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Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production

Yecong Li [a], Yi-Feng Chen [a], Paul Chen [a], Min Min [a], Wenguang Zhou [a], Blanca Martinez [b], Jun Zhu [b], Roger Ruan [a,*]

[a] Center for Biorefining, Department of Bioproducts and Biosystems Engineering, University of Minnesota, 1390 Eckles Avenue, St. Paul, MN 55108, USA
[b] Department of Bioproducts and Biosystems Engineering, University of Minnesota, 1390 Eckles Avenue, St. Paul, MN 55108, USA

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ABSTRACT

The feasibility of growing *Chlorella sp.* in the centrate, a highly concentrated municipal wastewater stream generated from activated sludge thickening process, for simultaneous wastewater treatment and energy production was tested. The characteristics of algal growth, biodiesel production, wastewater nutrient removal and the viability of scale-up and the stability of continuous operation were examined. Two culture media, namely autoclaved centrate (AC) and raw centrate (RC) were used for comparison. The results showed that by the end of a 14-day batch culture, algae could remove ammonia, total nitrogen, total phosphorus, and chemical oxygen demand (COD) by 93.9%, 89.1%, 80.9%, and 90.8%, respectively from raw centrate, and the fatty acid methyl ester (FAME) content was 11.04% of dry biomass providing a biodiesel yield of 0.12 g-biodiesel/L-algae culture solution. The system could be successfully scaled up, and continuously operated at 50% daily harvesting rate, providing a net biomass productivity of 0.92 g-algae/(L day).

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1. Introduction

Petroleum based fuels are considered unsustainable because of the declining supply of fossil oils and their association with greenhouse gas emission. Therefore, discovering and constructing renewable, carbon neutral transportation fuel systems are possibly two of the most vital issues for current society (Chisti, 2007). Biodiesel, a promising substitute for petroleum fuels, has the potential to address sustainability and energy security issues because it is derived from plant oils or animal fats, and has much lower greenhouse gas emission. Currently, soybean oil is the major feedstock for commercial biodiesel production; other oil feedstock including canola, corn, jatropha, waste cooking oil, and animal fats (Chisti, 2007) are also being tested. However, these processes have caused concerns of competing with food source, having little effect on green house gas emission (Fargione et al., 2008), and not capable of satisfying the existing demand for petroleum fuels (Chisti, 2007). Prior research suggested that microalgae, which has an areal productivity 20–40 times that of oil crops (Sheehan et al., 1998) and oil content up to 80% by weight of dry biomass (Spolaore et al., 2006; Chisti, 2007) has the potential to replace the current source for renewable biodiesel production (Sheehan et al., 1998; Banerjee et al., 2002; Chisti, 2007). However for this transaction to happen, algae biomass must be produced practically and economically. One of the methods to reduce costs of algae mass cultivation is to integrate wastewater treatment with algae biomass production, which was first suggested in 1960s (Oswald and Golueke, 1960).

The research of growing algae in municipal wastewaters has been under investigation for more than half a century (Oswald et al., 1978; Tam and Wong, 1989, 1990; Lau et al., 1995; Woertz et al., 2009). Some researchers also investigated the feasibility of algal–bacterial symbiotic processes for the treatment of industrial and livestock wastewaters (Muñoz and Guieysse, 2006). Municipal wastewater collected from various stages during treatment process has been tested for their ability of supporting algae growth in previous studies. These different types of wastewater include primary clarifier effluent (Tam and Wong, 1989, 1990; Woertz et al., 2009; Lau et al., 1995), activated sewage filtrates (Tam and Wong, 1989, 1990), and effluent from secondary treatment tank (Oswald et al., 1978). The centrate, which is the liquid from activated sludge thickening process, has the characteristics of rich nutrients including phosphorus, ammonium, and COD. With a daily process rate of one million gallons in the wastewater treatment plant located in Saint Paul, Minnesota and higher nutrients levels than liquid from other stages, centrate provided a potential source for algae cultivation. Up to now, rare studies have been conducted to test the suitability of growing algae in raw centrate, thus our current research effort in this area is necessary and important.

* Corresponding author. Tel.: +1 612 625 1710; fax: +1 612 624 3005.
E-mail address: ruanx001@umn.edu (R. Ruan).

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Lipid contents in algae cultivated in artificial media solution have been reported to exceed 80% with different lipid classes (Spolaore et al., 2006; Chisti, 2007). However, few reports have explored the lipid contents of algae grown in municipal wastewaters (Woertz et al., 2009).

The objectives of this project are herein to (1) determine the growth rate, biomass yield, nutrients removal efficiency, and biodiesel productivity for algae grown in centrate, and (2) test the scalability and growth stability of algae cultivated in centrate.

2. Methods

2.1. Experiment layout

The experiments in this project were carried out in two consecutive stages. The first stage was aimed at providing baseline information on performance of microalgae alone in wastewater, and thus conducted using autoclaved centrate to eliminate bacterial contamination and excess heterotrophic growth. In this stage, TAP media was used as positive control to justify the performance of autoclaved centrate as media for algae cultivation. The second stage was targeted on testing the feasibility of growing algae as a practical wastewater nutrients removal and biomass accumulation process, and thus conducted in raw centrate after removing solid particles. The cultivation time for each condition was 14 days with three replications. The effect of both autoclaved and raw centrate was studied in terms of algal growth, wastewater nutrients removal, and biodiesel productivity. Scale-up experiment and the stability of continuous operation was tested with raw centrate. The scale-up experiments were first carried out in batch mode with 7-day cultivation time and then in continuous mode with 50% of algae culture solution harvested and same amount of raw centrate replenished daily for a period of 7 days.

