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Impact of inorganic contaminants on microalgae productivity and bioremediation potential

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ABSTRACT

As underdeveloped nations continue to industrialize and world population continues to increase, the need for energy, natural resources, and goods will lead to ever increasing inorganic contaminants, such as heavy metals, in various waste streams that can have damaging effects on plant life, wildlife, and human health. This work is focused on the evaluation of the potential of *Nannochloropsis salina* to be integrated with contaminated water sources for the concurrent production of a biofuel feedstock while providing an environmental service through bioremediation. Individual contaminants (As, Cd, Cr, Co, Cu, Pb, Ni, Hg, Se, and Zn) at various concentrations ranging from a low concentration (1X) to higher concentrations (10X, and 40X) found in contaminated systems (mine tailings, wastewater treatment plants, produced water) were introduced into growth media. Biological growth experimentation was performed in triplicate at the various contaminant concentrations and at 3 different light intensities. Results show that baseline concentrations of each contaminant slightly decreased biomass growth to between 89% and 99% of the control with the exception of Ni which dramatically reduced growth. Increased contaminant concentrations resulted in progressively lower growth rates for all contaminants tested. Lipid analysis shows most baseline contaminant concentrations slightly decrease or have minimal effects on lipid content at all light levels. Trace contaminant analysis on the biomass showed Cd, Co, Cu, Pb, and Zn were sorbed by the microalgae with minimal contaminants remaining in the growth media illustrating the effectiveness of microalgae to bioremediate these contaminants when levels are sufficiently low to not detrimentally impact productivity. The microalgae biomass was less efficient at sorption of As, Cr, Ni, and Se.

1. Introduction

Microalgae have been identified as a promising organism for a variety of applications including food, dietary supplements, medicine, fuel, and wastewater treatment. In efforts to curb carbon dioxide emissions, various microalgae strains are being investigated for biofuel and bioproducts production due to their high productivity and synergistic relationships with various waste streams resulting in a positive environmental impact. The oil yield potential for microalgae at 30% dry weight lipids is 130 times greater than soybean and nearly 10 times greater than palm oil, which has the next highest oil yield potential (Chisti, 2007). The positive attributes of microalgae and the negative impacts from global population increase has renewed interest in alternative environmentally friendly microalgae based systems.

Population increase and world industrialization will lead to ever increasing anthropogenic waste. These wastes will include heavy metals and other inorganic contaminants due to mining operations, both for energy production and consumer goods. High levels of inorganic contaminants can lead to cell destruction affecting animals, plant life, and humans. For example, high levels of lead can cause brain and kidney damage (Martin and Griswold, 2009). In addition, arsenic has been known to cause problems with the cardiovascular system, such as decreased red and white blood cells and an abnormal heart beat (Martin and Griswold, 2009). Some metals have the ability to bioaccumulate which endangers humans and animals high on the food chain (El-Moselhy et al., 2014). The need for inexpensive and efficient methods of inorganic contaminant removal will become increasingly imperative.

Microalgae have been studied extensively for use as a biosorbent for heavy metals and other contaminants. However, sorption studies using *Nannochloropsis salina*, which represents a promising biofuel feedstock, are limited. Hala and Sjahrul (2013) studied the biosorption of Zn^{2+} and Cd^{2+} ions on *N. salina* with removal efficiencies of 95.77% and 92.92%, respectively. However, they did not study the effect on microalgae growth or lipid content. Dong et al. (2014) performed a

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study on the effects of Cu and Zn. Results from this study showed 2.64 mg L^{-1} Zn and Cu at 15.06 mg L^{-1} would reduce growth by 50%. However, growth was performed under low light conditions compared to solar light, inoculations densities were low (0.05 g L^{-1}), lipid content was not reported, and no sorption analysis was performed. Napan et al. (2015a) introduced 14 inorganic contaminants (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn) into the media at concentrations that would be expected to be found in the growth media after 7 days of bubbling flue gas through the system. They found that *N. salina* growth decreased by approximately 60% compared to the control. Trace contaminant analysis showed that Cd, Co, and Mn, were sorbed at efficiencies greater than 90%. As, Cr, Cu, Ni, and Pb were sorbed at efficiencies from 50% to 90%, and Sb and V were sorbed at less than 50%. However, this study does not assess the impact of the individual contaminants. The negative impacts observed could be caused by an individual contaminant. Results from previous studies highlight the need to better understand the impact of heavy metals on algal growth and lipid productivity for realistic assessment of waste stream integration.

Based on the survey of literature there is a need to directly quantify the impact of individual contaminants on a microalgae feedstock that represents a promising candidate as a feedstock for biofuel and bioproducts. This work is focused on the evaluation of the potential of *N. salina* to be integrated with contaminated water sources for the concurrent production of a biofuel feedstock while providing an environmental service through bioremediation. Experimental systems are developed and operated at multiple contaminant concentrations and light levels. Multiple contamination levels are evaluated based on existing contamination levels from industrial processes. Various light levels are explored to support the extrapolation of the data to large-scale systems and evaluate the performance of the system in outdoor cultures. Results show the impact of ten individual inorganic contaminants as a function of light intensity and contaminant concentration level. Discussion focuses on the bioremediation potential of *N. salina*, the end fate of the individual inorganic contaminants, physiological impacts of contaminants, and the potential for integration with various waste streams while producing a quality biofuel feedstock.

