



# Co-digestion of Wastewater-Grown Filamentous Algae With Sewage Sludge Improves Biomethane Production and Energy Balance Compared to Thermal, Chemical, or Thermochemical Pretreatments

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Wastewater algal treatment systems show improved economic viability and enhanced energy return on investment if integrated with biofuel production. One option is to anaerobically digest the algae to generate bio-methane. This method is appropriate for relatively low lipid filamentous algae typical of turf scrubbers<sup>®</sup>. However, an unbalanced algal biomass composition (e.g. carbon-to-nitrogen ratio, C/N) and the resistance of algae to biodegradation can limit biomass conversion into bio-methane. To evaluate options to enhance bio-methane production, an indigenous assembly of macro-algae was established and cultivated in CO<sub>2</sub>-infused secondary wastewater effluent, then harvested and either anaerobically digested using pretreatments or co-digested with sewage sludge. Results were used to develop methane production kinetic models and perform an anaerobic digestion (AD) system energy balance analysis to assess the feasibility of pretreatment and co-digestion for a scaled process. Flowways were dominated by *Ulothrix* and *Oedogonium* algae and had periphyton biomass production rates that averaged  $3.7 \pm 0.4 \text{ g VS m}^{-2} \text{ d}^{-1}$  ( $\pm 1 \text{ SD}$ ) during the initial 7-day colonization period. Biomass increased by 62% in the second half of the 14-day experiment. Ultimate methane yield from harvested biomass was improved relative to controls ( $306 \pm 13 \text{ mL gVS}^{-1}$ ) through thermal pretreatment by 15%, dilute acid by 5%, dilute alkali by 17%, and acid- and alkali-assisted thermochemical pretreatments by 23 and 27%, respectively. However, all pretreatment methods undermined the energy balance parameters including Net Energy Ratio (NER) and Net Energy Efficiency (NEE) due to the heat required for thermal pretreatments and the electricity needed to produce chemical reagents. In contrast, co-digestion of algal biomass with sewage sludge synergistically enhanced methane generation, yielding up to  $401 \pm 3 \text{ mL gVS}^{-1}$  at an algae-to-sludge ratio of 20% to 80%. Co-digestion with sludge also strongly improved AD system energy balance. NER and NEE increased from 2.8 and 73% for algae alone to 4.3 and 81% for a

20% to 80% algae-to-sludge mix, respectively. Moreover, the Net Energy Recovery during co-digestion reached 39% compared to 26 and 33% when algae or sewage sludge were processed as single-substrates. Thus, co-digestion of algae with sewage sludge serves as an attractive option for maximizing energy gain from AD of biomass harvested from filamentous algal treatment systems.

**Keywords:** filamentous algal treatment systems, indigenous algal polyculture, algal biomass pretreatment, algae co-digestion with sewage sludge, methane production models, scaled digester energy balance analysis

## INTRODUCTION

Nutrient over-enrichment has resulted in the impairment of more than 189,000 km of rivers and 6,800 km<sup>2</sup> of freshwater in the US (EPA, 2018). These nutrients cause eutrophication and contribute to the rise in harmful freshwater algal blooms (Heisler et al., 2008). Economic costs associated with freshwater eutrophication alone already exceed \$2 billion per year for the US economy (Dodds et al., 2009). Because wastewater effluent contributes substantially to the release of nutrient pollution into aquatic ecosystems (Carey and Migliaccio, 2009), advanced treatment technologies have been developed that can reduce nutrient pollution from wastewater effluent (Leo et al., 2011). However, only 32% of US wastewater treatment facilities use advanced nutrient removal technologies (EPA, 2013) due to their high capital and operation costs.

Treatment technologies using algae to remove nutrients from wastewater hold promise as a more sustainable alternative to traditional wastewater treatment (Park et al., 2011; Bohutskyi et al., 2015b, 2016b). While there are numerous methods for growing algae in wastewater (Prajapati et al., 2013), filamentous algal treatment systems are easy to harvest and dewater, operate under a broad range of environmental conditions, reduce nutrients to low concentrations, and produce large quantities of renewable biomass (Adey et al., 2011; Bohutskyi et al., 2016a). Indeed, algal turf scrubbers<sup>®</sup> can be used to treat aquatic ecosystems, contaminated groundwater, municipal sewage, and industrial/agricultural wastewater (Adey et al., 2011; Bohutskyi et al., 2016a).

Generally speaking, algal treatment systems show improved economic viability (Zamalloa et al., 2011) and enhanced energy return on investment (Zaines and Khanna, 2013) when the algal biomass is converted to valuable products or biofuel (Tang et al., 2015). One practical approach is anaerobic digestion (AD) of the algae to generate bio-methane (Bohutskyi and Bouwer, 2013; Bohutskyi et al., 2018b). For filamentous algae, typical of turf scrubbers, AD may be preferred to the extraction of lipids for biodiesel considering low lipid composition of macro-algae (Bohutskyi et al., 2016a). Because AD methane production can be inhibited by the unbalanced carbon-to-nitrogen (C/N) ratios and the resistance of algal cell walls to degradation (Prajapati et al., 2013), realistic and energy-effective methods for enhanced bio-digestion filamentous algae are needed to realize the full potential of coupled turf scrubber-biogas systems (Adey et al., 2011).

One option for improving algal bio-digestion is to pretreat algae using chemical, mechanical, or thermal processes (Bohutskyi and Bouwer, 2013). The effectiveness of these

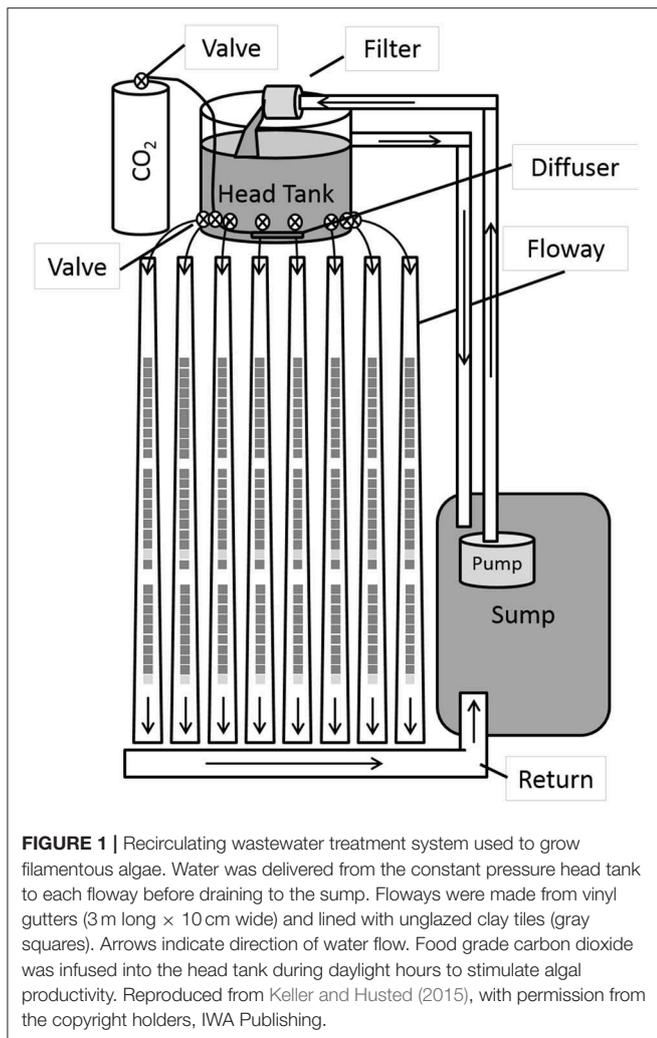
approaches depends on the pretreatment applied and on the biomass characteristics of the processed algal species (Bohutskyi et al., 2014). Alternatively, production of bio-methane can be improved through co-digestion with other types of low-cost co-substrates, which may include lignocellulosic residues (Bohutskyi et al., 2018a) or sewage sludge (Bohutskyi et al., 2019) available at wastewater treatment sites. Because pretreatment and co-digestion can require additional energy and increased volume of the digesters, these processes could decrease the net energy output, net energy ratio (NER) and other energy balance parameters of AD. These additional “costs” can reduce the economic attractiveness of the entire process. While pretreatment and co-digestion efficiency are typically evaluated using the final bio-methane yield, the assessment of their effects on the kinetics of methane production and AD system energy balance parameters is essential for evaluating the feasibility of scaling up the entire wastewater-algae-methane process.

Currently, wastewater treatment facilities in the U.S. and Puerto Rico use anaerobic digestion to treat half of the wastewater-derived sewage sludge for beneficial uses (Seiple et al., 2017). These wastewater treatment facilities have a pre-existing infrastructure for co-digestion of the filamentous algae with sewage sludge. Moreover, most of the existing digesters are not loaded to their full capacity (Pennington, 2018). Thus, the U.S. currently has a significant number of the treatment facilities capable of coupling filamentous algal treatment systems with anaerobic co-digestion. This study seeks to address a critical research and engineering challenge—namely, to evaluate the impact of various pretreatment methods and co-digestion with sewage sludge on the methane production potential from filamentous algae grown in wastewater for tertiary nutrient removal. These data were then used to develop methane production kinetic models and to compare the AD system energy balance parameters including net energy output, volume- and mass-specific energy output, net energy ratio (NER), net energy efficiency (NEE), and net energy recovery (NERec).