2.2. Pretreatments and characteristics of wastewaters

The centrate was collected from the Metropolitan Wastewater Treatment Plant located in Saint Paul, Minnesota, and large solid particles were removed by sedimentation and filtration with filter cloth (Wypall X70, Kimberly-Clark Professional). After filtration the centrate was divided into two equal portions. One portion was autoclaved at 121 °C, after which, the liquid was stored at 4 °C for 5 days to settle out any visible solid particles and the supernatant was used for algae cultivation. The other untreated portion was stored at 4 °C until use in experiments. Thus, there were two types of centrate used in this project, namely autoclaved centrate, and raw centrate. The characteristics of the two types of centrate are summarized in Table 1. It showed that similar nutrients characteristics were observed for both types of centrate.

2.3. Algae strain and culture condition

A microalgal mixture, named 72205, was obtained from local Lake Minnetonka, Minnesota, USA. Five unialgal strains, named 72205a–72205e, were purified from the mixture on TAP medium solidified with 1.5% agar. One of the strains (72205e) grew well, initially on 50% TAP + 50% centrate, then on 100% (full) centrate. The strain was morphologically identified as a *Chlorella* species, renamed *Chlorella* sp. 10b, and used in the entire investigations of this project. The algae strain was conserved in Tris–Acetate–Phosphorus (TAP) media (Harris, 1989) with the following solid ingredients: 400 mg/L NH_4Cl, 100 mg/L $MgSO_4{\cdot}7H_2O$, 50 mg/L $CaCl_2{\cdot}2H_2O$, 108 mg/L K_2HPO_4, 56 mg/L KH_2PO_4, and 2420 mg/L Tris (hydroxymethyl) aminomethane,. Liquid chemicals include: 1 mL/L glacial acetic acid, 1 mL/L trace elements solution consisted of 50 g/L Na_2EDTA, 22 g/L $ZnSO_4{\cdot}7H_2O$, 0.05 g/L $CaCl_2{\cdot}2H_2O$, 11.4 g/L H_3BO_3, 5.06 g/L $MnCl_2{\cdot}4H_2O$, 4.99 g/L $FeSO_4{\cdot}7H_2O$, 1.61 g/L $CoCl_2{\cdot}6H_2O$, 1.57 g/L $CuSO_4{\cdot}5H_2O$, 1.10 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 16 g/L KOH. The growth rate, nutrients consumption, lipid content and biodiesel productivity of the algae was studied with 250 mL Erlenmeyer flasks containing 100 mL TAP or two different centrate media. The 250 ml Erlenmeyer flasks were kept on a shaker with 100 rpm rotation speed. The growth characteristics of the algae on raw centrate in a larger scale were further studied with a coil reactor, which was constructed with two pieces of 16 m long clear polyvinyl tubing (19-mm ID, 25-mm OD, and 9.0-L volume) coiled around a metal frame of 1.4 m in height and 0.4 m in diameter. The total volume of the algae culture solution was 25 L, 9 L of which was contained in the coil and the rest (16 L) was in a storage container. Light was provided by six 1.2-m-high Gro-Lux fluorescent tubes (40 W/tube) residing on the inside of the coil. The tubes were placed 3 cm away from the reactor tubing. Dayton carbonator pump (4FG41, Dayton) was employed to circulate the solution of algae at 1.8-L min^{-1}. In all cases, *Chlorella sp.* were inoculated at 1:10 (v:v), and grown under 25 ± 2 °C with illumination at light intensity of 50 μmol m^{-2} s^{-1}.

Table 1
Characteristics of centrate.

Characteristics	Raw centrate	Autoclaved centrate
Ammonia (mg/L)	82.5 ± 2.2	85.9 ± 1.1
Total nitrogen (mg N/L)	116.1 ± 3.8	132.3 ± 5.1
Total phosphorous (mg PO_4^{3-}-P/L)	212.0 ± 7.0	215.1 ± 6.4
COD (mg/L)	2304.0 ± 2.5	2389.5 ± 57.3
Total suspended solid (TSS)	0.070 ± 0.014	0.180 ± 0.000
Total volatile suspended solid (TVSS)	0.050 ± 0.014	0.120 ± 0.000
Al (mg/L)	0.075	0.082
B (mg/L)	0.286	0.357
Ca (mg/L)	161.7	132.67
Cu (mg/L)	0.01	0.017
Fe (mg/L)	3.074	1.925
K (mg/L)	145.50	197.63
Mg (mg/L)	73.30	96.209
Mn (mg/L)	2.797	4.010
Na (mg/L)	160.70	199.34
Ni (mg/L)	0.0273	0.075
Zn (mg/L)	0.020	0.115

Note: *P* is short for phosphorus, N is short for nitrogen, COD is short for chemical oxygen demand, ND is short for not detected.

2.4. Analytical procedures

2.4.1. Sampling and nutrients analysis

A volume of 1.5 mL algae suspension was collected daily from each flasks or coil reactor for nutrient consumption analysis starting from inoculation. The samples were first centrifuged at 5000 rpm for 15 min and then the supernatants were properly diluted and analyzed for chemical oxygen demand (COD), total phosphorus, ammonium (NH_4-N), and total nitrogen following the Hach DR 5000 Spectrophotometer Manual (Hach, 2008).

2.4.2. Algal growth determination

Algal growth was monitored daily by the total volatile suspended solids (TVSS), which represents biomass concentration and was determined according to the standard method (APHA et al., 1995) using 5 mL of algae suspension from the flasks or coil reactor. For algae grown in centrate, the algae growth was determined by the total biomass minus the biomass in control experiments, where only treated or untreated centrate was placed under light. Algae growth in batch culture is usually characterized by five reasonably defined phases: (1) lag, (2) exponential, (3) declined, (4) stationary, and (5) death. The growth rate (k) is usually determined from the exponential phase by following this equation: