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Characterization of three *Chlorella sorokiniana* strains in anaerobic digested effluent from cattle manure ☆

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HIGHLIGHTS

- Chlorella strains were compared in cattle anaerobic digested effluent and Bold's Basel Media.
- Algae biomass production is dependent on nutrient provision and removal from growth media.
- Starch and protein production was greater than lipid accumulation in anaerobic digested effluent.
- Chlorella grown in anaerobic digested effluent may be more suitable for animal feed application.

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ABSTRACT

Chlorella sorokiniana CS-01, UTEX 1230 and UTEX 2714 were maintained in 10% anaerobic digester effluent (ADE) from cattle manure digestion and compared with algal cultivation in Bold's Basal Medium (BBM). Biomass of CS-01 and UTEX 1230 in ADE produced similar or greater than 280 mg/L after 21 days in BBM, however, UTEX 2714 growth in ADE was suppressed by more than 50% demonstrating a significant species bias to synthetic compared to organic waste-based media. The highest accumulation of protein and starch was exhibited in UTEX 1230 in ADE yielding 34% and 23% ash free dry weight (AFDW), respectively, though fatty acid methyl ester total lipid measured less than 12% AFDW. Results suggest that biomass from UTEX 1230 in ADE may serve as a candidate alga and growth system combination sustainable for animal feed production considering high yields of protein, starch and low lipid accumulation.

1. Introduction

Anaerobic digestion (AD) allows generation of bioenergy from organic wastes such as manure (Bohutskyi and Bouwer, 2013). The biogas product from AD contains methane which can be used as a bioenergy source as well as carbon dioxide which can serve as a carbon source for algae growth. AD also produces a digestate containing most the original nitrogen, phosphorus and micronutrients from the input material such as manure that can serve as an inexpensive, nutrient-rich, organic fertilizer (Field et al., 1984) and may serve as a nutrient source for algal cultivation (Singh et al., 2011). A major challenge in sustainably producing biofuels and feed from algae cultivation is the need to provide nitrogen and phosphate nutrients. To address this challenge the value of anaerobic digested effluent (ADE) as a low cost nutrient suppliement has been evaluated in a number of studies (Chinnasamy et al., 2010; Kebede-Westhead et al., 2006; Wilkie, 2002). Microalgae compare favorably to terrestrial plants as a renewable biomass feedstock for multiple reasons: (1) efficient productivity per unit area per unit time yielding up to 300 times more oil per acre per year than conventional crops such as rapeseed, palms, soybeans, or jatropha (Greenwell et al., 2009); (2) rapid growth cycle of 10–14 days permitting several harvests in a short time period and throughout the year; (3) ability to convert significant fraction of biomass to oil for biodiesel or biofuel (Dismukes et al., 2008); and (4) ability to readily utilize CO_2 generated from a point source such as flue gas; and (5) capable of utilizing wastewater by means of consuming the excess nutrients in natural environments such as ponds, rivers and oceans (Singh et al., 2011).

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Several algae species have the ability to produce energy dense biomass enabling liquid biofuels production. For example, Chlorella is a unicellular green microalga that produces substantial biomass rich in starch and lipids under minimum conditions requiring addition of only nitrogen, phosphorus and other minor nutrients in the presence of light and carbon dioxide. Nutrient uptake from different manure-based ADE products and industrial effluents have been evaluated for production of biomass, lipids, carbohydrates and proteins in Chlorella species. Effluent sources have (1) poultry litter (Singh et al., 2011); (2) citric acid effluent produced by fermentation (Li et al., 2013); (3) carpet mill industrial effluent (Chinnasamy et al., 2010) and (4) animal manure from different sources (Hu et al., 2012; Huo et al., 2012; Kebede-Westhead et al., 2004; Mulbry et al., 2008; Zhou et al., 2012).

The sources of nitrogen and carbon as well as availability affect algae growth and the amount of protein, carbohydrate and lipid composition of the algae biomass (Hu et al., 2008). Similar studies have identified gene expression patterns in algae that correspond to oil and carbohydrate accumulation during nitrogen deprivation in *Chamydomonas reinhardtii* and *Coccomyxa* sp. (Msanne et al., 2012). Lipid profiling has allowed assessment of algal oils from photoautotrophic and heterotrophic growth of *Chlorella zofingiensis* for biodiesel production (Liu et al., 2011). Recently four microalgae strains, including Chlorella species, demonstrated higher biomass production when cultured in poultry waste ADE. The harvested algae yielded high levels of carbohydrate (22%) and protein (39% w/w) (Singh et al., 2011). Additionally, another study showed higher biomass production and fatty acid contents (7.5–11% of dry weight) of *Chlorella* sp. grown in reduced total nitrogen and phosphorous effluent; however, fermented swine manure with different chemical additives served as the substrate (Hu et al., 2012).

