did it outperform other genera, the filaments can be harvested easily with a net, and it appears to be quick at adapting to culture conditions to obtain high biomass productivities, as demonstrated by its large increase in culture growth during week 2 and week 3. Microalgae may require more cost-intensive production and should be further researched in comparison to different species of filamentous algae. This research suggests *Oedogonium* is an efficient organism for carbon capture and can potentially be used for multiple sustainable practices, including nutrient removal from wastewaters and bioenergy production in the future.

Acknowledgements

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