## MATERIALS AND METHODS

### Algae Cultivation and Biomass Harvesting

Algae were cultivated in a pilot-scale, recirculating filamentous algal treatment system (Keller and Husted, 2015) operated outdoors at the South Columbus Water Resources Facility (Columbus Water Works, Inc., 32.41N, 84.97W) from Oct 10 to Oct 24, 2014. This treatment facility processes the municipal wastewater for Columbus and Fort Benning (Georgia,



USA), serving a combined population of more than 200,000 residents. The region exists in a temperate climatic zone, defined as humid subtropical (Peel et al., 2007).

The recirculating filamentous algal treatment system used to grow the algae for these measurements was composed of a constant-head feeder tank (180 L), 8 vinyl flowways (3 m long × 10 cm wide × 1 cm water depth) and a sump (560 L) (Figure 1). The system was filled with secondarily treated wastewater from a clarifier before it was neutralized or chlorinated. Algae were grown on 47 mm circular glass fiber filters (1 μm) attached to clay tiles (Figure 1).

Food-grade compressed carbon dioxide (100%) was infused into the head tank during daylight hours to prevent pH spikes (Mulbry et al., 2008). Gas delivery rates were set to ensure sump water pH remained below 8.0 during times of peak algal productivity. To characterize environmental conditions during the experiment, data on insolation, discharge, and nutrient dynamics were also collected. The modeled light incidence (DHI) was estimated using the National Renewable Energy Laboratory's Physical Solar Model (PSM v3, accessed 6/21/2018). Discharge,

volume per unit time (mL/s), was calculated after collecting effluent from each floway for 15–30 s and measuring volume/unit time. Nutrient dynamics in the filamentous algal treatment system were characterized prior to, during (day 7) and after the end of the 14-day experiment. Water samples were collected from each sump, chilled on ice, and analyzed for phosphate (US EPA 365.3), nitrate (US EPA 300A), and ammonia (US EPA 350.3) at the Columbus Water Works, Inc. water quality laboratory.

To quantify algal chlorophyll and total biomass, two randomly selected tiles were collected from each floway to measure chlorophyll a (CHL) and volatile solids (VS) on days 7 and 14. Algal samples were transported on ice and stored frozen until analysis. To characterize the biogas production potential, all the algal biomass remaining in each floway was collected, partially dewatered, and transported on ice. At the lab, samples were stored and shipped frozen (−20°C) for AD experiments.

To characterize changes in productivity over the course of the experiment, the volatile solids productivity rates (g/m<sup>2</sup>/day) were calculated and compared for day 7 and 14 using a paired *t*-test and JASP Version 0.9.1 software (JASP-Team, 2018). Paired *t*-test was used because each flume was sampled twice (i.e., day 7 and 14). Nutrient removal rates were calculated as loss of nutrient mass (g) from the beginning to the end of the experiment per unit growing area (i.e., 2.4 m<sup>2</sup>) per day (i.e., 14).

## Biomass Feedstock

Thawed samples of filamentous algal biomass were homogenized for 10 min using an Oster® 10-Speed blender equipped with a 25 mm radius blade at 27,000 rpm (A.J. Oster Corp., Warwick, CT, USA). Homogenized algal biomass was diluted to ~3% dry weight slurry and stored at 4°C prior to analysis (<48 h). Raw sewage sludge, representing a mix of primary and secondary sludge, was collected at the Back River Wastewater Treatment Plant. This facility processes domestic wastewater derived from nearly 1.3 million residents in Baltimore City and County (USA). It was sampled from the input to the anaerobic digester, stored at 4°C and used as a co-substrate for co-digestion experiments.

## Pretreatment and Co-digestion of Algal Biomass

### Alkaline and Acid Treatments

To characterize the effectiveness of alkaline and acid pretreatments, a known amount of either 50% NaOH or 37% HCl was added to 120 mL of ~3 % dry weight (dw) algal biomass slurry. This slurry had a final concentration of 10 g L<sup>-1</sup> of the reagent (NaOH or HCl). The samples were mixed carefully and transferred into 60 ml bottles for either thermal or room temperature pretreatment. After ambient temperature samples were incubated for 2 h at room temperature (22°C), the pH of the remaining solution was measured and neutralized to 7.2 ± 0.1 using either 37% HCl or 50% NaOH. Neutralized samples were used in biomethane potential tests.

### Thermal and Thermochemical Treatment

Thermal and thermochemical pretreated samples were autoclaved at 121°C, 10 bar for 30 min and cooled to ambient

temperature (~1 h). Samples were neutralized to pH 7.2 ± 0.1 prior to their use in biomethane potential assays.

### Co-digestion of Algal Biomass With Sewage Sludge

The effect of co-digestion of algal biomass with sewage sludge was evaluated by digesting mixes of algae and sludge at different ratios. The algae-to-sewage sludge ratios are presented based on biomass volatile solids. Nine algae (A)-to-sludge (S) ratios were tested: A100%:S0%, A90%:S10%, A80%:S20%, A65%:S35%, A50%:S50%, A35%:S65%, A20%:S80%, A10%:S90%, and A0%:S100%.

### Biomethane Potential Testing and Theoretical Methane Production

The biomethane potential (BMP) test procedure was adapted from Owen et al. (1979) with minor modification as described previously (Bohutskyi et al., 2014). In all experiments, the total dose of substrate and inoculum in the final anaerobic medium equaled 2 gVS L<sup>-1</sup>. All samples were analyzed in triplicate to estimate sample mean and standard deviation. The maximum theoretical yield of methane was estimated using a modified Buswell Equation (1) (Buswell and Mueller, 1952):



where:  $x = (4c + h - 2o - 3n)/8$  and  $y = (4c - h - 2o + 3n)/4$

### Biogas and Methane Production Kinetic Models

In the current study, four kinetic models were compared for their accuracy in describing biogas and methane production dynamics including 1<sup>st</sup>-order rate (Equation 2), pseudo-parallel, 1<sup>st</sup>-order rate (Equation 3), modified Gompertz (Equation 4), and transference function (Equation 5).

$$Y_i = Y_m (1 - e^{-k t_i}) \quad (2)$$

$$Y_i = Y_{th,m} (1 - P_{bd} e^{-k_{bd} t_i} - P_{rs} e^{-k_{rs} t_i}) \quad (3)$$

$$Y_i = Y_m \exp \left( - \exp \left( \frac{K e (\lambda - t_i)}{Y_m} + 1 \right) \right) \quad (4)$$

$$Y_i = Y_m \left( 1 - \exp \left( - \frac{K (t_i - \lambda)}{Y_m} \right) \right) \quad (5)$$

where  $Y_m$  and  $Y_{th,m}$  are the ultimate and theoretical maximum cumulative biogas or methane yields (mL gVS<sup>-1</sup>), respectively;  $k$ ,  $k_{bd}$  and  $k_{rs}$  are the 1<sup>st</sup>-order rate constants (day<sup>-1</sup>) for algal biomass, readily biodegradable fractions and resistant fractions, respectively;  $P_{bd}$  and  $P_{rs}$  are the fractions of the biodegradable and resistant fractions of the algal biomass, where  $P_{rs} = 1 - P_{bd}$ ;  $K$  is the specific rate constant (mL gVS<sup>-1</sup> d<sup>-1</sup>);  $\lambda$  is the lag phase time constant (day); and  $t_i$  is incubation time (day).

Model parameters were estimated using MS Excel's built-in optimization tool (i.e., Solver) by minimizing the root mean square deviations (RMSD, see Table S1 of Supplementary Information for details). The agreement between the measured and model-predicted values was evaluated by comparing the RMSD and the coefficient of determination  $R^2$ .

### Synergistic Effects of Co-digestion

The synergy in methane yield from co-digestion of wastewater algae with sewage sludge was estimated using the following Equation 6:

$$E_{Synergy} = \frac{Y_{exper.co-digestion}}{Y_{exper.algae} \times F_{algae} + Y_{exper.SS} \times F_{SS}} 100 \quad (6)$$

where:  $Y_{exper.co-digestion}$  is the experimentally measured biogas or methane production from co-digested samples;  $Y_{exper.algae}$  and  $Y_{exper.SS}$  are experimentally measured biogas or methane production from the digestion of algae and sewage sludge as single-substrates; and  $F_{algae}$  and  $F_{sewage sludge}$  are fractions of algae and sewage sludge substrates used in the co-digestion experiment. Positive values of  $E_{synergy}$  indicate the cdigestion improved methane production whereas negative values indicate inhibition.

