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Cultivating the marine microalga *Nannochloropsis salina* under various nitrogen sources: Effect on biovolume yields, lipid content and composition, and invasive organisms

Herman Campos [a], *Wiebke J. Boeing* [a,*], *Barry N. Dungan* [b], *Tanner Schaub* [b]

[a] *Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Las Cruces, NM 88003, USA*
[b] *Chemical Analysis and Instrumentation Laboratory, New Mexico State University, Las Cruces, NM 88003, USA*

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ABSTRACT

Algae can be a viable source for biofuel production, but the source of nitrogen used to cultivate could affect algae yields. Here, we observe how various nitrogen treatments can impact the growth and biovolume of microalga *Nannochloropsis salina* as well as invasion of undesired organisms. Invading organisms increase the likelihood of crashes of the desired microalgae culture. Experiments were conducted over 28 days in open aquaria in a greenhouse. We used five different nitrogen treatments; ammonium chloride (NH_4Cl), ammonium hydroxide (NH_4OH), sodium nitrate ($NaNO_3$), urea (CH_4N_2O), and a mixture of all these sources. Highest values for Maximum Sustainable Yield (MSY), a measure of potential harvest rate based on population productivity, were observed in the urea treatment, but cell size was smaller compared to other treatments. Sodium nitrate and the mixture of nitrogen sources also had high MSY values but larger cell sizes, making them the treatments with highest total biovolume. The highest percentages of lipid by weight, but also highest densities of invading organisms were observed in the mixed treatment. Our results suggest that tradeoffs between biovolume and lipid yields as well as culture success can ultimately decide what nitrogen sources to use.

1. Introduction

The current energy crisis results from the excessive consumption of non-renewable fossil fuel and greenhouse gas emissions are causing negative environmental repercussions [1]. Thus, development of modern energy sources like biofuels are important. These are fuels that may be derived from plant oil or animal lipid and their reaction with alcohol to form mono-alkyl ester [2]. Microalgae are at the forefront as a biofuel candidate because they can be grown all year around on non-arable land, they require less space, yields are higher compared to traditional crops, and novel technologies are making rapid improvements [3,4]. However, at this point, microalgae for biofuels are not an economic viable commercial enterprise [5]. Next to using various products from algae,

* *Corresponding author*. Tel.: +1 575 646 1707; fax: +1 575 646 1281.
E-mail address: wboeing@nmsu.edu (W.J. Boeing).
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lowering the cost of algae lipids is a major goal of current research.

While maximum algae growth occurs typically under ample supply of nutrients and light and optimum temperature conditions, lipid accumulation is enhanced when microalgae cells are stressed. The maximization of oil yield in microalgae strains and cultures through environmental manipulation can be less expensive and easier to achieve in the short-term than alternatives such as gene modification. There is potential to increase yields by manipulating environmental factors, which cause stress for microalgae and induce maximum accumulation of lipids [6]. Sources of stress include manipulating environmental conditions such as salinity [7,8], pH [9,10], temperature [11,12], and nutrients [13,14].

It has been well established, that nitrogen limitation in general is beneficial for increasing lipid accumulation in some microalgae [15,16] and impacts the type of lipids produced [17]. Specifically, nitrogen replete cultures will often produce more triacylglycerols (TAG) in contrast to nitrogen deplete batch cultures, where lipid C16:0 chains are favored [18]. However, lipid productivity is dependent on lipid accumulation as well as microalgae biomass. Wan et al. [16] were able to demonstrate that highest lipid productivity might occur under higher nitrogen concentrations. Not only the amount of nitrogen, but also the source of nitrogen is likely to have an impact on biomass and lipid productivity as well. To date, this topic is not as well studied [13,19,20].

Among 30 microalgae, *Nannochloropsis* spp. was identified to have among the highest biomass and lipid productivity [15]. These are marine microalgae that are tolerant to a large range of environmental conditions. Thus, various *Nannochloropsis* species have become popular model systems. However, to our knowledge, the effects of different nitrogen sources on growth rate and lipid production on *Nannochloropsis* have not been tested. Additionally, despite the general knowledge of contaminating invading organisms being a major challenge [7,15,21,22], little is known about what organisms invade production systems and what environmental conditions might limit their occurrence [8,10].