2. Materials and methods

2.1. Growth system

2.1.1. Inoculum setup

The salt water species *Nannochloropsis salina* (UTEX 1776) was obtained from The Culture Collection of Algae at the University of Texas at Austin. The microalgae were initially cultivated on solid nutrient rich medium and 3% (w/v) agar in sterile petri dishes under 24 h of low light. The microalgae colonies were then stepped up to baffled Erlenmeyer flasks containing 200 mL of nutrient rich medium and continuously illuminated on a shaker table. Finally, the microalgae were stepped up again to 1.1 L sterile glass photobioreactors (PBR) containing nutrient rich medium. The inoculum in the PBRs were temperature controlled to 23 °C ± 1 °C by water submersion and subject to 200 μmol m^{-2} s^{-1} of photosynthetic active radiation (PAR) on a 16/8 h on/off duty cycle. This media in the inoculum was replaced with fresh media every 1–2 weeks.

The growth medium is a modified F/2 and consists of NaCl (299.5 mM), $CaCl_2{\cdot}2H_2O$ (1.0 mM), KCl (6.4 mM), $Na_2SiO_3{\cdot}9H_2O$ (0.2 mM), $MgSO_4{\cdot}7H_2O$ (6.0 mM), KNO_3 (10.1 mM), KH_2PO_4 (0.5 mM), Ammonium Ferric Citrate (2.0*10–2 mM), H_3BO_3 ($1.5*10^{-2}$ mM), $Na_2MoO_4{\cdot}2H_2O$ ($5.0*10^{-5}$ mM), $MnCl_2{\cdot}4H_2O$ ($1.5*10^{-3}$ mM), $ZnSO_4{\cdot}7H_2O$ ($2.1*10^{-4}$ mM), $CuSO_4{\cdot}5H_2O$ ($8.0*10^{-5}$ mM). Prior to inoculation the medium was autoclaved at 120 °C for 30 min, and sterile biotin (0.1 mM), vitamin B12 (0.135 mg mL^{-1}), and thiamine (6.5 mM) were added after the medium had cooled to room temperature.

2.1.2. Experimental growth system

The experimental growth system consisted of a temperature controlled enclosure, light racks, shaker table, and gas supply (see Supplementary Material). The New Brunswick Scientific Innova 2300 shaker table was operated at 120 rpm with 32 individual small PBRs equally spaced on the system. Illumination was provided by T5 fluorescent bulbs. Temperature was actively controlled to 27 °C with air circulation. The pH of the system was controlled through active feedback with a pH sensor and controlled at 7.3 ± 1.0. Air and carbon dioxide introduced into the system was humidified and filtered. Prior to inoculation, the PBRs were washed with soap and rinsed with deionized water and then placed in 10% nitric acid (HNO_3) over night to remove all contaminants adsorbed to the glass surface. The PBRs were then rinsed with deionized water and autoclaved at 120 °C for 20 min.

The growth systems were inoculated at approximately 1 g L^{-1} (ash free dry weight). Experimentation took place under 3 light levels, which correspond to PAR of approximately 200 μmol m^{-2} s^{-1}, 450 μmol m^{-2} s^{-1}, and 800 μmol m^{-2} s^{-1}. These light levels are referred to as low, medium, and high light intensities, respectively. Under each light intensity, microalgae were grown in the presence of inorganic contaminant concentrations of 1X (baseline), 10X, and 40X, more details below. Each growth trial lasted approximately 4.2 days.

Biomass density was measured via optical density (OD) at 750 nm using a GENESYS 5 spectrophotometer. OD was correlated to ash free dry weight. The OD of each system was measured everyday including at the start and end of each growth trial. The correlation coefficient was determined through dry mass with $R^2 = 0.987$.

2.2. Inorganic contaminants

The following inorganic contaminants were chosen to be studied: As (III), Cd (II), Cr (VI), Co (II), Cu (II), Pb (II), Ni (II), Hg (II), Se (IV), and Zn (II) as they are common contaminants in various wastewater systems. The baseline concentration (referred to as 1X hereafter) of each contaminant represents the concentrations that are commonly found in various wastewaters (Acheampong et al., 2010; Avila-Pérez et al., 1999a; Bujdoš et al., 2005; Gallup and Strong, 2006; Lee et al., 2002; Mansour and Sidky, 2002; Mansouri and Ebrahimpour, 2011; Matlock et al., 2002). Growth trials were conducted at 10X and 40X the baseline concentration in order to evaluate other highly contaminated wastewaters. For example, the Cu concentration reported at the Jose Antonio Alzate Reservoir in Mexico (Avila-Pérez et al., 1999b) is similar to the 1X concentration in this study. However, a goldmine in Ghana (Acheampong et al., 2010) reported Cu concentrations similar to the 40X concentration in this study. Table 1 shows the concentrations of each contaminant in terms of mg L^{-1} and the source of the inorganic contaminant. All of the contaminants were of analytical grade and fully solubilized prior to introduction into the experimental system.

Inorganic contaminants were added to the cultures at inoculation to simulate the microalgae growth with the media being derived from contaminated waters.

Table 1
Inorganic contaminant concentrations at 1X, 10X, and 40X concentrations and the contaminant salt source.

Contaminant	Source	1X, mg L^{-1}	10X, mg L^{-1}	40X, mg L^{-1}
As (III)	$NaAsO_2$	0.078	0.78	3.12
Cd (II)	$CdCl_2$	0.015	0.15	0.6
Cr (VI)	$Na_2Cr_2O_7{\cdot}2H_2O$	0.13	1.3	5.2
Co (II)	$CoCl_2{\cdot}6H_2O$	0.016	0.16	0.64
Cu (II)	$CuCl_2{\cdot}2H_2O$	0.13	1.3	5.2
Pb (II)	$PbCl_2$	0.054	0.54	2.16
Ni (II)	$NiCl_2{\cdot}6H_2O$	0.25	2.5	10
Hg (II)	$HgCl_2$	0.01	0.1	0.4
Se (IV)	Na_2SeO_3	0.01	0.1	0.4
Zn (II)	$ZnCl_2$	0.44	4.4	17.6