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Anaerobic digestates are useful nutrient sources for microalgae cultivation: functional coupling of energy and biomass production

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Abstract We investigated the extent to which nitrogenous and phosphorus nutrients from liquid anaerobic digestates could be recycled for photosynthetic growth of a microalga, *Scenedesmus* sp. AMDD. Digestates recovered from the anaerobic digestion of cow manure and swine manure and a co-digestion of swine manure and algal biomass were diluted in distilled water and used for algal growth with and without supplemental CO_2 addition. Nutrient assimilation and final biomass yield were retarded in all but the swine manure/algae co-digestate cultures supplemented with high CO_2. Swine manure digestate cultures supplemented with the typical complement of micronutrients normally added with a commonly used growth medium or with Fe/EDTA failed to grow any better than unamended controls. When the culture medium was prepared by blending swine manure digestate with 25 or 50 % algal biomass digestate, diluting it with lake water or by supplementing with magnesium, nutrient assimilation and final algal biomass yields were maximized, indicating that magnesium was critically limiting for algal growth in swine manure digestates. Magnesium amendment thus appears to be essential if nutrients from swine manure digestates are recycled for algal growth. No such requirement is necessary for recycling nutrients from digestates generated wholly or in part from algal biomass.

Keywords Nutrient recycling · Anaerobic digestates · Biomass · Photosynthesis

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Introduction

There has been a resurgence of interest in the alternative energy potential of microalgae. Most of the renewed research focus has involved the manipulation of physiological parameters to increase the intracellular lipid content of microalgae followed by the extraction of the oils and subsequent conversion to biodiesel. Economics is considered a key barrier to full-scale algal biodiesel production as a drop-in fuel, energy source, and commodity (Chisti 2007). The developing consensus is that there is a need to couple microalgal oil production with another revenue stream or with another form of energy production, such as anaerobic digestion (AD), to become a viable alternative energy production pathway (Chisti 2007; Sialve et al. 2009; Collet et al. 2011).

Anaerobic digestion of biomass is a relatively mature industrial process which utilizes bacterial populations in a controlled, anoxic environment to produce biogas, which can be further upgraded to methane for combustion to generate energy or compressed to a liquid fuel. Anaerobic digestion of biomass creates a nutrient-rich waste stream, called digestate, which can be spread as crop fertilizer but has the potential to be used as a nutrient source for microalgal growth. Recycling the nutrients from AD and assimilating them into algal biomass can result in further feedstock for the process without incurring the monetary or environmental costs of using nitrogenous or phosphorus fertilizers

while simultaneously remediating the liquid waste stream from the process. Studies assessing the integration of algal growth and AD were performed as far back as the 1950s (Golueke et al. 1957), and the topic has once again gained momentum in the bioenergy sector. Recently, Ras et al. (2011) performed experiments with *Chlorella vulgaris* in an integrated algal cultivation and digestion system, Vergara-Fernández et al. (2008) digested a variety of marine algae to assess their biogas generating potential, and Wang et al. (2010) observed efficient nitrogen and phosphorus remediation from anaerobic digestates of dairy manure using *Chlorella* sp.

We were interested in investigating the degree to which remineralized nutrients found in anaerobic digestates from different organic substrates, such as animal manures and microalgal biomass itself, could be reused for microalgae cultivation. In our view, linking the energy production pathway to the supply of nutrients could improve the overall sustainability of biofuel production from microalgae by lessening its dependence on commercial fertilizers.

Material and methods

Liquid anaerobic digestates were provided by the Centre for Agricultural Renewable Energy and Sustainability located at the University of Guelph, Ridgetown Campus (Ridgetown, Ontario, Canada). Various digestates were supplied from different agricultural feedstocks including vegetable wastes, cow manure, and swine manure and from a large-scale co-digestion of swine manure with dried *Nannochloropsis granulata*, a marine microalga. Liquid digestates from the AD of *Scenedesmus* sp. AMDD, a favorite model freshwater alga in our lab (McGinn et al. 2011, 2012), were provided by B. Tartakovsky (NRC Montreal). For one experiment, water from a nearby lake (Kearney Lake, Halifax, Nova Scotia, Canada) was sampled and filter-sterilized for use in algal growth.