Here, we test different nitrogen sources (ammonium chloride (NH_4Cl), ammonium hydroxide (NH_4OH), sodium nitrate ($NaNO_3$), urea (CH_4N_2O), and a mixture of all these sources) on growth and lipid accumulation of *Nannochloropsis salina*. Furthermore, we identify organisms that invaded our open cultivation systems in the various treatments. While we found three other studies, that looked at effects of different nitrogen sources on growth of various microalgae [13,19,20], only one study [13] included lipid accumulation and no other studies considered invading organisms or tried to use different nitrogen sources simultaneously (mixed treatment).

2. Methods

2.1. Microalgae cultures and experimental set-up

N. salina (strain 1776) was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. The experiment was conducted in a greenhouse located at New Mexico State University's Fabian Garcia Science Center in Las Cruces, New Mexico, USA. We grew *N. salina* in an outdoors raceway at Fabian Garcia in standard f/2 medium for marine microalgae [23] and subsequently inoculated cultures in a greenhouse where aquaria were subjected to natural light and temperature conditions. The culture medium used during experiments was also f/2 medium, with an alternative source of nitrogen for each treatment. We had a total of five nitrogen treatments (ammonium chloride, NH_4Cl; ammonium hydroxide, NH_4OH; sodium nitrate, $NaNO_3$; urea, CH_4N_2O; and a mixture of all these sources). In order to acquire identical amounts of nitrogen from these different sources we calculated the g mol^{-1} of nitrogen. The standard f/2 medium contains 75 g of sodium nitrate per liter of which 14.0067 g mol^{-1} is nitrogen. Each treatment consisted of this same concentration of nitrogen. This amounted to 41.21 g ammonium chloride, 53.1 cm^3 ammonium hydroxide, and 26.50 g urea per liter of water. The mixture treatment consisted of an equal amount from each treatment. Experiments were conducted in aquaria with 30 L working volume of media and each nitrogen treatment was replicated five times. Aquaria were left open to the environment so that they represent open raceways, typically used for microalgae cultivation and remained susceptible to invading organisms. Salinity was kept between 29 and 33 PSU. Water was circulated using air stone aerators, adding atmospheric CO_2. Three times a week, absorbance readings were taken and samples were collected and preserved using Lugol's solution. Cultures were inoculated with 250 cm^3 of microalgae so that an initial cell density for all tanks was approximately $2.5 \cdot 10^{12}$ m^{-3}. The experiment ran for 28 days from September 27–October 25, 2012. Growth parameters were monitored throughout the duration of the experiment.

2.2. Water quality measurements and nitrogen measurements

Three times per week, during sample collection, we also monitored temperature, pH, and salinity in the aquaria with a Hydrolab (model MS 5, HachHydromet, Loveland CO). At the beginning, middle and end of the experiment, the values were also measured at night to account for day–night fluctuations. Temperature of the aquaria over the duration of the experiment ranged from 16 to 32 °C, salinity from 29 to 33 PSU and pH fluctuated between 7.9 and 9.3. These values are well within the tolerance levels of *N. salina* [8,10] and did not differ among treatments.

Using Colorimeter DR/890 (Hach Company, USA) we monitored concentration levels of detectable nitrate and ammonia in the water. Using NitraVer Reagent, we analyzed in 16 mm test-and-tube vials 1 cm^3 of sample from each tank to measure NO_3–N. Similarly, in test-and-tube vials using Ammonia Reagent we measured 0.1 cm^3 of sample to measure NH_3–N. We took readings until there was no more detectable available nitrogen on day six.

2.3. Cell density evaluation

Cell density was estimated in two ways: (1) Fresh samples were evaluated using optical density of cultures by using a spectrophotometer, at $\lambda = 740$ nm. (2) Samples preserved in

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