### Estimation of AD System Energy Balance Parameters

AD process kinetic models were used to evaluate the impact of algal biomass pretreatment and co-digestion with sewage sludge on the energy balance of a full-scale completely-mixed continuous AD system (CSTR AD). Models were constructed using the methodology described earlier (Bohutskyi et al., 2018a), with modifications accounting for additional energy input for pretreatment. Briefly, the model assesses CSTR AD performance assuming the processing of 25 wet metric tons of algal biomass per day (details in Table S1). The generated biogas was utilized onsite using a boiler and a combined heat-and-power unit (CHP). The equations used for calculation of selected energy balance parameters are the following:

$$Net\ Energy\ Output\ (NE_{Output}) = E_{Production} - E_{Input} \quad (7)$$

$$Volume-specific\ Net\ Energy\ Output\ (NE_{Output}^{mass}) = E_{Output} / m_{VS} \quad (8)$$

$$Mass-specific\ Net\ Energy\ Output\ (NE_{Output}^{volume}) = E_{Output} / V_{AD} \quad (9)$$

$$Net\ Energy\ Ratio\ (NER) = E_{Output} / E_{Input} \quad (10)$$

$$Net\ Energy\ Efficiency\ (NEE) = [E_{Output} / E_{Production}] \times 100\% \quad (11)$$

$$Net\ Energy\ Recovery\ (NERec) = [E_{Output} / HHV_{biomass}] \times 100\% \quad (12)$$

where  $E_{Production}$  is the energy produced from burning the biogas in a boiler (for heat) and CHP (for heat and electricity);  $E_{Input}$  is the input of energy (as heat and electricity) required to operate the biomass pretreatment system, AD bioreactor and CHP unit;  $m_{VS}$  is the digested feedstock in metric tons of volatile solids (organic matter);  $V_{AD}$  is the volume of AD bioreactor, m<sup>3</sup>; and  $HHV_{biomass}$  is the high heating value (calorific) of processed biomass. Energy balance parameters were calculated in watts for heat energy, electrical energy, and combined heat + electrical energy.

The AD system total energy input and production were calculated as follows:

$$E_{Input}^{Total} = E_{Input}^{Heat} + E_{Input}^{Electricity} \quad (13)$$

The heat input was estimated as heat for biomass preheating, heat losses through AD walls and heat required for the production of the chemical reagents used for pretreatment:

$$E_{Input}^{Heat} = m\gamma(T_d - T_a)/(24 \times 3,600) + k_i A_i (T_d - T_a) + d m p \quad (14)$$

where:  $m$  is the wet mass of processed biomass (ton);  $\gamma$  is the specific heat of the biomass ( $4.19 \text{ kJ kg}^{-1} \text{ }^\circ\text{C}^{-1}$ );  $T_a$  and  $T_d$  are the ambient and digestion temperatures of the biomass ( $10^\circ\text{C}$  and  $35^\circ\text{C}$ , respectively);  $k_i$  is the heat transfer coefficient ( $\text{W m}^{-2} \text{ }^\circ\text{C}^{-1}$ );  $A_i$  is the area of the digester surface (walls, bottom or cover), ( $\text{m}^2$ );  $d$  is the dose of chemical reagent (1%); and  $p$  is the power for production of chemical reagent ( $200 \text{ kW ton}^{-1}$ ) (Worrell et al., 2000; Scheme, 2010).

The electricity input was calculated as follows:

$$E_{Input}^{Electricity} = E_{Input}^{pumping} + E_{Input}^{mixing} + E_{Input}^{recycling} + E_{Input}^{other} + E_{Input}^{CHP} + E_{Input}^{reagents} \quad (15)$$

where:  $E_{Input}^{pumping}$  is the electricity for biomass pumping in and out of the digester (head losses);  $E_{Input}^{mixing}$  is the electricity for biomass mixing ( $3.8 \text{ W per m}^3$  of digester) (Lue-Hing, 1998);  $E_{Input}^{recycling}$  is the electricity for biomass circulation for heating ( $2.4 \text{ W m}^{-3}$ );  $E_{Input}^{other}$  is the other electricity consumption (e.g. lighting, automation), ( $3.6 \text{ W m}^{-3}$ );  $E_{Input}^{CHP}$  is the electricity for operation of the CHP unit ( $74 \text{ W per m}^3$  of methane) (Naegele et al., 2012); and  $E_{Input}^{reagents}$  is the electricity for production of chemical reagent ( $1,500 \text{ kW ton}^{-1}$ ) (Worrell et al., 2000; Scheme, 2010).

The AD system total energy production was calculated using the following equation:

$$E_{Production}^{Total} = E_{Production}^{Heat} + E_{Production}^{Electricity} \quad (16)$$

Both heat and electrical energy productions were calculated based on the biogas and methane production at various digester HRT times (from 3 to 45 days) using one of the models (Table 2) with the highest fitting accuracy as follows:

$$E_{Production}^{Heat} = E_{Boiler}^{Heat} + E_{CHP}^{Heat} \quad (17)$$

where:  $E_{Boiler}^{Heat}$  is the production of heat using boiler and  $E_{CHP}^{Heat}$  is the production of heat using CHP.

$$E_{Boiler}^{Heat} = 0.05 Y_{CSTR} \xi \eta_{boiler}^{heat} \quad (18)$$

$$E_{CHP}^{Heat} = 0.9 Y_{CSTR} \xi \eta_{CHP}^{heat} \quad (19)$$

$$E_{Production}^{Electricity} = 0.9 Y_{CSTR} \xi \eta_{CHP}^{electricity} \quad (20)$$

where: 0.05 and 0.9 account for 5% of the produced biogas utilized in the boiler (90% in CHP, and the remaining 5% of produced biogas is flared);  $Y_{CSTR}$  is the methane production in the CSTR AD system which was estimated using the most appropriate model (Equations 2–5), ( $\text{m}^{-3}$ );  $\xi$  is the methane lower calorific value ( $36.6 \text{ MJ m}^{-3}$ ); and  $\eta_{boiler}^{heat}$ ,  $\eta_{CHP}^{heat}$ ,  $\eta_{CHP}^{electricity}$  are the energy conversion efficiency for heat in boiler, for heat and electricity in CHP unit (85, 55, and 30%, respectively).

The biogas and methane production in the CSTR AD system for various HRTs were estimated using the most accurate kinetic model and CSTR residence time distribution by applying the segregation model described by Fogler (2016):

$$Y_{CSTR} = \int_0^\infty Y_{model}(t) E(t) dt \quad (21)$$

where:  $Y_{CSTR}$  is the estimated biogas or methane production in CSTR AD system;  $Y_{model}(t)$  is the kinetic equation of biogas or methane production (Equations 2–5); and  $E(t)$  is the residence time distribution function.

The residence time distribution function for the CSTR bioreactor (Fogler, 2016) is calculated as follows:

$$E(t) = \frac{1}{\tau} e^{-t/\tau} \quad (22)$$

where:  $\tau$  is the bioreactor hydraulic residence time (days) and  $t$  is the solid residence time for a selected portion of biomass (days).

The final equations for the estimation of the CSTR AD biogas and methane production ( $\text{mL gVS}^{-1}$ ) are shown in Equation 23 (uses analytical solution) for the 1<sup>st</sup>-order kinetic model and in Equation 24 for other kinetic models (calculated by numerical integration using trapezoidal rule). The detailed calculations are shown in Table S2 of Supplementary Information.

$$Y_{CSTR} = Y_m \int_0^\infty (1 - e^{-k t}) \frac{1}{\tau} e^{-t/\tau} dt = Y_m \frac{\tau k}{1 + \tau k} \quad (23)$$

$$Y_{CSTR} = Y_m \int_0^\infty Y_{model}(t) E(t) dt = \sum_{n=0}^{N-1} \frac{1}{2} (Y_{model,n} E_n + Y_{model,n+1} E_{n+1}) (\Delta t_n) \quad (24)$$

## Analytical Techniques

### Algal Cultivation

Temperature and pH were recorded twice per hour using a calibrated YSI 6920-V2 multi-parameter water quality sonde submerged in the sump. Insolation was estimated using the National Renewable Energy Laboratory Physical Solar Model v3 model (DHI field). DHI models solar radiation on a horizontal surface received from the sky excluding the solar disk. Soluble phosphate (filtered,  $0.45 \mu\text{m}$ ) and total phosphorus (digested) in wastewater were measured using the ammonium molybdate-antimony potassium tartrate colorimetric method based on US

**TABLE 1** | Biochemical composition of filamentous algal biomass (I-IV) and sewage sludge (S), and its effect on theoretical and observed ultimate methane yields from biomass as mono-substrates.

Parameter	Relative content in harvested algal biomass, % dw <sup>A</sup> (mean ± 1 S.D.)								
	I-a	I-b	II-a	II-b	III-a	III-b	IV-a	IV-b	S
Ash	7.8 ± 1.2	7.8 ± 0.7	9 ± 2	8.0 ± 0.3	8.0 ± 0.3	7.2 ± 0.3	8.3 ± 0.7	8.3 ± 0.1	32.9 ± 0.7
Volatile solids (VS)	92 ± 1	92.2 ± 0.7	91 ± 2	92.2 ± 0.3	92 ± 0.3	92.8 ± 0.3	91.7 ± 0.7	91.7 ± 0.1	67.1 ± 0.7
Lipids	19 ± 1	20 ± 2	19 ± 2	17 ± 1	18 ± 2	19 ± 2	17 ± 2	18 ± 1	n/a
Protein <sup>B</sup>	27.6	26.3	27.4	29.5	29.4	26.9	28.6	27.5	31.5
Carbohydrates <sup>C</sup>	45.6	45.9	44.6	45.5	44.6	46.9	46.1	46.2	n/a
Carbon	46.1	47.1	46.5	46.6	46.8	45.7	46.7	46.6	39.4
Hydrogen	7.03	7.22	7.2	7.13	7.1	6.9	7.07	7.24	5.04
Oxygen <sup>D</sup>	34.6	33.7	33.0	33.6	33.4	35.9	33.3	33.5	6.7
Nitrogen	4.42	4.21	4.38	4.72	4.71	4.31	4.58	4.4	16.1
C/N ratio	10.4	11.2	10.6	9.9	9.9	10.6	10.2	10.6	7.81
Empirical formula	C <sub>11.9</sub> H <sub>21.7</sub> O <sub>6.52</sub> N		C <sub>12.7</sub> H <sub>23.2</sub> O <sub>7.14</sub> N		C <sub>12.1</sub> H <sub>22.3</sub> O <sub>6.48</sub> N		C <sub>11.9</sub> H <sub>22.1</sub> O <sub>6.43</sub> N		C <sub>9.11</sub> H <sub>18.6</sub> O <sub>2.79</sub> N
Theoretical CH <sub>4</sub> yield <sup>E</sup> , mL gVS <sup>-1</sup>	526		523		538		535		697
Observed CH <sub>4</sub> yield <sup>F</sup> , mL gVS <sup>-1</sup>	343 ± 2		309 ± 16		306 ± 13		319 ± 1		395 ± 2
CH <sub>4</sub> yield from theoretical, %	65		59		57		60		57

<sup>A</sup>Dry weight percent; <sup>B</sup>Calculated as  $6.25 \times N$ ; <sup>C</sup>Calculated by subtracting the lipid, protein, and ash contents from the total weight; <sup>D</sup>Calculated as  $(100 - C - N - H)$ ; <sup>E</sup>Calculated using modified Buswell Equation 1; <sup>F</sup>Mean ± 1 S.D. of three replicates.

EPA Method 365.3 (Murphy and Riley, 1962). Ammonia was determined using a calibrated ion selective probe following US EPA Method 350.3 (Thomas and Booth, 1973) while nitrate (filtered, 0.45 μm) was measured using ion chromatography following US EPA method 300.0A (Pfaff, 1993). Alkalinity was determined using an acid titration following Standard Methods SM 2320B (Eaton et al., 2005).

### Algal Biomass and Pigment Analysis

To characterize the algal portion of the biomass, chlorophyll-a samples were analyzed using a modified US EPA Method 150.1 (EPA, 1991). Samples were vacuum filtered onto glass fiber filters (1 μm) before being extracted using 90% acetone buffered with magnesium carbonate. Samples were extracted in a dark freezer for 48 h prior to analysis using a Hach DR 2700™ spectrophotometer. Samples were acidified and re-analyzed with the spectrophotometer to correct for phaeophytin before calculating chlorophyll-a using equations described in EPA Method 150.1 (EPA, 1991).

To estimate total volatile solids, algal biomass was transferred to pre-weighed aluminum trays and oven-dried (105°C) until a constant mass (>24 h). Volatile solids were calculated as the dry mass change after ignition at 550°C following US EPA Method 340.2 (EPA, 1993).

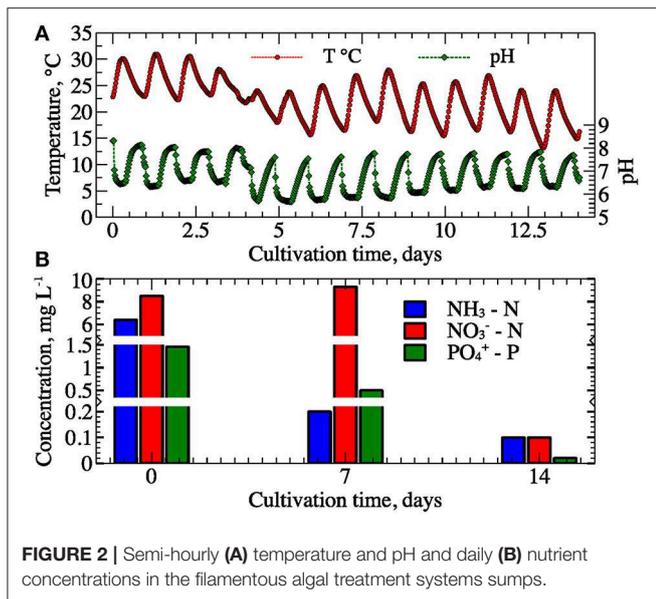
Prior to the biogas and methane potential (BMP) tests, algal biomass was assayed for total solids (TS), and volatile solids (VS) according to Standard Methods (Eaton et al., 2005). Total lipid content was assessed using the classical Bligh and Dyer method (Bligh and Dyer, 1959). The carbohydrate content was achieved by subtracting the lipid, protein, and ash contents from the total dry weight of the sample (Zhang et al., 2017a). CHN elemental composition of lyophilized algal biomass was determined by Micro Analysis, Inc. (Wilmington, DE) with a %CHN Analyzer.

## RESULTS AND DISCUSSION

### Cultivation and Composition of Filamentous Algal Biomass

Environmental conditions varied during the course of the algal growth period. Daytime light intensity averaged 100 W m<sup>-2</sup> h<sup>-1</sup> and showed a coefficient of variation of 0.6. Temperature and pH showed periodic daily fluctuations (Figure 2A). The temperature peaked in the afternoons (maximums: 24–31°C) and sagged in the mornings (minimums: 13–23°C). Unlike temperature, pH peaked during nighttime hours after carbon dioxide infusions had ceased (pH < 8). The daily minimum pH values (i.e., 5.7–6.7) were documented in the afternoon after carbon dioxide had been continuously infused for more than 6 h (~14:00). Discharges were adjusted to minimize variation among floways (129 ± 4 cm<sup>3</sup> s<sup>-1</sup>, mean ± 1SD).

Thick mats of green algae, dominated by filaments of *Ulothrix* and *Oedogonium*, developed during the 2-week growing period. Benthic diatoms were also present. Periphyton productivity ranged from 1.06 to 4.22 g VS m<sup>-2</sup> d<sup>-1</sup>. Daily algal productivity increased 62% from day 7 to 14 (paired *t*-test, *p* < 0.001) and averaged 3.7 ± 0.4 g VS m<sup>-2</sup> d<sup>-1</sup> during the experimental period. Final chlorophyll-a (corrected) in the floways ranged from 120 to 285 mg m<sup>-2</sup>. Daily chlorophyll-a (corrected) productivity averaged 16.3 ± 0.4 mg m<sup>-2</sup> d<sup>-1</sup>. The metabolic activity of the *de novo* assembled polyculture of filamentous algae was likely responsible for the near-complete removal of nutrients from the wastewater (Figure 2B). Removal rates of phosphate (0.033 g m<sup>-2</sup> d<sup>-1</sup>), nitrate (0.19 g m<sup>-2</sup> d<sup>-1</sup>), and ammonia (0.14 g m<sup>-2</sup> d<sup>-1</sup>) were low given the relatively low initial concentrations of the floways. These nutrient removal rates were comparable to the values reported for algal turf scrubber® systems (Bohutskyi et al., 2016a), but lower than the values observed in high rate algal



ponds (Park and Craggs, 2010; Gutiérrez et al., 2016) and vertical-algal-biofilm enhanced raceway ponds (Zhang et al., 2018c). In the initial portion of the experiment, nitrate concentrations increased while ammonia concentrations declined. This pattern suggests that the biofilm supported nitrifying bacteria. Similar evidence for nitrification and potentially denitrification processes have been reported for biofilm and planktonic algal-bacteria communities assembled during wastewater treatment (Bohutskyi et al., 2015b, 2018b; Zhang et al., 2018b).

The biomass productivity observed in this study was lower than the productivities reported for high-rate algal ponds (HRAP) or for algal turf scrubbers<sup>TM</sup> (ATS). Mean planktonic algal productivities in HRAPs have been reported to be 30–54 during winter and 150–200 mg Chl-a m<sup>-2</sup> d<sup>-1</sup> during summer (Sutherland et al., 2014). Productivity measured as volatile solids has been reported to be 15–25 g biomass VS m<sup>-2</sup> d<sup>-1</sup> (Park and Craggs, 2010; Gutiérrez et al., 2016). Biomass productivities observed in ATS populated by periphytic algal communities varies widely, ranging from 1 to 20 g VS m<sup>-2</sup> d<sup>-1</sup> (Kebede-Westhead et al., 2006; Mulbry et al., 2008; Bohutskyi et al., 2016a), and observed productivities are strongly regulated by nitrogen and phosphorus loading rates (Craggs et al., 1996b; Kebede-Westhead et al., 2006; Mulbry et al., 2008). The lower biomass productivity observed in this study may be partially explained by the fact that the experiments were started *de novo* and, thus, had no previously established algae to support prolific production of biomass. Additionally, the high productivities observed in previous studies were obtained in single pass ATS that processed wastewater containing elevated concentrations of nitrogen and phosphorus (Craggs et al., 1996a,b). In this study, the recirculating ATS was operated as a batch reactor where nutrient concentrations declined over time, providing >98.5% removal of nitrogen and phosphorus (Figure 2B). Indeed, similar biomass productivities have been reported when

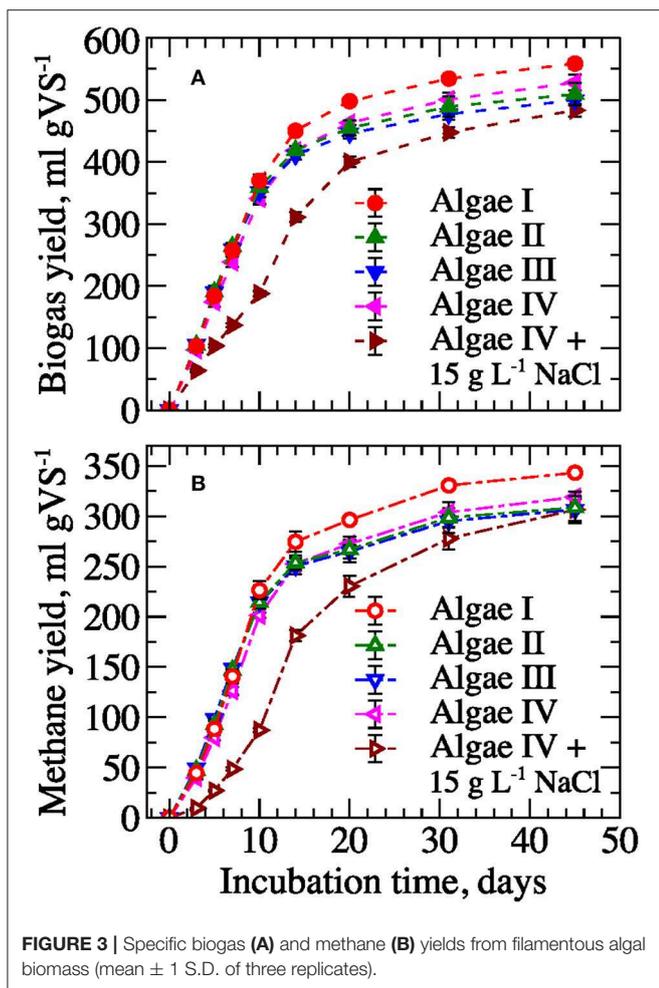
using comparable nutrient loads in previous investigations (Keller and Husted, 2015).

The biochemical composition of harvested algal biomass and sewage sludge (SS) showed relatively low variability among samples (Table 1). These results suggest that there existed high stability and consistency of the periphyton community composition. Volatile solids (i.e., organic fraction) represented more than 90% of the dry mass, indicating that the periphyton has high potential for conversion into biofuels or bioproducts. The ~8% ash content is considerably lower than the >30% ash content reported for periphyton biomass cultivated in a filamentous algal treatment system dominated by *Rhizoclonium* and *Cladophora* (Ehimen et al., 2013; Bohutskyi et al., 2016a). While carbohydrates were not measured directly, they likely represented the major biochemical constituent in the periphyton biomass (~40%). If true, anaerobic digestion appears to represent a viable option for the conversion of this low-cost wastewater-grown algae into bio-methane. In contrast, crude lipids composed <20% of the dry mass, and thus this biomass had low applicability as a feedstock for biodiesel production.

## Biodegradability and Methane Yield From Filamentous Algal Biomass and Sewage Sludge as Single-Substrates

To evaluate digestibility, algal biomass from different flowways was assessed for biogas and methane production (Figure 3). The consistent composition of the biomass resulted in relatively similar ultimate biogas and methane yields among samples. Yields varied only ~12% with maximum values of 559 ± 8 and 343 ± 2 mL gVS<sup>-1</sup> for biogas and methane yield, respectively. More than 85% of the gas produced was generated during the first 20 days of incubation and reflected the high biodegradability of the harvested algae. Indeed, the yields observed in this study are significantly higher than the methane yield of about 160–200 mL gVS<sup>-1</sup> reported earlier for periphyton biomass, represented mostly by green filamentous alga *Rhizoclonium* (Ehimen et al., 2013), *Hydrodictyon reticulatum* (Lee et al., 2014) or polyculture of *Cladophora* sp., *Rhizoclonium* sp., *Melosira* sp., *Hydrodictyon* sp., and *Spirogyra* sp. (Bohutskyi et al., 2016a). However, similarly high methane yields (350–480 mL gVS<sup>-1</sup>) have been reported for batch anaerobic digestion of mix of *Ulva*, *Cladophora*, and *Chaetomorpha* (Hansson, 1983). It appears that the algal species diversity and their associated biochemical composition strongly influence biomass biodegradability and methane production, which can vary more than 2-fold.

Observed methane yields ranged from 57 to 65% of their theoretical maximums (Table 1), with the highest yield for sample I. The superior biodegradability of this sample may be related to its slightly higher lipid and lower protein contents. This result is consistent with earlier studies showing that lipids have a high energy density, and their digestion yields more methane compared to proteins or carbohydrates (Bohutskyi and Bouwer, 2013; Bohutskyi et al., 2015a, 2016a). Other studies have confirmed that proteins contribute to the recalcitrant fraction in complex biomass (Bougrier et al., 2007; Park and Li, 2012). The fact that nearly 40% of algal biomass is not bioprocessed

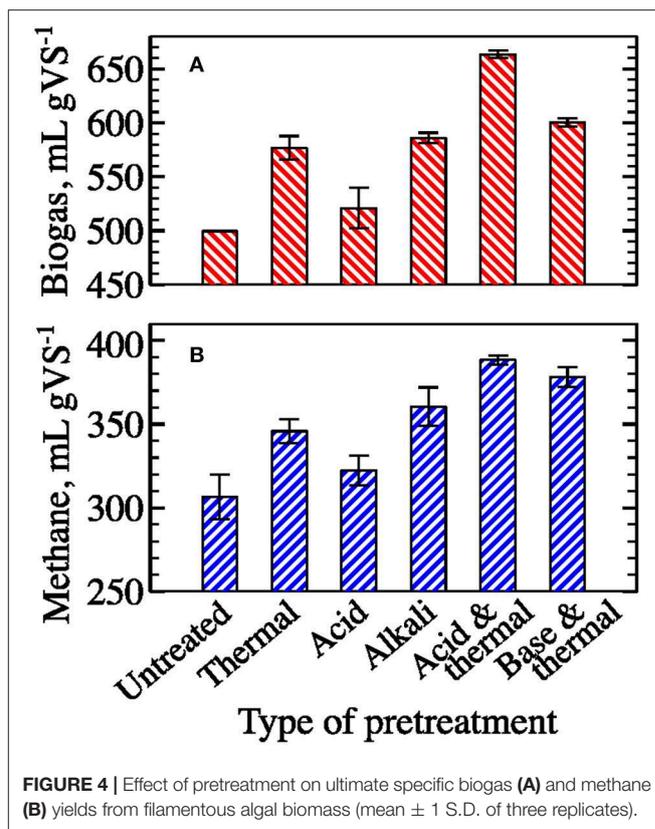


into methane (theoretical vs. observed yields) indicates that pretreatment of the biomass has the potential to improve gas production during anaerobic digestion.

### Pretreatment of Filamentous Algal Biomass to Improve Methane Yield

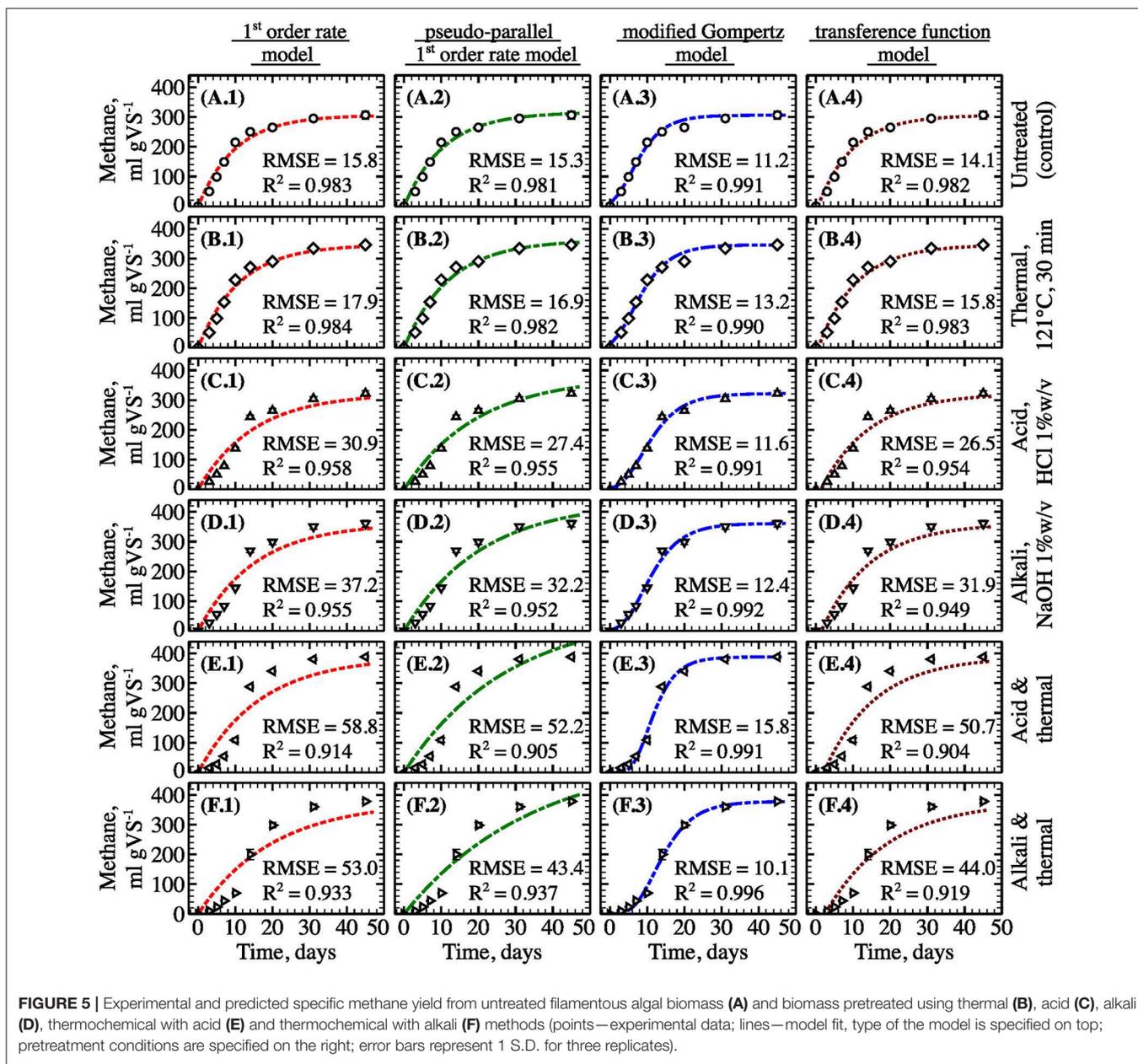
While initial AD tests indicated that this wastewater-grown filamentous algae biomass was well-suited for anaerobic digestion, the methane production was still limited to  $\sim$ 60% of its theoretical maximum. Therefore, various biomass pretreatment methods including thermal, dilute acid, dilute alkali, combined acid and thermal, and combined alkali and thermal were assessed for their potential to enhance methane ultimate yield and production rate.

Dilute acid pretreatment resulted in no statistically significant change in ultimate gas yield ( $\sim$ 5% increase after 45 days of digestion, **Figure 4**). In contrast, biomass pretreatment with dilute alkali improved ultimate gas yield by about 17%. Comparable improvement of nearly 15% was observed after thermal pretreatment without chemicals. However, combined thermal and chemical pretreatments showed the greatest boost in methane yields—23 and 27% for acid and alkali, respectively.



To quantify the effect of pretreatment methane and biogas production rates as well as to identify the most appropriate kinetic model, the biogas and methane data were fit using 1<sup>st</sup>-order rate, pseudo-parallel 1<sup>st</sup>-order rate, modified Gompertz, and transference function models (**Figure 5** and **Figure S1**, respectively). While all assessed methane and biogas production models demonstrated strong agreement with experimental data for untreated biomass ( $R^2 > 0.981$  for all), the modified Gompertz model provided a slightly better fit for biogas production across pretreatments ( $R^2 > 0.988$  for all). Kinetic coefficients for all assayed models are summarized in **Table 2**. Based on the best performing Gompertz model, the thermochemical pretreatment with acid had the strongest favorable effect on methane production rate by boosting the specific rate constant by almost 50%. However, both thermochemical pretreatments delayed the onset of biogas and methane production (from 0.8 to  $>6$  days according to the modified Gompertz model, **Table 2**). Relative to the untreated control, all types of pretreatment led to increased gas yield after day 31 of incubation; however, the initial gas production (during the first 15 days) dropped by 20–80% for all pretreatments except thermal (**Figure 6**).

The lag-phase (i.e., delay in gas production) was nearly two times longer for methane compared to biogas production (**Table 2**; **Figure 6**). Methanogenic archaea are typically found to be the most sensitive members of the anaerobic microbial community involved in the AD process (Garcia et al., 2000; Hori et al., 2006). Also, biomass treated with alkali and



alkali-assisted thermal pretreatments showed longer lag-phase or greater inhibition than acid and acid-assisted thermal pretreatments, respectively. Sodium cations could be responsible for the observed time delays. The concentration of  $\text{Na}^+$  ( $\sim 0.25$  M) added into the alkali pretreated samples was  $\sim 20\%$  higher than the  $\text{Na}^+$  added to the acid pretreated samples for pH neutralization ( $\sim 0.2$  M). Similar  $\text{Na}^+$  concentrations have been reported to inhibit methanogenic ruminal and AD cultures (Zhou et al., 2011; Zhang et al., 2017b). In addition, comparable inhibition in gas production was also observed in the control sample supplied with  $15\text{ g L}^{-1}$  of  $\text{NaCl}$  or  $\sim 0.25$  M of  $\text{Na}^+$  (see Figure 3), which provided additional

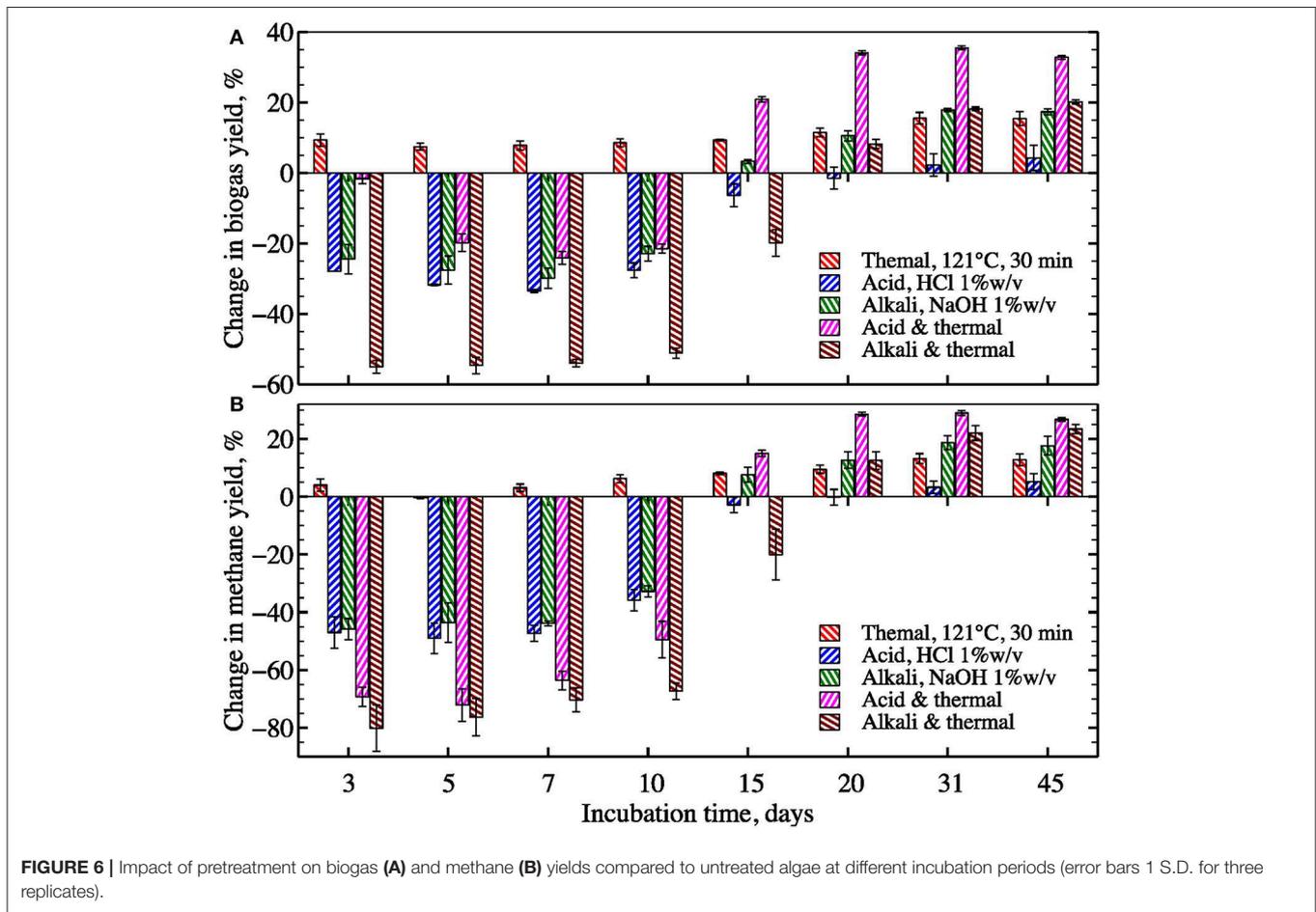
evidence supporting the hypothesis that  $\text{Na}^+$  was responsible for methanogenesis inhibition. Sodium inhibition can be addressed through adaptation of the methanogenic community to high  $\text{Na}^+$  content or through an application of alternative alkali reagents such as  $\text{KOH}$ ,  $\text{Ca}(\text{OH})_2$ , and  $\text{Mg}(\text{OH})_2$ . However, a systematic investigation would be required to identify the optimal alkali type and pretreatment conditions, since they have been reported to be substrate- and reagent-specific (Neyens, 2003; Monlau et al., 2012; Zhang et al., 2018a).

Sodium toxicity cannot be the only explanation for the methane inhibition observed in these experiments because the lag-phase after thermochemical pretreatment with  $\text{NaOH}$  was

**TABLE 2** | Effect of algae pretreatment on biogas and methane production kinetic parameters.

Sample and type of pretreatment	1 <sup>st</sup> -order rate model	Pseudo-parallel 1 <sup>st</sup> -order rate model			Modified Gompertz model		Transference function model	
	k, d <sup>-1</sup>	k <sub>1</sub> , d <sup>-1</sup>	k <sub>2</sub> , d <sup>-1</sup>	P <sub>biodeg</sub>	k, mL gVS <sup>-1</sup> d <sup>-1</sup>	λ, d	k, mL gVS <sup>-1</sup> d <sup>-1</sup>	λ, d
<b>BIOGAS PRODUCTION</b>								
Untreated	0.107	0.104	0.0	0.533	36.3	0.08	55.6	0.30
Thermal <sup>A</sup>	0.097	0.093	0.0	0.619	37.6	0	58.0	0.29
Acid <sup>B</sup>	0.074	0.064	0.0	0.596	28.7	0.92	41.8	0.75
Alkali <sup>C</sup>	0.072	0.058	0.0	0.698	32.3	1.24	46.0	0.87
Acid + thermal <sup>D</sup>	0.074	0.057	0.0	0.803	38.9	1.7	53.5	0.93
Alkali + thermal	0.055	0.032	0.0	0.883	30.3	3.43	37.6	1.65
<b>METHANE PRODUCTION</b>								
Untreated	0.100	0.094	0.0	0.587	23.3	0.83	32.7	0.53
Thermal	0.091	0.083	0.0	0.672	23.8	0.82	33.8	0.6
Acid	0.067	0.051	0.0	0.704	20.9	2.97	24.8	1.38
Alkali	0.066	0.048	0.0	0.808	23.8	3.26	27.6	1.48
Acid + thermal	0.060	0.036	0.0	1.000	34.5	6.04	28.4	2.13
Alkali + thermal	0.050	0.029	0.0	1.000	24.6	6.29	22.8	2.49

<sup>A</sup>121°C, 30 min; <sup>B</sup>HCl 1%w/v; <sup>C</sup>NaOH 1%w/v; <sup>D</sup>Combined treatment.

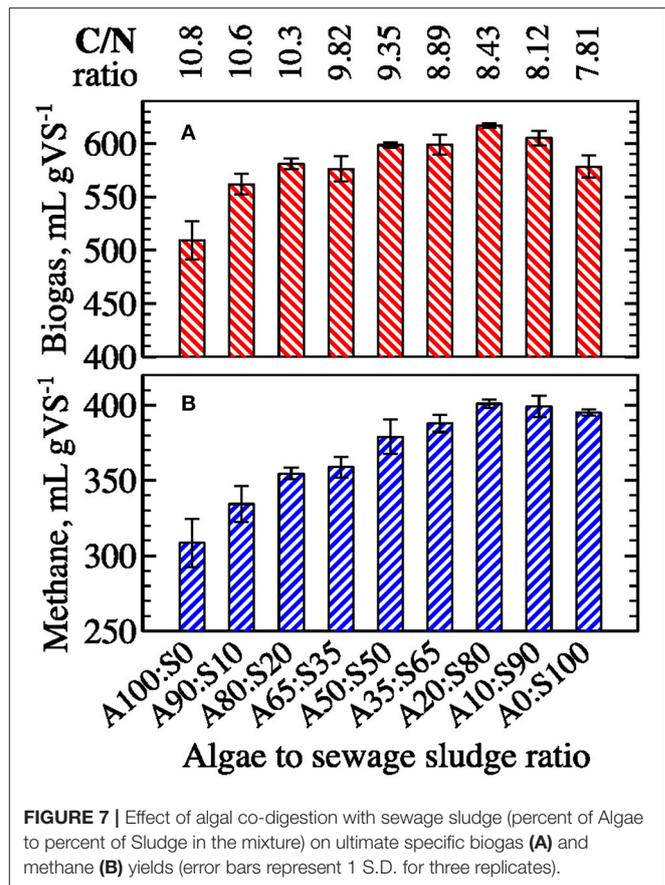


nearly 2-fold longer than the NaOH-treated samples without thermal pretreatment. One additional contributing factor could be that the inhibition of methanogens by pretreatment generated fatty acids that cause a reduction in pH. Short and long chain fatty acids, as well as soluble proteins and sugars (that can be promptly fermented to fatty acids by acidogenic anaerobic bacteria), are abundant products of biomass thermochemical hydrolysis (Samson and Leduy, 1983; Wilson and Novak, 2009; Fdez.-Güelfo et al., 2011). In addition, high temperature can promote conversion of readily biodegradable compounds into refractory and even toxic products, but this transformation only becomes significant at temperatures above 175°C (Stuckey and Mccarty, 1984).

### Co-digestion Algal Biomass With Sewage Sludge

To evaluate the efficacy of co-digestion as an alternative to pretreatments for improving AD processes, biogas and methane yields were measured for samples of algal biomass mixed with different proportions of sewage sludge (Figure 7). Compared to filamentous algae, sewage sludge has a higher energy density and theoretical and observed methane production when digested as a single-substrate (Table 1). Sludge addition boosted biogas and methane production when mixed with filamentous algae biomass. Final gas yields increased concomitantly with increasing sewage sludge fraction and peaked at a ratio of A20%:S80% (i.e.,  $617 \pm 2$  mL biogas per  $\text{gVS}^{-1}$  and  $401 \pm 3$  mL methane per  $\text{gVS}^{-1}$ ). The maximum yield at A20%:S80% mix indicates the presence of synergistic effects on gas production from co-digestion of algal biomass with sewage sludge (Figure 8). Interestingly, the mixtures containing 50–20% of algae with 50–80% of sewage sludge showed the greatest synergy during the initial period of fermentation (i.e., 3–10 days). In contrast, the mixtures of 90–80% of algae with 10–20% of sewage sludge had negative synergies during the first week. However, the synergy values equalized after ~15 days of digestion to 7–12% for biogas and to 3–8% for methane for all algae-to-sewage sludge ratios. These results are consistent with other published studies that have reported relatively small improvements in methane production associated with co-digestion of algal biomass with food waste or cellulose (Zhen et al., 2016; Bohutskyi et al., 2018a). Mixing algae with sludge in the AD slightly elevated the carbon-to-nitrogen ratio (C/N) and somewhat shifted it toward the optimal C/N ratios of 20–30 (Yen and Brune, 2007; Calicioglu and Demirer, 2017). While an improvement in the C/N ratio may have contributed to the observed synergy in gas production (Figures 7, 8), this shift in C/N was likely too minor to explain the synergies measured. Characterizing the fundamental mechanisms that cause synergistic effects of co-digestion is an important avenue of future research.

To identify the most appropriate kinetics model, methane and biogas production over time were analyzed using the four models described previously (Figure 9 and Figure S2). In general, all models showed a relatively tight fit to the observed data ( $R^2 \geq 0.941$  for all). The modified Gompertz model provided a slightly better fit for samples with a high fraction of algal biomass (e.g.,

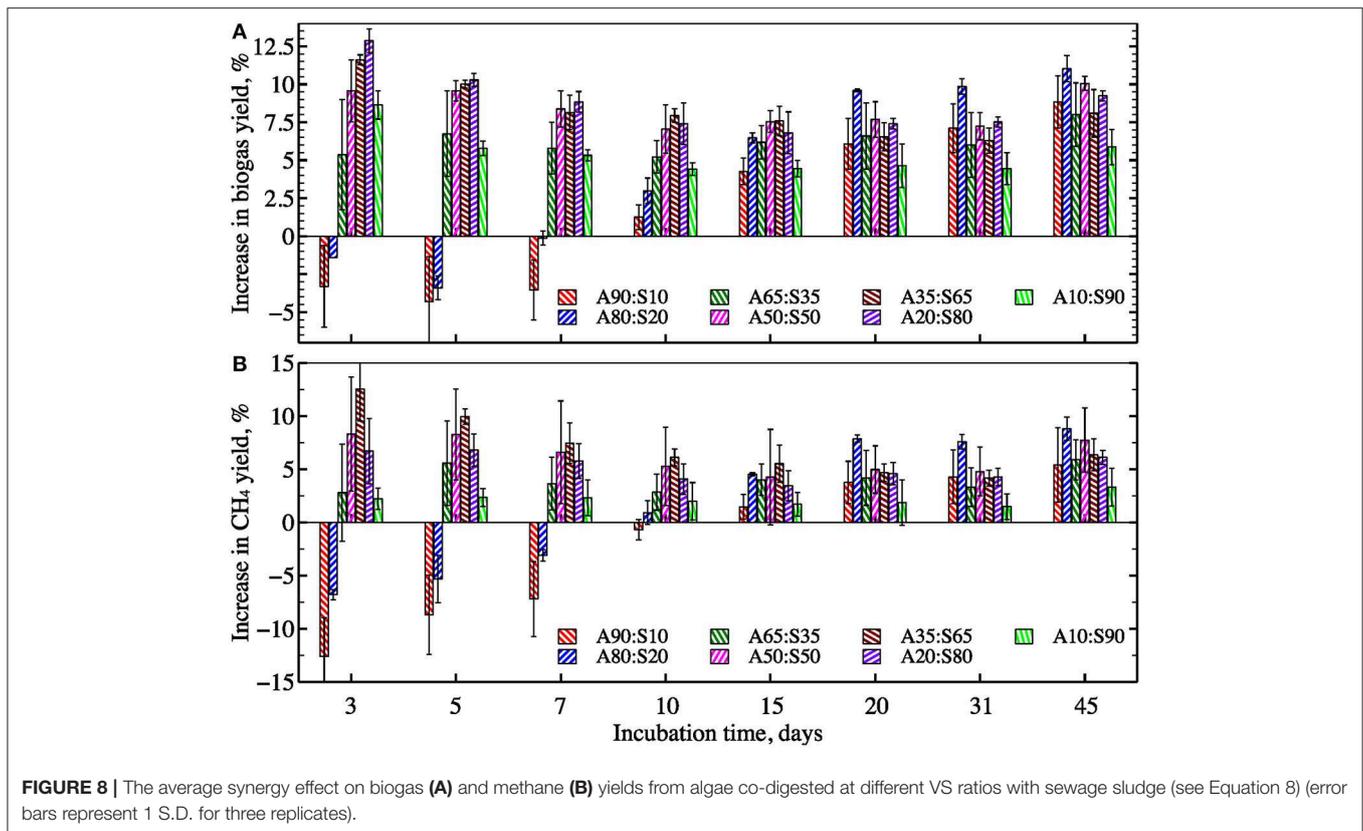


**FIGURE 7** | Effect of algal co-digestion with sewage sludge (percent of Algae to percent of Sludge in the mixture) on ultimate specific biogas (A) and methane (B) yields (error bars represent 1 S.D. for three replicates).

