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Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production

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HIGHLIGHTS

- Cultivation of *Nannochloropsis salina* with effluent of anaerobic digestion (AD).
- The highest biomass yield was obtained at 6% AD effluent loading.
- Lipid content and productivity decreased with increased effluent loading from 3% to 18%.
- Biomass productivity increased by up to 49% as harvest ratio increased from 25% to 50%.

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ABSTRACT

The biomass and lipid productivities and the nutrient removal capacity of microalgae *Nannochloropsis salina* grown using anaerobically digested municipal wastewater effluent as a nutrient source were evaluated in this study. Results from bench-scale batch reactors showed that *N. salina* grew well under 3%, 6%, 12%, and 18% (v/v) anaerobic digestion (AD) effluent loading with the highest growth rate being 0.645 d^{-1} obtained at 6% AD effluent loading. The growth of *N. salina* decreased when the effluent loading was increased to 24%. The highest biomass productivity of 92 mg l^{-1} d^{-1} was obtained with 6% effluent loading. Three harvesting frequencies (1, 2, and 3 d intervals) and two harvesting ratios (25% and 50%, v/v) were tested in semi-continuous bench-scale reactors with 6% effluent loading. The highest lipid productivity of 38.7 mg l^{-1} d^{-1} was achieved with a 2-d harvesting interval and 50% harvesting ratio, where nitrogen and phosphorus were removed at rates of 35.3 mg l^{-1} d^{-1} and 3.8 mg l^{-1} d^{-1}, respectively. The fatty acid (FA) profile showed that palmitic acid (C16:0), palmitoleic acid (C16:1), and eicosapentaenoic acid (C20:5) were the major components, accounting for 32.1%, 26%, and 15.7% of the total FAs, respectively.

1. Introduction

Concerns about the depletion of global crude oil reserves [1,2] and the environmental deterioration [3] resulting from the overconsumption of fossil fuels have prompted the development of renewable and environmentally friendly energy sources [4,5]. Microalgae-based biodiesel is one of the promising alternatives to fossil fuels [6]. Some species of microalgae can accumulate more than 50% of their dry weight in the form of lipids which can be extracted and converted into biodiesel through similar processes used for vegetable oil [7]. Compared to terrestrial oilseed crops, microalgae grow rapidly, providing more than 20 times the oil per area [8]. In addition to their high biomass and lipid productivities, microalgae also have potential environmental benefits, such as mitigation of CO_2 through photosynthesis [9] and bioremediation of wastewater by removing large amounts of nutrients and heavy metals [8,10]. Although microalgae-based biodiesel is currently not commercially feasible, strategies have been proposed to reduce the production costs. One of them is to grow microalgae using wastewater as nutrients, since the costs of commercial nutrients can be saved and the production costs can be further offset by wastewater treatment revenues.

Anaerobic digestion (AD) is a technology used to decompose organic matter under oxygen-free conditions and produce biogas [11,12]. The residual effluent from the digestion process contains high amounts of nutrients, such as ammonium and phosphate. Superfluous amounts of effluent may create concerns of pollution when applied to agricultural land [13], or economic issues if additional treatment is required prior to discharge [6]. However, the nutrient-rich effluent can be utilized as a growth medium for photosynthetic microalgae [14–17]. Therefore, existing processes for AD may be coupled with microalgae cultivation to mitigate costs associated with effluent treatment.

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The performance of a coupled microalgae cultivation and AD effluent treatment process depends on a variety of factors which can be grouped into three categories: characteristics of algal species, properties of the AD effluent, and operational parameters during algal growth. Among the wide variety of microalgae that can be utilized for nutrient removal, species that have potentially high growth rate and high lipid content are favored. In this study, *Nannochloropsis salina* was selected as an exemplary species because of its high biomass productivity (up to 24.5 g m^{-2} d^{-1} in outdoor ponds) [18] and high lipid content (up to 50% of its dry weight during the stationary phase) [19].

The level of inhibitors – mainly ammonia – in the AD effluent is usually too high to be tolerated by microalgae. As a result, dilution is generally needed before the effluent is fed to the microalgae. The AD effluent composition may vary as it is dependent on the incoming material that is being digested. Nutrients may be added to achieve a desired mixture of nutrients required for microalgal growth, although in general the composition of diluted AD effluent makes it a favorable medium for microalgae [6]. Wang et al. [14] studied the effectiveness of using digested dairy manure as a nutrient supplement for the cultivation of microalgae *Chlorella* sp. It was reported that biomass productivity, lipid content, and nutrient removal efficiency were all dependent on the dilution ratio.

Enhanced lipid accumulation in nitrogen-limited microalgae has been frequently reported. For example, the lipid content of *N. salina* can be increased up to 70% of its dry weight by nitrogen starvation [20]. However, high lipid content was often offset by a low growth rate, which led to low overall lipid productivity [21,22]. Therefore, to achieve maximum overall lipid production during continuous microalgae cultivation, it is important to determine an optimal feeding frequency and harvesting ratio which consider the growth rate and lipid content of the microalgae.

