Microalgae to biofuels: Life cycle impacts of methane production of anaerobically digested lipid extracted algae

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HIGHLIGHTS

- BMP of whole Nannochloropsis salina: 430 cm³CH₄ g-VS⁻¹ (σ = 60).
- BMP of lipid extracted Nannochloropsis salina: 140 cm³CH₄ g-VS⁻¹ (σ = 30).
- Current LCA modeling is dramatically overestimating methane production from LEA.
- Study results change baseline environmental impact from −21.3 to 18.4 g-CO₂-eq MJ⁻¹.

ABSTRACT

This study presents experimental measurements of the biochemical methane production for whole and lipid extracted Nannochloropsis salina. Results show whole microalgae produced 430 cm³CH₄ g-volatile solids⁻¹ (g-VS) (σ = 60), 3 times more methane than was produced by the LEA, 140 cm³CH₄ g-VS⁻¹ (σ = 30). Results illustrate current anaerobic modeling efforts in microalgae to biofuel assessments are not reflecting the impact of lipid removal. On a systems level, the overestimation of methane production is shown to positively skew the environmental impact of the microalgae to biofuels process. Discussion focuses on a comparison results to those of previous anaerobic digestion studies and quantifies the corresponding change in greenhouse gas emissions of the microalgae to biofuels process based on results from this study.

1. Introduction

The current instability in global oil prices has motivated researchers and entrepreneurs to search for alternative solutions to transportation fuel and energy needs. Biofuels represent a promising long-term alternative to petroleum fuels, but current feedstocks have many well-researched drawbacks including questionable land use impacts, insufficient scalability, minimal net energy reductions, and marginal greenhouse gas (GHG) reductions. Biofuels produced from microalgae are asserted to have the potential to address these shortcomings based on the findings of recent life cycle assessments (LCA). The foundational process for the production of biofuels from microalgae integrates an anaerobic digester to recycle nutrients from the lipid extracted algae (LEA) and produce on-site heat and power. Limited experimental work on the composition and digestibility of LEA has led to uncertainty in various microalgae to biofuel scalability assessments including life cycle, technoeconomic, and resource assessments that integrate anaerobic digestion as a functional process step.
Previous studies have investigated the methane yields of microalgae through anaerobic digestion but uncertainties in experimental procedures, microalgae species, and the use of whole microalgae make the use of the data in microalgae to biofuel LCA modeling challenging (Collet et al., 2011; Ehimen et al., 2011; Ras et al., 2011; Samson and Leduy, 1982; Sialve et al., 2009; Yen and Brune, 2007). Sialve et al. (2009) discuss the theoretical methane yield of microalgae based on elemental composition, and tabulate the results from a variety of studies with methane yields ranging from 90 to 450 cubic centimeters per gram volatile solid (cm$^3$/g-VS) at a gas fraction of between 62% and 72% methane. Variability in the reported data stems from differences in reactor type, temperature, load rate, retention time, and microalgae species (Spirulina, Dunaliella, Tetraselmis, and Chlorella were investigated). Ras et al. (2011) evaluate the feasibility of the anaerobic digestion of whole Chlorella biomass with the results indicating that the samples have limited digestibility and low bio-availability due to the cell wall. Samson and Leduy (1982) studied the anaerobic digestion of whole Spirulina maxima reporting a maximum methane production of 260 cm$^3$/g-VS at a methane gas fraction of 70%. Their primary conclusions asserted non-optimal conditions and results. Yen and Brune (2007) performed anaerobic digestion of combined microalgae sludge and waste paper in an effort to optimize the C:N ratio for maximum methane production. Methane production results from this study ranged between 80 and 330 cm$^3$/g-VS. Ehimen et al. (2011) is the only study identified that evaluates the methane production of a LEA sample (Chlorella). Results were found to be very sensitive to process parameters with methane yields ranging from <50 to 310 cm$^3$/g-VS. Based on this survey of the literature there exists a large variability in the reported biogas potential from microalgae, and a distinct lack of data for the biogas potential from LEA.

In an effort to evaluate the environmental impact of the large-scale production of biofuels derived from microalgae, a variety of LCAs have been performed (Aresta et al., 2005; Baliga and Powers, 2010; Batan et al., 2010; Beal et al., 2010; Brentner et al., 2011; Campbell et al., 2011; Clares et al., 2011, 2010; Collet et al., 2011; Cooney et al., 2011; Frank et al., 2011; Hirano et al., 1998; Jorquera et al., 2010; Kho et al., 2011; Lardon et al., 2009; Liu et al., 2012; Luo et al., 2011; Menger-Krug et al., 2012; Murphy and Allen, 2011; Razon and Tan, 2011; Sander and Murthy, 2010; Sevgi-Itoz et al., 2012; Shirvani et al., 2011; Sills et al., 2013; Stephenson et al., 2010; Xu et al., 2011). However, due to the immaturity of various steps within the microalgae to biofuels production process including the performance of an anaerobic digester operated on LEA (Brentner et al., 2011; Campbell et al., 2011; Clares et al., 2011; Collet et al., 2011; Frank et al., 2011; Menger-Krug et al., 2012; Sills et al., 2013; Stephenson et al., 2010), the majority of microalgae LCA analyses contain simplifying assumptions that have yet to be verified experimentally. Anaerobic digestion is a desirable process step as it enables the production of onsite power from the digestion of LEA to methane gas with the added bonus of recovering nitrogen which has been shown to be a scale limiting resource (Batan et al., 2010). In general, the models describing the function of anaerobic digestion that are included in these LCAs have lacked a strong experimental basis with production values typically representative of whole microalgae and not LEA due to limited published data. For example, Lardon et al. (2009) discuss anaerobic digestion of LEA but do not present data on product consumption or reactant yields. Stephenson et al. (2010) and Campbell et al. (2011) base the methane yields for their anaerobic digestion process off of those of previous studies, Sialve et al. (2009) and Benemann and Oswald (1996), respectively, both of which only consider hypothetical yields. Frank et al. (2011) and Collet et al. (2011) base their anaerobic digestion yields on a synthesis of studies which include the yields of both LEA and whole microalgae digestion. Clares et al. (2011) directly evaluate the conversion of all biomass to methane for electricity production through anaerobic digestion with a methane productivity derived from that of wastewater systems. To date, the performance of anaerobic digesters as modeled in the literature of microalgae LCA has been based on the limited results present in microalgae anaerobic digestion literature and typically based on experimental results from the digestion of whole microalgae.

This study experimentally determines the methane production potential of whole and lipid extracted *Nannochloropsis salina* through biochemical methane potential (BMP) with the objective of informing the microalgae biofuels LEA literature of the difference. A set of experiments to characterize the whole microalgae and LEA are performed which included elemental analysis and lipid profiles of the extracted microalgae oil. The discussion compares the properties of the whole microalgae to LEA, compares the methane production potential of *N. salina* to other previously reported microalgae species, illustrates the impact of anaerobic digestion on environmental impact of the microalgae to biofuels process on a systems level, and integrates the results from this study into previous environmental assessments of the microalgae to biofuels process to illustrate the sensitivity of LCA results to anaerobic digester performance assumptions.

2. Methods

The following section details the microalgae growth facility and large-scale lipid extraction process, the methods for biomass and lipid characterization, the analytics used to determine microalgae composition, the BMP experimental setup for determining methane potential, and the details for theoretical methane production potential calculations.

2.1. Microalgae growth facility and large-scale lipid extraction

Microalgae culture was obtained by Solix BioSystems from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), specifically *N. salina* (CCMP 1776). A limited number of microalgae species are being investigated for the production of biofuel with *N. salina* representing a promising strain due to characteristically high lipid yields and culture stability. Photobioreactors were utilized for microalgae cultivation in f/2 growth media modified to contain 5.0 mM nitrate and 0.368 mM phosphate per liter at a salinity of 20 g L$^{-1}$. Microalgae cultures were batch cultivated from an initial dry weight density of 1 g L$^{-1}$ to a final dry weight density of 3 g L$^{-1}$. Nitrogen stressing was the mechanism used to increase lipid content in the biomass. Samples were collected from two growth facilities: (1) Solix BioSystem’s Research and Development facility (8000 L of cultivation) located in Fort Collins, Colorado and (2) Solix BioSystem’s pilot plant facility (150,000 L of cultivation) located outside Durango, Colorado. Detailed descriptions of the growth facilities are presented in the supporting information. Microalgae cultures were harvested at the pilot plant facility via a continuous clarifier operated at 45,000 L hr$^{-1}$ (Westfallia) and frozen for transport to extraction facilities. Whole microalgae samples for this study were collected from this frozen stock prior to extraction. LEA samples were collected post-extraction.

Extraction was performed in a two-step process: (1) microalgae drying (thermal) and (2) hexane solvent extraction. Due to the immaturity of the microalgae to biofuels process, there are few large-scale extraction technologies available. Dry hexane extraction has proven to be an effective (although energetically intensive) process (Batan et al., 2010; Frank et al., 2011). This
A spitless injection was used requiring 1 of Schutter and Dick (2000). A mass of 5 mg of microalgae sample activated cultures. Full evaporation of the hexane solvent. Due to the large-scale of the whole microalgae with a byproduct of LEA. The LEA used in this technique was used to remove the triacylglycerol (TAG) from the sample. A 1:1 ratio of inoculum to nutrient solution resulted in theoretical methane production (data not shown). Results from positive control syringes indicated that the inoculum was capable of hydrolysis and methanogenesis. The carbon and nitrogen ratio was determined by combustion utilizing a 30 m/250°C to 208°C at 70°C min⁻¹, then ramped to 230°C at 3°C min⁻¹, and finally to 240°C ramped at 2°C min⁻¹ and held for 1 min. Prior to running samples, a blank was run followed by the generation of a 4 point standard curve using a GLC-461 standard obtained from NU-CHEK PREP, Inc.

2.2. Whole microalgae and lipid characterization methods

Biomass samples were analyzed for lipid content and overall composition. The following section details the analytics used to determine the lipid composition and profile of the samples and elemental composition including carbon to nitrogen ratio.

2.2.1. Lipid content and characterization

Assays of lipid profile and content based on fatty acid methyl ester (FAME) analysis were performed on material collected at the end of each batch culture. Lipid fractions were determined using an in-situ transesterification technique based on the methods of Schutter and Dick (2000). A mass of 5 mg of microalgae sample was centrifuged at 4000g for 5 min followed by the removal of the supernatant. An auto-pipette was used to dispense 2.5 ml of 0.2 M KOH in methanol onto the 5 mg microalgae pellet. Samples were pipette mixed and transferred to a glass test tube previously washed in 1% HCl acid. An additional 2.5 ml of 0.2 M KOH in methanol was added and pipette mixed. Samples were then aggressively mixed using a VWR Analog Vortex Mixer on a speed setting of 10 for 20 s followed by heating to 37°C for 30 min. One ml of acetic acid and 2 ml of high-performance liquid chromatography grade heptane was then added and the samples were mixed using a VWR Analog Vortex Mixer on a speed setting of 10 for 20 s and then centrifuged at 2000g for 5 min. The organic layer was then removed and processed in a gas chromatograph (GC) to determine lipid content and composition. The single heptane extraction reproducibly reproduced 80% (σ = 4.3%) of the overall lipid content in the cells as determined by standard addition methods using C15:0 TAG.

Transesterified samples were prepared for GC analysis by first diluting the sample 1:10 with heptane. An internal standard (23:0 FAME) obtained from NU-CHEK PREP, Inc. was added to the sample and the head space was then filled with nitrogen. Samples were analyzed with an Agilent Technologies 7890A GC machine utilizing a 30 m × 0.32 mm × 0.25 um Restek FAMEWAX column. A splitless injection was used requiring 1 μL of sample. Helium at 1.5 mL min⁻¹ was used as the carrier gas. The oven was operated at 90°C for 0.5 min and then ramped to 208°C at 70°C min⁻¹, then ramped to 230°C at 3°C min⁻¹, and finally to 240°C ramped at 2°C min⁻¹ and held for 1 min. Prior to running samples, a blank was run followed by the generation of a 4 point standard curve using a GLC-461 standard obtained from NU-CHEK PREP, Inc.

2.2.2. Biomass composition

Wet whole microalgae and dry LEA samples were supplied to the Colorado State University Soil, Water, and Plant Testing Laboratory for elemental analysis to determine the carbon to nitrogen ratio. The elemental analysis was performed by adding 5 mL of nitric and 5 mL of perchloric acid to 1 g of sample. The mixture was well mixed and placed in an oven at 125°C until all of the nitric acid was evaporated. The samples were then placed in an oven at 200°C for 2 h. Samples were cooled and the volume was increased to 50 mL. Samples were then decanted and auto-analyzed by inductively coupled plasma.

The carbon and nitrogen ratio was determined by combustion using a Leco TruSpec CN furnace. Prior to performing tests, the machine was calibrated and a drift correction was performed. Samples were weighed in a tin foil cup and sealed. The combustion furnace was operated at 950°C with the afterburner temperature of 850°C. Samples between 0.1 and 0.5 g were analyzed with an atmosphere blank to ensure accuracy.

The total solids (TS), fixed solids (FS), and volatile solids (VS) for the samples were determined for the whole microalgae and LEA. The TS for each sample were determined by placing 5–10 g of the homogenized sample in sampling dish. Mass of the dish before and after placing the sample in an electric oven operated at 103°C was recorded. The dish was kept in the oven until the weight stabilized (approx. 2–6 h). The final mass of the dish was recorded and the FS were calculated. The FS were determined by placing samples in a furnace at a temperature of 550°C. The sample dish was kept in the furnace until the weight of the dish stabilized (approx. 1 h) with the mass recorded. VS were calculated by the mass difference between the TS and the FS.

2.3. Biochemical methane production potential

A BMP test was performed to obtain the kinetics of substrate utilization, and to evaluate the potential biogas production efficiency (methanogenic performance) of the anaerobic digestion process with whole microalgae and LEA feedstocks. The viability of feedstocks for anaerobic digestion is commonly determined by the BMP assay (Owen et al., 1979). BMP results have been adopted for understanding the preliminary screening of feedstocks, toxicity testing, as well as the design and prediction of methane production from full-scale operational digesters. Standard methods were adopted from Owen et al. (1979).

The BMP tests were conducted in 140 mL luer lock plastic syringes to maintain a controllable anaerobic environment. The test involved the addition of three ingredients, substrate, inoculum, and nutrient solution. Substrate is the biodegradable carbon source (whole microalgae, LEA sample, or glucose), inoculum is the stream of anaerobic bacteria which utilize the substrate to produce biogas (collected from an anaerobic digester at the Drake water reclamation facility, Fort Collins, CO) and a nutrient solution with all the essential nutrients required by the methanogens to efficiently grow in the environment (Owen et al., 1979). The constituents were added to clean, dry, graduated, 140 mL luer lock plastic syringes each fitted with a three way valve. The quantity of substrate added to the syringe was normalized based on chemical oxygen demand (COD) with all tests performed in biological triplicate. COD was quantified through sealed digestion of samples (Hach Method 8000) followed by spectrometry (Hach DR 2500). An amount of 0.050 g-COD L⁻¹ of microalgae sample corresponding to 0.080 g of whole microalgae or 0.030 g of LEA was added to each of the syringes followed by 25 mL of inoculum (22.7 g-COD L⁻¹, σ = 2.6) and 25 mL of nutrient solution. The mass of microalgae added was determined such that an appropriate quantity of biogas would be formed for measurement in the test syringes. Addition of nutrient solution was based on previously developed methods (Owen et al., 1979). Previous experiments demonstrated that a 1:1 ratio of inoculum to nutrient solution resulted in theoretical methane production (data not shown).

Positive control syringes containing glucose and negative control syringes were also run in triplicate. Positive control syringes with glucose as the sole carbon source instead of microalgae sample were used to ensure the inoculums were efficiently digesting and producing biogas. Positive control syringes were inoculated with 0.050 g-COD L⁻¹. The syringes containing glucose produced 490 cm³ g-COD⁻¹ with a standard deviation (σ) of 50 cm³ g-COD⁻¹. Results from positive control syringes indicated that the inoculum was capable of hydrolysis and methanogenesis.

Negative control syringes were set up without a substrate to detect the production of gas from inoculum and nutrient solution alone. Biogas produced by the negative control syringes was
subtracted from the biogas production of test samples to account for background methane production. Results indicated that the inoculum contributed to approximately 42.8% (σ = 1.4%) of total biogas produced from positive control syringes, 55.5% (σ = 3.3%) of total biogas produced from syringes with whole microalgae and 74.0% (σ = 0.5%) of total biogas produced from syringes with LEA.

All test syringes were placed in an incubator (LAB-Line® Orbit Environ-shaker ATRIX ID#0805) set to 35 °C. The volume of biogas produced was monitored daily by recording the change in syringe plunger extension length using a digital caliper (measurement error of ±0.02 mm contributing to <0.4% gas volume measurement error). The tests were concluded when the change in syringe plunger extension length was negligible over a 24 h period. Biogas was periodically sampled from the syringe through the 3 way valve and analyzed for methane concentration using a GC (Hewlett Packard 5890 Series II). The GC was calibrated prior to gas sample testing by injecting methane gas concentrations of 20, 40, 80, and 100%. The coefficient of regression was greater than 0.98 for all calibration curves.

2.4. Theoretical methane yield

The theoretical methane yield was determined based on the lipid, protein, and carbohydrate composition of the whole microalgae and the LEA. The theoretical methane yield can be calculated based on the adaptation of the formula from Bushwe and Neave (1930) which balances the total conversion of organic material to CH₄ and CO₂ with H₂O under anaerobic conditions:

\[
\text{C}_n\text{H}_m\text{O}_b + \frac{4n - a + 2b}{4}\text{H}_2\text{O} \rightarrow \frac{8}{4}\text{CH}_4 + \frac{8}{4}\text{CO}_2
\]

Eq. (1) represents a theoretical calculation of the methane production potential and does not include anaerobic cell maintenance or anabolism or bioavailability of the feedstock. The availability of intercellular components can be limited if microalgae cells are not ruptured prior to anaerobic digestion. The extraction of lipids, for example, disrupt the cell wall and makes the remaining biomass more accessible to anaerobic bacteria but at the cost of removing the energy rich lipids. The specific methane yields for carbohydrates ((C₆H₁₀O₅)n), lipids (C₇H₁₂O₈n), and proteins (C₉H₁₆O₅N₄A₀n) based on Eq. (1) are 415, 1014, and 851 cm³ g⁻¹VS⁻¹, respectively. It is assumed the nitrogen from the protein is converted to NH₃.

3. Results and discussion

The results and discussion are divided into three sections: (i) whole microalgae and LEA characterization which includes elemental analysis and lipid profile, (ii) methane potential (BMP) results, and (iii) comparison of results to previously published data and current LCA modeling assumptions and the impact of study results on systems level LCA results.

3.1. Whole microalgae biomass and lipid extracted algae characterization

Whole microalgae and LEA prior to hexane extraction were characterized for lipid percentage and content. Lipid percentage and FAME profiles are presented in Table 1. The fatty acid composition presented is indicative of the N. salina species with the exception of 18:3 chain. The 18:3 chain is symptomatic of an invasive species with the relatively low quantities of 18:3 illustrating a relatively homogeneous culture of N. salina. The total FAME content of ~35% is indicative of the cultures being cultivated under nitrogen stress conditions for high lipid yield. Analysis of the LEA was performed to verify that the extraction process had removed all of the lipids from the microalgal cultures.

Elemental analyses of the whole microalgae and LEA are presented in Table 2 on a TS basis. All of the elements measured, with the exception of sodium, decreased in concentration in the LEA compared to the whole microalgae due to their removal during the extraction process. The higher sodium concentration in the LEA is attributable to the precipitation of sodium salts during the drying of the microalgae prior to extraction. The whole microalgae samples were approximately 33% solids and 66% salt water at 20 g L⁻¹ concentration. During the drying process prior to extraction, sodium chloride precipitates. Extraction does not remove the highly polar sodium chloride precipitate as hexane is a non-polar solvent.

Results from the carbon, nitrogen, and total, fixed, and volatile solids tests are presented in Table 3. The TS for the biomass sample of 33.4% is expected based on the sample being harvested using a batch type centrifuge which can concentrate the culture to 20–40% solids. The LEA sample has very high TS because it has been kiln dried prior to extraction removing all external and internal cell water. The carbon to nitrogen ratio for the whole microalgae and LEA are 20.4:1 and 8.7:1, respectively. Previous detailed carbon to nitrogen studies have shown this ratio can vary depending on growth phase, with the reported value for whole microalgae being consistent with previous studies (Frank et al., 2011). The decrease in the carbon to nitrogen ratio between the biomass and LEA samples is a result of the removal of the TAGs during extraction. TAGs are composed of three long chain lipids connected to one glycerol molecule. Both components are predominantly composed of carbon with no nitrogen thus their removal increases the carbon to nitrogen ratio. The LEA had an average lipid mass percentage of 32% corresponding to a total TAG mass of approximately 40%. The extraction process removed the TAGs thereby decreasing the carbon to nitrogen ratio in the LEA.

3.2. Methane production results

So as to perform the BMP tests on the basis of equivalent sample COD, the COD of the whole microalgae and LEA were measured. The total COD for the whole microalgae was measured at 0.63 g (g-microalgae)⁻¹ with a 0.01 standard deviation. The total COD for the LEA was measured at 1.67 g (g-LEA)⁻¹ with a 0.04 standard deviation.

Methane production over the 19 day test period for the whole microalgae and LEA are presented in Fig. 1 with raw tabulated data presented in the supporting information. The methane percentage for the whole microalgae and LEA were 61.8% (σ = 1.74%) and 49.5 (σ = 2.50%), respectively. A positive control sample consisting of glucose as the carbon source was inoculated to verify the anaerobic bacteria were effectively producing biogas. Total biogas results are presented in Table 4 along with the theoretical methane yield for the whole microalgae and LEA. Due to potential sodium toxicity above 11 g L⁻¹, total dissolved solids (TDS) concentration in the BMP syrings were measured to evaluate toxicity. The BMP syrings with whole microalgae and LEA had an average TDS concentration of 5.4 g L⁻¹ (σ = 0.1) and 5.5 g L⁻¹ (σ = 0.2) respectively, indicating that sodium was well below the toxicity limit.

As shown in Table 4, the whole microalgae produced 3 times more methane than then LEA, as might be expected based on the decrease in the overall energy composition of the LEA that results from the extraction of the TAGs. Assuming the whole microalgae is composed of 40% protein, 20% carbohydrate, 35% lipid and 5% ash, the theoretical maximum methane production from the whole microalgae and the LEA is 1.8 and 4 times greater than the
experimental results reported here, respectively. These experimental results confirm that the removal of the TAGs (lipids and glycerol) from the microalgae biomass reduces the methane productivity potential of the remaining biomass through a decrease in the biomass energy content and net biodegradability.

### 3.2.1. Comparison to previous studies

A variety of researchers have evaluated the methane production potential from microalgae (Collet et al., 2011; Ehimen et al., 2011; Ras et al., 2011; Samson and Leduy, 1982; Sialve et al., 2009; Yen...

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### Table 1
FAME percentages and profile for whole microalgae sample and lipid extracted algae (LEA) samples prior to extraction. The LEA sample is a mixture of 22 batch harvests due to the scale of the extraction process.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>FAMESa</th>
<th>Std. Dev.</th>
<th>14:0 (%)</th>
<th>16:0 (%)</th>
<th>16:1 (%)</th>
<th>17:1 (%)</th>
<th>18:0 (%)</th>
<th>18:1 (%)</th>
<th>18:2 (%)</th>
<th>18:3 (%)</th>
<th>20:4 (%)</th>
<th>20:5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole microalgae</td>
<td>34.9</td>
<td>0.9</td>
<td>2.1</td>
<td>39.9</td>
<td>36.2</td>
<td>0.0</td>
<td>0.0</td>
<td>8.4</td>
<td>0.0</td>
<td>0.0</td>
<td>3.6</td>
<td>9.7</td>
</tr>
<tr>
<td>LEA (prior to extraction)</td>
<td>32.0</td>
<td>9.03</td>
<td>2.3</td>
<td>32.1</td>
<td>21.4</td>
<td>2.7</td>
<td>1.6</td>
<td>19.4</td>
<td>3.2</td>
<td>7.3</td>
<td>2.1</td>
<td>6.5</td>
</tr>
<tr>
<td>STDEV</td>
<td></td>
<td></td>
<td>1.8</td>
<td>7.7</td>
<td>12.6</td>
<td>3.7</td>
<td>0.4</td>
<td>12.3</td>
<td>2.6</td>
<td>8.6</td>
<td>1.4</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a Total FAME results are the average of triplicate samples.

### Table 2
Elemental analysis of the whole microalgae and lipid extracted algae (LEA). All values are presented in units of mg (kg TS)\(^{-1}\).

| Sample          | Ca   | Mg   | Na   | K    | P    | Al   | Fe   | Mn    | Ti   | Cu   | Zn   | Ni   | Mo   | Cd   | Cr   | Sr   | Ba   | Be   | Pb   | V    | Sb   | Ag   |
|-----------------|------|------|------|------|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Whole microalgae| 3982 | 9943 | 135.2| 16,886| 4937 | 26.9 | 311  | 32.6  | 1.68 | 3.11 | 7.78 | 1.23 | <0.01 | 0.299| <0.01 | 18.0 | 2.99 | <0.001| 0.389| 7.19 | <0.01| 2.33 | 4.56 |
| LEA             | 1770 | 3915 | 5877 | 7966 | 2986 | 5.98 | 15.8 | 15.5  | 0.304| 2.33 | 4.56 | 0.781| 1.22  | 0.142| 0.274| 9.6   | 0.923| <0.001| 0.051| 2.43 | <0.01| <0.01|      |

### Table 3
Results for the carbon (C), nitrogen (N), total solids (TS), fixed solids (FS), volatile solids (VS), and ash. TS, FS, and VS were tested in triplicate with the standard deviation (\(\sigma\)) presented.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Whole microalgae</th>
<th>LEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>20.2</td>
<td>50.1</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.988</td>
<td>5.73</td>
</tr>
<tr>
<td>TS (%)</td>
<td>33.4 ± 0.30</td>
<td>98.6 ± 0.05</td>
</tr>
<tr>
<td>FS (%)</td>
<td>1.9 ± 0.03</td>
<td>10.0 ± 0.03</td>
</tr>
<tr>
<td>VS (%)</td>
<td>31.5 ± 0.03</td>
<td>88.5 ± 0.80</td>
</tr>
<tr>
<td>Ash (g·VS g·TS(^{-1}))</td>
<td>0.94</td>
<td>0.89</td>
</tr>
</tbody>
</table>

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### Fig. 1
Cumulative methane production for the whole microalgae and lipid extracted algae (LEA) samples over 19 day test period.

### Table 4
Biogas production for the whole microalgae and lipid extracted algae (LEA) samples, compared to calculated theoretical yields. Standard deviation (\(\sigma\)) is presented in parenthesis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total biogas ((\sigma))</th>
<th>Methane ((\sigma))</th>
<th>Theoretical methane yield(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole microalgae</td>
<td>(L g-COD(^{-1})) 0.33 (0.06)</td>
<td>0.21 (0.04)</td>
<td>0.39</td>
</tr>
<tr>
<td>(L g-VS(^{-1})) 0.70 (0.13)</td>
<td>0.43 (0.08)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>LEA</td>
<td>(L g-COD(^{-1})) 0.15 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.32</td>
</tr>
<tr>
<td>(L g-VS(^{-1})) 0.29 (0.02)</td>
<td>0.14 (0.02)</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Sialve et al. (2009).
LCAs have assumed methane production potential based on either the methane production in the anaerobic digestion process. Past impact of the extraction of lipids from microalgae biomass on temper. The majority of the studies surveyed do not consider the on the energy balance of a microalgae to biofuel production sys-

tegration of an anaerobic digester has a systems-level effect this sub-process has on the overall life cycle of the biofuel system. The integration of an anaerobic digester has a systems-level effect on the performance of the anaerobic digester based on the impact differences in digestibility due to details of the lipid extraction pro-