Flask-level growth

Scenedesmus sp. AMDD was grown in an environmentally controlled plant growth chamber set at 22 °C in 250-mL Erlenmeyer flasks which contained approximately 100 mL of algal culture. Growth light was supplied from fluorescent bulbs (Philips 65 W F72T8 warm white) fixed at the top of the chamber to give an irradiance of 85–90 μmol photon $m^{-2}s^{-1}$ measured with a flat quantum sensor at the surface of the algal cultures. To promote more efficient gas exchange with the headspace and ensure that the algae remained suspended, the cultures were mixed with PTFE magnetic stir bars. Digestates from three different AD feedstocks were used to prepare algal growth media: a co-digestion of algal biomass and swine manure, cow manure, and vegetable wastes. The digestates were diluted in deionized water (DI H_2O) to obtain a final nitrogen (N) concentration of approximately 1.5×10^{-3} mol L^{-1} (measured as NH_3-N) and various phosphorus (PO_4^{3-}) concentrations depending on the digestate source, passed through a 0.45-μm nominal-pore-size glass fiber filter, and followed by filtration through a 0.22-μm polycarbonate membrane filter. The NH_3 concentration of 1.5 mM obtained after dilution was chosen based on previous work which showed it to be appropriate for growth of the same algal strain in municipal wastewater (McGinn et al. 2012). The strength of the required dilution varied between 1 and 6.5 % (balance DI H_2O) depending on the strength of the undiluted digestate. One hundred mL of diluted, filtered digestate was transferred into a sterile flask and inoculated (1 % (*v*/*v*)) from an exponentially growing culture of *Scenedesmus* sp. AMDD. The cell density of the cultures was determined daily using a particle enumerator (Multisizer III Counter, Beckman Coulter, USA). A spectrophotometric nitrate (NO_3^-) assay indicated that the digestates were NO_3^--depleted to levels below the assay detection limit. Ammonia and phosphate assays were performed using a benchtop spectrophotometer and commercially available assay kits (Hach, USA).

Bottle experiments under CO_2 supplementation

Culture experiments were performed in 1-L glass bottles which allowed for the provision of supplemental CO_2 through a pH-stat system. All other growth conditions were the same as for the flask-level experiments. Cultures of *Scenedesmus* sp. AMDD were inoculated (1 % (*v*/*v*)) into digestates from algal biomass and swine manure co-digestion, swine manure, cow manure, and digestion of algal biomass alone, diluted to approximately 1.5×10^{-3} mol L^{-1} NH_3-N in DI H_2O. Further digestate treatments for growth experiments included swine manure digestate blended with algal biomass digestate (25 and 50 %), swine manure digestate supplemented with $MgSO_4$ and with MgCl (3.04×10^{-4} mol L^{-1}), swine manure digestate with Fe/EDTA addition (1.17×10^{-5}), and swine manure digestate with trace metal and vitamin addition (Guillard 1975). The microalgal cultures were aerated through a 0.22-μm in-line PTFE filter, and pH was monitored and controlled using Omega brand pH controllers and automated carbon dioxide (CO_2) injections. In order to prevent carbon limitation of algal growth, CO_2 was periodically injected into the culture through the air line in response to a transient rise in pH triggered by algal demand for CO_2. Cell density and growth rates, nutrient levels, and final biomass were monitored and determined as described for the flask-level growth experiments. Upon reaching the stationary growth phase, a 5–15-mL

aliquot of algal culture was filtered onto a precombusted glass fiber filter (550 °C), oven-dried at 120 °C, weighed, and combusted in a Vario MICRO Cube elemental analyzer (Elementar, USA) to determine the C and N content of the biomass. All culture experiments were performed in triplicate.

Results

Deionized water dilutions

The maximum yields attained in our flask cultures ranged from 0.21 g dw L^{-1} in diluted vegetable waste digestate to a maximum of 0.27 g dw L^{-1} in diluted swine/algae digestate and cow digestate (Fig. 1a, $n=3 \pm$ SD). In all three sources of digestate, the growth rate of the culture was greatest between inoculation and day 1, slowing considerably between days 1 and 3 (Fig. 1a). In all treatments, growth ceased by the third day of culture before the onset of N and P limitations. By the end of the sixth day, relatively high levels of residual N and P remained in the culture media (Fig. 1b, c). These results suggested that the cultures had excess macronutrients for growth and that some other factor was limiting algal growth in these diluted digestate cultures.

One-liter culture experiments were performed at pH 7 with CO_2 supplementation in diluted swine/algae digestate. We observed an increase in final biomass yield to 0.42 g L^{-1} relative to the initial flask-level growth experiments (Table 1, treatment 1). As in our flask experiments, exponential growth had ceased by day 3 of the experiment; however, NH_3-N and PO_4^{3-} continued to be drawn down to near depletion. By the sixth day of cell growth, the levels of NH_3-N and PO_4^{3-} in the culture media had been reduced to 6.89×10^{-6}mol L^{-1} NH_3-N and 1.56×10^{-6}mol L^{-1} PO_4^{3-}. Additional 1-L culture experiments with diluted swine and cow manure digestates supplemented with CO_2 resulted in final biomass yields of 0.14 and 0.11 g dw L^{-1}, respectively (Table 1, treatments 2 and 3). In both experiments, neither NH_3-N nor PO_4^{3-} was depleted by algal growth.

To investigate the lower biomass yields that we observed in swine digestates compared to the co-digested algae/swine digestates, *Scenedesmus* sp. AMDD was grown in diluted swine manure digestate amended with algae digestate obtained from separate experiments where algal biomass was the only substrate for the anaerobic digestor. Swine manure and algae digestates were blended at ratios of 3:1 and 1:1 swine/algae. In parallel experiments, swine manure digestates were amended either with the full complement of trace metals and vitamins typically used in f/2 media (Guillard 1975), with Fe/EDTA, or with Mg^{2+}, added as either $MgSO_4$ or MgCl. *Scenedesmus* sp. AMDD grown in algal biomass digestates achieved a final biomass yield of 0.45 g dw L^{-1} with complete N drawdown (Table 1, treatment 4). The algal cultures grown in mixed digestates (3:1 and 1:1 swine/algae digestate) obtained final

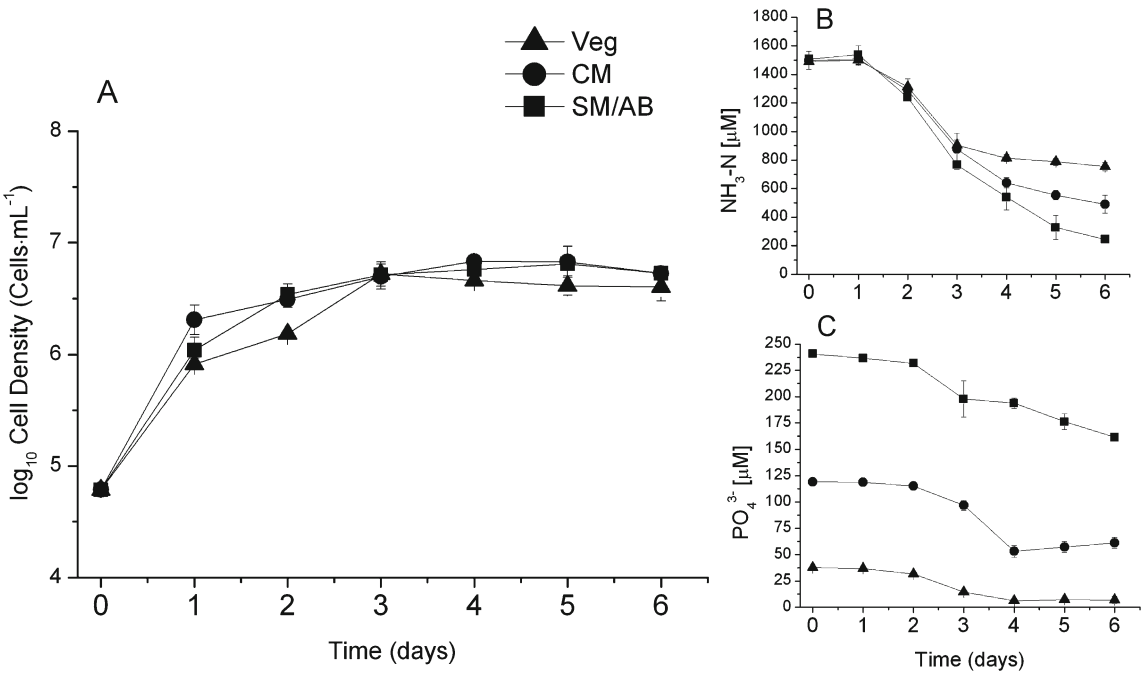

Fig. 1 **a** Growth curves of *Scenedesmus* sp. AMDD in anaerobic digestates from algae/swine manure (SM/AB), cow manure (CM), and vegetable waste (Veg). Digestates were diluted to an initial NH_3-N concentration of 1.5×10^{-3}mol L^{-1}. **b** Residual NH_3-N and **c** PO_4^{3-} concentrations (μM) in cultures from **a** supporting the growth of *Scenedesmus* sp. AMDD over 6 days of algal cultivation ($n=3 \pm$ SD)

Table 1 Nutrient drawdown and algal growth parameters from a variety of digestate sources diluted in DI H_2O

Treatment no.	Anaerobic digestate source	Amendment	NH_3-N (mol L^{-1})	PO_4-P (mol L^{-1})	N/P (mol/mol)	%NH_3-N drawdown	%N assimilation (particulate N/N provided)	%$P0_4$-P drawdown	Final cell density (cells mL^{-1})	Biomass (g dw L^{-1})	μ (day^{-1})
1	SM/AB	–	$1.58\pm0.04\times10^{-3}$	$2.01\pm0.05\times10^{-4}$	7.9	99.6	95.2	92.2	$12.37\pm2.2\times10^{6}$	0.42±0.07	1.62
2	SM	–	$1.50\pm0.06\times10^{-3}$	$2.00\pm0.05\times10^{-4}$	7.4	57.7	37.5	45.5	$4.08\pm0.6\times10^{6}$	0.14±0.04	1.34
3	CM	–	$1.48\pm0.01\times10^{-3}$	$0.92\pm0.00\times10^{-4}$	16.3	47.1	ND	68.8	$2.94\pm0.7\times10^{6}$	0.11±0.04	1.38
4	AB	–	$1.40\pm0.01\times10^{-3}$	$0.98\pm0.04\times10^{-4}$	14.4	99.4	105.0	96.8	$9.65\pm1.0\times10^{6}$	0.45±0.03	1.58
5	SM	AB 25 %	$1.41\pm0.02\times10^{-3}$	$1.71\pm0.08\times10^{-4}$	8.2	99.3	90.3	89.5	$8.89\pm0.2\times10^{6}$	0.37±0.06	1.56
6	SM	AB 50 %	$1.43\pm0.05\times10^{-3}$	$1.45\pm0.10\times10^{-4}$	10.0	99.2	100.7	81.5	$8.31\pm0.9\times10^{6}$	0.42±0.02	1.46
7	SM	Trace metals+ vitamins	$1.58\pm0.2\times10^{-3}$	$2.23\pm0.03\times10^{-4}$	7.0	41.0	35.4	25.2	$2.69\pm0.7\times10^{6}$	0.09±0.01	0.98
8	SM	EDTA+Fe	$1.40\pm0.12\times10^{-3}$	$2.18\pm0.02\times10^{-4}$	6.4	22.9	33.0	13.5	$2.15\pm0.6\times10^{6}$	0.07±0.02	0.85
9	SM	$MgSO_4$	$1.62\pm0.04\times10^{-3}$	$2.10\pm0.01\times10^{-4}$	7.6	99.9	108.4	65.2	$13.33\pm1.0\times10^{6}$	0.33±0.01	1.59
10	SM	MgCl	$1.64\pm0.03\times10^{-3}$	$1.47\pm0.03\times10^{-4}$	9.5	92.3	96.7	82.8	$10.21\pm0.6\times10^{6}$	0.40±0.02	1.40
11	SM	Lake water	$1.65\pm0.03\times10^{-3}$	$2.17\pm0.01\times10^{-4}$	7.5	100.0	103.4	99.8	$15.12\pm2.2\times10^{6}$	0.55±0.02	1.66

Values represent the average of three replicate samples ± standard deviation of the mean

SM/*AB* swine manure and algal biomass co-digestion, *AB* algal biomass, *SM* swine manure, *CM* cow manure, *ND* not determined

cell densities of 0.37 and 0.42 g dw L^{-1}, respectively, coincident with near total removal of NH_3-N from the media (Table 1, treatments 5 and 6). Algal cultures grown in swine digestates amended with trace metals and vitamins or with Fe/EDTA supported final biomass yields of 0.09 and 0.07 g dw L^{-1} respectively, and NH_3-N drawdown was incomplete in both (Table 1, treatments 7 and 8). Cultures grown in swine manure digestates amended with Mg^{2+} (added as either $MgSO_4$ or MgCl) assimilated virtually all of the N in the growth medium and obtained final biomass yields of 0.33 and 0.40 g dw L^{-1}, respectively (Table 1, treatments 9 and 10).

Freshwater experiments

Scenedesmus sp. AMDD was cultivated in swine manure digestates diluted to 1.5×10^{-3} mol L^{-1} NH_3-N in 0.22 μm of filtered lake water in 1-L bottles with CO_2 supplementation. Final biomass yield was 0.55 g dw L^{-1}, and nearly all of the NH_3-N and PO_4^{3-} were removed from the growth medium (Table 1, treatment 11).

Particulate nitrogen analysis

Elemental analysis of filtered dried algal biomass from our batch culture experiments indicated a high fraction of nitrogen uptake by *Scendesmus* sp. AMDD. In biomass from cultures where NH_3-N appeared to be drawn down from the growth medium, the fraction of measured particulate N in the biomass compared to the N provided in the growth medium was as high as 1.08 (mol/mol; Table 1, treatment 9), with the additional N likely the result of residual transfer from the inoculum culture. In cultures where we measured a significant level of residual NH_3-N in the culture medium at the cessation of growth, the fraction of N in the biomass relative to the N provided in the growth medium ranged from 0.33 to 0.38 (Table 1, treatments 2, 7, and 8).

Discussion

Growth and nutrient drawdown by *Scenedesmus* sp. AMDD was greatest when cultivated in swine manure digestates diluted in lake water. Growth in digestates that contained a fraction of digested algal biomass resulted in good nutrient assimilation and high biomass yields. Animal manure-only digestates in DI H_2O did not support complete NH_3-N drawdown; however, when supplemented with Mg^{2+}, algal growth and nutrient drawdown were increased in these media.

Research has been conducted on a variety of microalgae grown on digestates from various sources. Golueke and Oswald (1959) were successful in cultivating *Scenedesmus* sp. in digestate in an integrated system of digestion and algal growth. Our trials focused more on the growth and nutrient drawdown characteristics of *Scenedesmus* sp. AMDD and attempted to use a 'cleaner' source of digestate, by diluting in DI H_2O followed by filtration to reduce the bacterial load, to investigate the potential of the digestates alone as a source of nutrients for sterile algal cultures. Olguin et al. (1994) demonstrated efficient uptake of both NH_4^+ and PO_4^{3-} by *Spirulina maxima* in swine manure digestates that had been diluted with seawater. Similar to our study, they observed an increase in cell yields upon supplementation with CO_2, and they concluded that a coupled system of AD and algal cultivation is a viable means of treating effluent and recycling nutrients. Blier et al. (1995) observed complete inorganic nutrient removal in separate batch cultures of the cyanobacterium *Phormidium bohneri* and the microalga *Micractinium pusillum* grown in anaerobic effluent from a cheese factory. Ammonium was drawn down to insignificant levels by the fourth day of algal growth, which is a similar rate to what we observed in our most productive cultures.

The results from initial experiments with digestates diluted in DI H_2O suggested limitation by a factor other than the macronutrients N and P. Supplementation with CO_2 allowed for complete drawdown of NH_3-N and PO_4^{3+} in cultures grown in algae/swine manure co-digestates and in blended algae/swine manure digestates but not in cultures grown in either swine or cow manure digestates as the only nutrient sources. This suggested that for optimal growth, there was at least one other factor in addition to carbon required which was apparently available in algae digestates but missing in animal manure digestates. Supplementation of swine manure digestate with trace metals and vitamin stocks or with Fe/EDTA did not improve growth rates or biomass yields (in fact, both decreased slightly), which suggested that micronutrients were not the missing requirement. Further experiments indicated that cell yields were limited when cultures of *Scenedesmus* sp. AMDD were grown in Bold's Basal Medium (Bold 1949) in which the Mg^{2+} had been omitted (results not shown). Subsequent experiments showed that algal growth rates and biomass yields in swine manure digestates approached those achieved in optimal treatments only when supplemented with Mg^{2+}, indicating that this element was likely the key nutrient required for high biomass yields and which was present in adequate quantities in algae digestates. Dilution of swine manure digestate in lake water resulted in the highest biomass yields of all of the treatments, suggesting that Mg^{2+} concentrations were likewise sufficient to meet the growth requirements of the algae. Although we did not conduct elemental analysis on the lake water used in our experiments, the concentration of Mg^{2+} in this lake has averaged 0.03±0.02 mM since 2006 (n=13; Stantec 2012), which suggested that this level of Mg^{2+} is adequate for optimal growth. Park et al. (2010) also observed an increase in cell growth following the addition of

Mg^{2+} to semi-continuous cultures of *Scenedesmus acuminatus* grown in piggery effluent. Magnesium is an essential macronutrient for algal growth and is an essential constituent of the chlorophyll molecule. A deficiency of Mg^{2+} could prevent the algal culture from accumulating sufficient chlorophyll to sustain photosynthesis and growth and could therefore limit overall productivity. Complete N drawdown by *Scenedesmus obliquus* in AD wastes from swine manure at different dilutions in tap water has been observed (de la Noüe and Bassères 1989), and it is possible that municipal tap water contained sufficient Mg^{2+} for algal growth if, as in our digestate, Mg^{2+} levels were not sufficiently high. Swine manure digestates can support substantial algal growth and nutrient recycling, with minor supplementation of Mg^{2+} or dilution with algae-derived digestates if required, and could be a viable source of nutrients to support industrial-scale growth of algal biomass. In addition, if AD wastes were to be used as a supplement to municipal wastewater as a medium for algal growth, there would likely be no need to add trace elements such as Mg^{2+} to achieve high biomass and efficient nutrient drawdown (McGinn et al. 2012). This further supports the idea that AD wastes could be useful in a closed loop system of algal biomass and energy production, and wastewater remediation.

The modest enhancement in growth rate and biomass yield in cultures grown in lake water compared to the most productive cultures prepared by dilution in DI H_2O may have been due to a form of mixotrophic growth via the assimilation of dissolved organic carbon compounds, like tannins and small DOCs, which were shown to be significant in samples of the lake water (Stantec 2012). In a separate study, mixotrophic growth with acetate and glycerol has been shown to boost productivity in *Scenedesmus* sp. AMDD compared to autotrophic media (Park et al. 2011).

This study is unique in that it examined the potential of a variety of agriculturally derived digestates along with digestate from microalgal biomass subjected to AD. It allowed us to investigate suitable substrates for algal cultivation for a biorefinery system and supports the idea that co-digestion can not only provide energy production in the form of methane gas, but also provides evidence that algal-derived digestates may enhance the remediation potential of animal waste-derived digestates through enhanced algal productivity and nutrient assimilation.

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