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Cultivation of marine microalgae using shale gas flowback water and anaerobic digestion effluent as the cultivation medium

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HIGHLIGHTS

- Flowback water and AD effluent were examined as potential water and nutrient sources.
- *N. salina* and *D. tertiolecta* grew well in the flowback water and AD effluent.
- The highest average biomass productivity was obtained with 6% AD effluent.
- Algae growth in effluent and flowback water was comparable to commercial nutrients.
- Fatty acid profiles using flowback water and effluent were comparable to commercial equivalents.

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ABSTRACT

The potential of shale gas flowback water and anaerobic digestion (AD) effluent to reduce the water and nutrient requirements for marine microalgae cultivation was evaluated with the following strains: *Nannochloropsis salina*, *Dunaliella tertiolecta*, and *Dunaliella salina*. *N. salina* and *D. tertiolecta* achieved the highest biomass productivity in the medium composed of flowback water and AD effluent (6% v/v). Growth in the above unsterilized medium was found to be comparable to that in sterilized commercial media with similar initial inorganic nitrogen concentrations, salinity, and pH levels. Specific growth rates of 0.293 and 0.349 day^{-1} and average biomass productivities of 225 and 275 mg L^{-1} day^{-1} were obtained for *N. salina* and *D. tertiolecta*, respectively. The lipid content and fatty acid profile of both strains in the medium were also comparable to those obtained with commercial nutrients and salts.

1. Introduction

The continued growth in the demand for energy has made microalgae an increasingly attractive option for use as a biofuel feedstock. Studied extensively by the U.S. Department of Energy, microalgae have the capability of growing on non-arable land, producing high-value byproducts, and yielding more lipids per acre than terrestrial crops (Sheehan et al., 1998). Meeting the water and nutrient requirements for algal cultivation, however, remains a significant barrier to the economic viability of algal biofuels. More abundant water resources for inland sites are required to address water conservation concerns as the location of microalgae production facilities can impact the cost, availability, and transportation of water (Ferrell and Sarisky-Reed, 2010).

Flowback water from shale gas exploration is one of the promising water resources to meet the water demand of marine microalgae production. Shale formations extend throughout the continental U.S. and contain an estimated 3.29×10^{13} m^3 of technically recoverable shale gas (U.S. Energy Information Administration, 2013). To make shale gas production more economically viable, an aqueous fluid is injected into the formation at high pressures to create fissures and interconnected cracks in a process called hydraulic fracturing. Flowback water – the aqueous fluid that returns to the surface after hydraulic pressure is relieved – is the primary wastewater associated with shale gas production (Gregory et al., 2011). Produced at a volume of 7570–22,700 m^3 per well (Blauch et al., 2009), flowback water can contain high concentrations of total dissolved solids (up to 261,000 mg L^{-1}), primarily soluble chloride salts (Gregory et al., 2011). Because of high handling costs and the scarcity of brine disposal facilities (Blauch et al., 2009), flowback water is an increasingly available wastewater

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resource that may meet the water demand of commercial marine microalgae cultivation.

Additionally, researchers have proposed a way to reduce costs associated with microalgae cultivation by using anaerobic digestion (AD) effluent as a nutrient supplement. Used to treat a variety of organic wastes, AD converts organic matter into biogas and produces a nutrient-rich effluent. Because of its high levels of total phosphorus and total nitrogen, AD effluent has been considered a potential agricultural fertilizer. Over-applying AD effluent to agricultural fields, however, can result in environmental impacts such as watershed eutrophication. These concerns have made AD effluent an attractive nutrient source for algal cultivation (Cai et al., 2013a; Sheets et al., 2014; Wang et al., 2010). However, no report was found on the use of AD effluent in conjunction with flowback water for the cultivation of marine microalgae.

Various marine microalgal strains have been previously studied for their ability to adapt to different salinities, produce large quantities of intracellular lipids, and produce high-value pigments. For example, *Nannochloropsis salina* is a marine microalga with a high lipid production rate (4.0 g m^{-2} day^{-1}) (Boussiba et al., 1987) and a high content of the nutritionally-valuable eicosapentaenoic acid (Krienitz and Wirth, 2006). The *Dunaliella* genus of microalgae has also been extensively studied and is unique in its robust tolerance to varying salinity levels. For example, *Dunaliella tertiolecta* is known to tolerate high salinity levels (up to ~1.0 M NaCl) as well as accumulate high levels of intracellular lipids (~67%) (Takagi et al., 2006). Similarly, *Dunaliella salina* is known to tolerate hypersaline conditions (up to ~5.5 M NaCl) as well as accumulate large amounts of the commercially-valuable pigment, β-carotene (Chen et al., 2011a). While some microalgal strains, such as *N. salina*, have been successfully cultured in media composed of AD effluent (Cai et al., 2013a,b; Sheets et al., 2014), currently, no studies have been reported on the culturing of *N. salina*, *D. tertiolecta*, or *D. salina* in media composed of flowback water.