The wide range of results is due to a variety of experimental differences and feedstock inputs. Only one study has been identified that uses LEA, specifically lipid extracted Chlorella. Methane production results of 245 (σ = 15) cm³ g-VS−1 were reported (Ehimen et al., 2011), whereas the results from this study are 140 (σ = 30) cm³ g-VS−1, or 42% less methane production than the results from Ehimen et al. (2011). The difference in biogas production could stem from different microalgal composition, use of a salt water species (N. salina) compared to a freshwater species (Chlorella), or differences in digestibility due to details of the lipid extraction process.

The results from literature and from this study both show that the total methane produced from LEA is much less than the total methane produced from whole microalgae biomass.

### 3.3. Application to life cycle assessment

A variety of literature LCAs on microalgae based biofuel systems have been performed with net energy ratio (NER, defined here as energy in over energy out) metrics of performance varying from between <50 to 330 cm³ g-VS−1. The wide range of results is due to a variety of experimental differences and feedstock inputs. Only one study has been identified that uses LEA, specifically lipid extracted Chlorella. Methane production results of 245 (σ = 15) cm³ g-VS−1 were reported (Ehimen et al., 2011), whereas the results from this study are 140 (σ = 30) cm³ g-VS−1, or 42% less methane production than the results from Ehimen et al. (2011). The difference in biogas production could stem from different microalgal composition, use of a salt water species (N. salina) compared to a freshwater species (Chlorella), or differences in digestibility due to details of the lipid extraction process.

The results from literature and from this study both show that the total methane produced from LEA is much less than the total methane produced from whole microalgae biomass.

Previous LCA studies by Frank et al. (2011), Campbell et al. (2011), and Menger-Krug et al. (2012) have included anaerobic digestion process models, and have assumed a methane production of 330 cm³ g-VS−1, 500 cm³ g-VS−1, and 569 cm³ g-VS−1, respectively. The results from this study show that if the anaerobic digesters are operated on whole microalgae biomass then the range of the modeled production rates includes the results of this study, however if the process is extracting TAGs for conversion to fuel (which is the case for the three studies presented) then the assumptions for the methane production from the anaerobic digest-
gection of the LEA are modeled as more than two times too high. Considering that the anaerobic digester is a critical component used to generate onsite power, process heat, and recycle nutrients, an overestimation of the methane production will underestimate the life cycle impacts of the microalgae to biofuels process. For example, the baseline global warming potential (GWP) of the microalgae biofuels life cycle for the study of Frank et al. (2011) is −21.3 g of CO₂-equivalent per MJ of biofuel (g-CO₂-eq MJ⁻¹). Replacing the methane production assumptions in Frank et al. (2011) (330 cm³ g-VS⁻¹) with those from this study (140 cm³ g-VS⁻¹) increases the GWP of the microalgae to biofuel processes by 185% to +18.4 g-CO₂-eq MJ⁻¹ (details on the GREET inputs and results are presented in the supporting information along with results for soy based biofuel and conventional diesel). This analysis shows the environmental impact of a microalgae to biofuels process that integrates an anaerobic digester is very sensitive to the methane production assumptions. Reducing the methane production assumed by Frank et al. (2011) to be in-line with the experimental results of this study, result in the net GHGs increasing and changing signs from negative (desirable) to positive (undesirable).

### 4. Conclusions

This study illustrates the importance of considering the effects of the removal of lipids from microalgae for modeling of anaerobic digestion performance in the LCA of microalgae biofuels. BMP...
results show that lipid extracted *N. salina* produces 3 times less methane than raw whole biomass. The removal of energy rich lipids for fuel production dramatically decreases the methane yield.

Comparison of the results from this study to the modeling assumptions present in most LCA and TEA shows consistent over-estimation of anaerobic digestion performance which skews environmental impact and economic results in favor of microalgae-based biofuels production.

**Acknowledgements**

The authors greatly acknowledge support from Solix Biosystems, Debbie Weddle and James Self from the Soil, Water, and Plant Testing Lab at Colorado State University, and Danna Quinn.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.08.037.

**References**