The aim of this study was to investigate nutritional utilization and lipid production by algae grown in cattle manure ADE. *Chlorella sorokiniana* CS-01, *C. sorokiniana* UTEX 1230 and *C. sorokiniana* UTEX 2714 were selected based on their high biomass and lipid productivity. The objective was to monitor and compare production of algal biomass, protein, carbohydrate and lipid in two different media types: chemically formulated Bold's Basal Media (BBM) and cattle ADE in large-scale 80 L hanging-bag (HB) cultures. Nitrogen and phosphorus composition was evaluated during the course of algae growth. The behavior of algae in growth medium differing in nutrient composition will aid in identifying the resources imperative for healthy algae cultivation and the accumulation of lipid, carbohydrate and protein. The results obtained will be used to develop a more comprehensive understanding of nutritional utilization by algae regarding growth and production of lipid, protein and carbohydrate using cattle ADE to promote both sustainable animal and biofuel production.

2. Methods

2.1. Anaerobic digester effluent (ADE)

ADE was obtained from the Animal Science Department at the University of Nebraska–Lincoln. Cattle waste was collected from crossbred steers consuming a diet consisting of 47.5% dry rolled corn, 40% wet distillers grains plus soluble, 7.5% alfalfa hay, and 5% supplement (minerals, vitamins, and feed additives with fine ground corn as a carrier). Total manure (feces and urine mixture) was collected for a 5 day period from six steers fed this diet. The collected composite manure slurry was used to feed an anaerobic digester from which ADE was collected daily. ADE was pretreated prior to the introduction of alga by centrifugation at 3,500*g* for 5 min thereby removing sediment. 10% ADE was added with distilled water to total 80 L HBs to accommodate Chlorella cultivation. ADE was stored at 4 °C.

2.2. Algae strains and growth conditions

Samples of *C. sorokiniana* UTEX 1230 and *C. sorokiniana* UTEX 2714 were obtained from the Culture Collection of Algae at the University of Texas at Austin. *C. sorokiniana* CS-01 was provided by Minxi Wan at Johns Hopkins University. All algae strains were transferred to Bold's Basal Media (Bold, 1949) (BBM) sterile agar plates containing 100 μg/mL tetracycline and 10 μg/mL ampicillin and grown at 25 °C under continuous illumination at 160 μmol m^{-2} s^{-1}. Liquid culture was initiated by inoculation of a single isolated colony into 5 mL of sterile BBM. The 5 mL cultures were shaken at 250 rpm for 7 days under continuous illumination (160 μmol m^{-2} s^{-1}) at 25 °C.

2.3. Hanging bag (HB) cultivation

Cultures used for HB inoculation included 3 L aerated photobioreactors set up according to Kobayashi et al. (2013). The 3 L cultures were divided equally in two 80 L polyethylene HBs (each 4×10^6 cell/mL), one containing BBM and another composed of 10% ADE. Air stones with tubing were inserted in adjacent columns providing vigorous and ascending aeration supplied from an external compressed air source (~30 L/min). Cell growth was measured by hemacytometer (Cole–Parmer, Neubauer improved bright-line 0.1 mm depth). Algae growth was monitored by collecting 1 L samples per species during three stages of the algal growth cycle in both BBM and ADE treatment: early and late logarithmic phases and stationary phase. Samples were harvested by centrifugation at 5,000*g* for 5 min. The supernatant was stored at 4 °C and used for nutrient analysis. The pellet was lyophilized overnight and used for the biomass analysis and the analysis of biochemical compositions. The biomass of dry weight and ash were determined gravimetrically and were subtracted as ash free dry weight (AFDW).

2.4. Nutrient analysis

Measurement of nutrient uptake in the media was performed by AQ1 Discrete Multi-Chemistry Analyzer (SEAL Analytical WI, USA). The AQ1 is a computer controlled multi-chemistry discrete wet chemistry analyzer. Algal nutrition consumption was measured corresponding to the uptake of ammonia, ortho-phosphate, nitrate+nitrite, total phosphorus and total nitrogen concentrations in BBM and 10% ADE media. Ammonia and total nitrogen were detected at 660 nm as the indophenol blue converted ammonia with two phenols under alkaline conditions. The AQ1 method number was EPA-129-B Rev. 0 with the detection limit of 0.05 mg N/L (range: 0.2 to 10 mg N/L). Ortho-phosphate and total phosphorus were detected at 660 nm as phosphomolybdenum blue converted ortho-phosphate to molybdate under acid conditions. The AQ1 method number was EPA-146-B Rev. 0 with the detection limit 0.007 mg P/L (range: 0.125 to 12.5 mg P/L). Nitrate+nitrite was detected at 520 nm as the colored azo compound reduced nitrate to nitrite via cadmium coil followed by a sulfanilamide reaction under catalyst conditions. The AQ1 method number was EPA-114-B Rev. 0 with the detection limit of 0.04 mg N/L (range: 0.25 to 15 mg N/L).

The supernatant sampled from BBM and 10% ADE media was diluted 25× and 50×, respectively for ammonium, ortho-phosphate and nitrate+nitrite measurements with deionized water into 2 mL sampling cups to improve measurement accuracy. All samples were centrifuged in 1.5 mL microtubes for 10 s. For the total phosphorus measurement the supernatant was digested with acid-persulfate following the USEPA method 365.1 and for total nitrogen copper(II) was used as a digestion catalyst following the USEPA methods 351.2 Rev. 2.0 in the AQ1 procedure. The ammonia, ortho-phosphate, nitrate+nitrite, digested total phosphorus