The purpose of this study was to test the biomass and lipid productivities and nutrient removal capacity of *N. salina* in the effluent from the AD of municipal wastewater. The AD effluent was collected from a commercial anaerobic digester after the removal of solids using a centrifuge. The performance of *N. salina* was evaluated by comparing its growth rate and nutrient removal efficiency at different effluent loadings. Batch experiments were conducted to determine the optimal effluent loading. The optimal harvesting ratio and frequency were determined via semi-continuous laboratory experiments in order to provide basic reference data for future pilot scale production.

Table 1
Chemical composition of undiluted AD effluent used as a nutrient source for the growth of *N. salina*.

Characteristic	Unit	Mean ± standard error
TS	%	0.287 ± 0.005
Volatile solids	%	0.208 ± 0.002
TC	mg l^{-1}	2014 ± 65
TN	mg l^{-1}	2667 ± 30
TAN	mg l^{-1}	2276 ± 45
TP	mg l^{-1}	381 ± 6
COD	mg l^{-1}	2661 ± 75
Na	ppm	89 ± 4
K	ppm	121.7 ± 3.5
Ca	ppm	32.95 ± 1.95
Mg	ppb	680 ± 26
Al	ppb	1166 ± 43
Fe	ppb	4141 ± 58
Mn	ppb	151.4 ± 19.5
Ni	ppb	25.69 ± 3.64
Co	ppb	<0.000
Cu	ppb	26.75 ± 2.39
Zn	ppb	105.7 ± 12.3
Mo	ppb	20.93 ± 0.56

2. Materials and methods

2.1. AD effluent and microalgae cultures

AD effluent was collected from a commercial-scale wet anaerobic digester (KB Compost Services, Akron, OH, USA) coupled with a D5LL solid bowl decanter centrifuge (ANDRITZ AG, Graz, Austria). The feedstock for this digester was the municipal wastewater from Akron, OH, USA. The centrifuge ran continuously at 3200 rpm. The effluent was kept at 4 °C before use. The chemical composition of the AD effluent is shown in Table 1. Total carbon (TC), total nitrogen (TN), and total phosphorus (TP) concentrations in the undiluted AD effluent were 2014 mg l^{-1}, 2667 mg l^{-1}, and 381 mg l^{-1}, respectively – higher concentrations than those in the original municipal wastewater. About 85% of the nitrogen was found as total ammonia nitrogen (TAN), a term referring to nitrogen in the form of both ammonia and ammonium. The nitrogen-to-phosphorus (N/P) ratio of the effluent was 7, which was lower than the atomic ratio of 16 for *N. salina*, indicating a nitrogen limitation for microalgal growth [23]. The relatively low total solids (0.287%) of the effluent indicated low turbidity [24], which was beneficial to the photosynthesis of *N. salina*. The effluent also contained other essential ions for microalgal growth, such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Al^{3+}.

The marine microalgae *N. salina* (CCAP 849/6) was obtained from the Culture Collection of Algae and Protozoa (CCAP, Oban, Scotland). Cultures were cultivated in *f*/2 medium, a general enriched seawater medium originally formulated by Guillard and Ryther [25], containing 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, while the vitamin stock solution contained cyanocobalamin (vitamin B_{12}) and thiamine HCl (vitamin B_1). These solutions were used in the media following recipes provided in the CCAP website [26]. Seed cultures were cultured under continuous light (200 μmol m^{-2} s^{-1}) at 25 ± 1 °C.

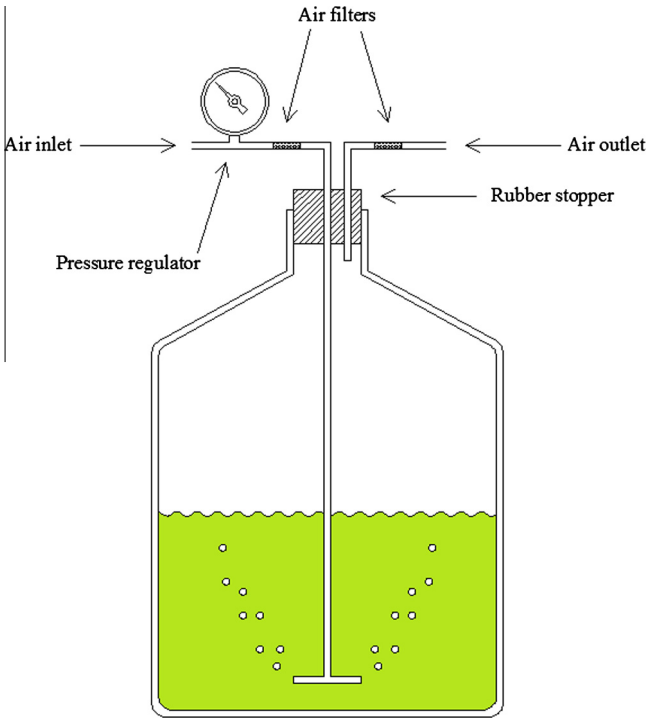

Fig. 1. Photobioreactor schematic for the cultivation of *N. salina*.