The purpose of this study was to evaluate biomass and lipid production from marine microalgae cultivated in the flowback water from shale gas exploration supplemented with AD effluent from a municipal wastewater treatment facility. To address this goal, three specific objectives were studied: (1) the effect of AD effluent loading on microalgal growth in flowback water, (2) the effect of nutrient and salinity sources on microalgal growth, and (3) the effect of nutrient and salinity sources on lipid production. A batch study was performed in which three marine microalgal strains were cultivated in media composed of flowback water and AD effluent at different loadings. The optimal effluent loading and the most robust strains were further evaluated against a modified commercial medium at similar inorganic nitrogen, salinity, and pH levels. The performance of the microalgal strains was evaluated by comparing the growth rate, lipid content, and fatty acid production between the two media.

2. Methods

2.1. Microalgal strains and seed cultures

Marine microalgae *N. salina* (849/6), *D. tertiolecta* (19/27), and *D. salina* (19/18) were obtained from the Culture Collection of Algae and Protozoa (Oban, Scotland). Seed cultures of these strains were maintained in 2-L reactors (1-L working volume) at 25 °C under constant illumination (200 μmol m^{-2} s^{-1}) using 32-watt fluorescent lamps (GE Lighting, Ravenna, OH, USA). The photosynthetic photon flux of the light was measured by a BQM quantum meter (Apogee Instruments, Logan, UT, USA). Reactors were placed in a white coated chamber and each reactor was equipped with a rubber stopper and two 4.76-mm diameter stainless steel tubes for the air inlet and outlet. Ambient air (0.039% CO_2) blown through a 0.2-μm Whatman PTFE Puradisc filter (GE Healthcare, Maidstone, UK) at an airflow rate of 650 mL min^{-1} was used to provide mixing and CO_2 for algal growth.

N. salina cultures were cultivated in a commercial medium originally formulated by Guillard and Ryther (1962), using Proline *f*/2 Algae Feed (Pentair Aquatic Eco-Systems, Apopka, FL, USA), which contained the following ingredients: 0.075 g L^{-1} $NaNO_3$, 0.00565 g L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 1 mL L^{-1} trace elements stock solution, and 1 mL L^{-1} vitamin mix stock solution. The minor ingredients in the trace element stock solution included Na_2EDTA, $FeCl_3{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, $Na_2MoO_4{\cdot}2H_2O$, and biotin. The vitamin stock solution contained cyanocobalamin and thiamine HCl. The salinity of the culture medium was maintained with 40 g L^{-1} of Instant Ocean® sea salt (Spectrum Brands, Madison, WI, USA).

D. tertiolecta was cultured in a modified artificial seawater (ASW) medium, originally formulated by McLachlan (1964), that contained the following ingredients: 3.75 mL L^{-1} extra salts stock solution, 2.50 mL L^{-1} vitamin stock solution, 25 mL L^{-1} soil extract, and 0.50 g L^{-1} tricine. The extra salts stock solution contained 30.0 g L^{-1} $NaNO_3$, 1.20 g Na_2HPO_4, and 1.0 g L^{-1} K_2HPO_4. The vitamin stock solution contained biotin, calcium pantothenate, cyanocobalamin, folic acid, inositol, nicotinic acid, thiamine HCl, and thymine. The medium was further enriched with Proline *f*/2 Algae Feed using the concentration mentioned previously for *N. salina*. Instant Ocean® sea salt was added to the ASW medium as described previously. Soil extract was prepared by sieving 500 mL of air-dried deciduous woodland soil (Akron, OH, USA) through a 2–4 mm mesh. Deionized (DI) water at a volume of 1000 mL was then added, and the resulting solution was autoclaved at 121 °C at 103 kPa for 2 h. The supernatant was then filtered using Whatman No. 1 filter paper (GE Healthcare, Maidstone, UK) to remove remaining particulates and then refrigerated at 4 °C until use. *D. salina* was cultured in an ASW medium enriched with Proline *f*/2 Algae Feed, as described previously, with the addition of 40 g L^{-1} of Instant Ocean® sea salt and 35 g L^{-1} NaCl.

2.2. Shale gas flowback water and anaerobic digestion effluent

Shale gas flowback water was provided by Weatherford International LTD (Houston, TX, USA), a commercial oil and natural gas company, and stored at 4 °C. Prior to use, the flowback water was centrifuged for 15 min at 8000 rpm using a Sorvall RC 6+ tabletop centrifuge (Thermo Scientific, Waltham, MA, USA) to reduce the presence of suspended solids. Municipal wastewater AD effluent was collected from a commercial-scale liquid anaerobic digester (KB Compost Services, Akron, OH, USA), centrifuged at 3200 rpm by a D5LL continuous solid bowl decanter centrifuge (Andritz AG, Graz, Austria), and stored at 4 °C prior to use.

The chemical composition of the flowback water and the AD effluent are shown in Table 1. The concentrations of total dissolved solids (TDS), chloride, and bromide in the flowback water were 41,714; 23,787; and 192 mg L^{-1}, respectively, which were comparable to the concentrations reported by Hayes (2009) for flowback water samples obtained from the Marcellus shale formation. Additionally, the concentrations of sodium, magnesium, and calcium were 11,455; 3575; and 472 mg L^{-1}, respectively, which were within the range of randomly sampled Marcellus shale flowback water samples reported by Gaudlip and Paugh (2008). These comparisons indicated that the flowback water used in this study was a realistic representation of the flowback water commonly produced by shale gas exploration.