80–100%). In contrast, the other models described experimental data better for sewage sludge as single-substrate and samples with fractions of algae from 10 to 50%. The kinetic coefficients for all assayed models are summarized in Table 3. Generally, models showed that gas production rate constants increased concomitantly with increasing sewage sludge fraction in the feedstock mixture. However, based on the pseudo-parallel 1<sup>st</sup>-order rate model estimation, the biodegradable fraction reached a maximum value at A80%:S20%, indicating that co-digestion improved biomass biodegradation in the mix. This result could be partially explained by the increased diversity and enhanced enzymatic machinery of the AD microbial community. These microbes may best utilize the complex substrates associated with the combination of algae and sludge biomass. Indeed, studies have demonstrated a strong connection between the substrate composition, matrix complexity, C/N ratio, and microbial community structure and lytic activity (Hernández and Hobbie, 2010; Regueiro et al., 2012; Ziganshin et al., 2013; Li et al., 2015).

### Effect of Pretreatment vs. Co-digestion on AD System Energy Balance

While pretreatment and co-digestion enhanced ultimate methane yields, their implementation requires additional energy inputs for AD system operation. The magnitude of this additional energy requirement could exceed the relatively moderate gain in



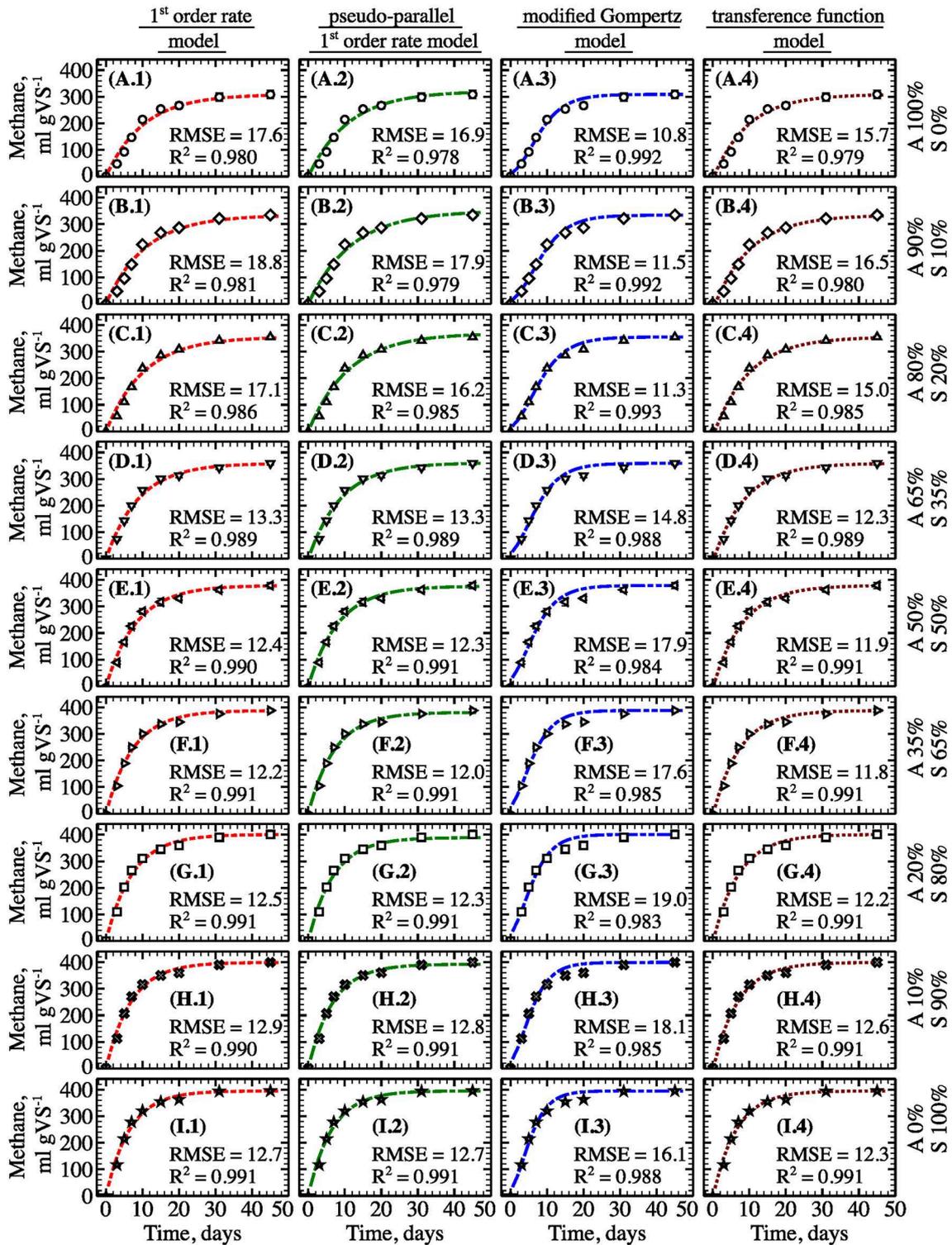
energy production due to the enhanced methane yields. Thus, to more fully compare pretreatment and co-digestion, these options were evaluated in terms of their effect on the energy balance parameters assuming an operational scale AD system that processes 25 wet metric tons per day of wastewater-grown filamentous algal biomass (other major parameters of assessed AD system are shown in **Table S3**).

### Effect of Algae Pretreatment on Energy Balance

Generally speaking, all algal pretreatment techniques impaired energy balance (**Figure 10**). The heat energy output increased slightly only for alkali-pretreated algal biomass (up to 10%) at long HRTs above 30 days (**Figure 10A.1**). All other pretreatments resulted in reduced heat energy outputs compared to untreated algae. These results can be attributed to the high heat requirements for thermal pretreatments and the negative effects of thermochemical pretreatments on methane yield during the initial 20 days of fermentation (**Figure 6**). The highest electrical energy output was observed for thermally pretreated biomass that showed a 5–10% improvement over the untreated algal biomass controls (**Figure 10A.2**). In contrast, chemical and thermochemical treatments reduced electrical energy output by up to 20–50%. Such dramatic reductions were caused by the high amount of electrical energy required for the production of alkali and acid using industrial electrolysis (Worrell et al., 2000; Scheme, 2010). The trends for total energy output (i.e., combined heat and electricity, **Figure 10A.3**) were somewhat

consistent with the results for heat energy. However, none of the pretreatments tested improved total energy output compared to untreated algal biomass despite improvements in ultimate methane production (**Figure 4**). Similar findings were observed for volume-specific (**Figure 10B**) and mass-specific (**Figure 10C**) energy outputs. All pretreatment strategies caused a reduction in specific energy outputs compared to untreated biomass. Total volume- and mass-specific energy outputs from untreated algae reached maximum values of  $0.25 \text{ MW m}^{-3}$  at an HRT of 10 days (**Figure 10B.3**) and  $1.5 \text{ MW ton VS}^{-1}$  at an HRT of 45 days (**Figure 10C.3**), respectively. The highest energy outputs from pretreated algae were only  $0.16 \text{ MW m}^{-3}$  (thermal and alkali treatment, HRT 14 days) and  $1.3\text{--}1.45 \text{ MW ton VS}^{-1}$  (same, HRT 45 days).

The highest Net Energy Ratio (NER) and Net Energy Efficiency (NEE) for combined heat and electricity were observed for untreated algal biomass, which achieved maximum values of 2.8 and 73% at HRT of 45 days, respectively (**Figures 10D.3,E.3**). NER and NEE for thermally and chemically pretreated biomass were under 1.8 and 65%, respectively. Moreover, NER for thermochemically pretreated biomass dropped below 1. Similarly, the highest NERec of up to 26% was observed for untreated algae compared to 25% for alkali treatment, 22% for thermal, and 20% or less for other methods. It is worth noting that these estimates indicate that only about a quarter of energy in the algal biomass higher heating value (HHV) was recovered as usable heat and electricity in the best-case scenario.



**FIGURE 9** | Experimental and predicted specific methane yield from filamentous algal biomass as a single-substrate (A), algae co-digested in different ratios with sewage sludge (B–H) and sewage sludge as a single-substrate (I) (points—experimental data; lines—model fit, type of the model is specified on top; algae (“A”) to sewage sludge (“S”) ratio are shown on the right; error bars represent 1 S.D. for three replicates).

**TABLE 3** | Effect of algae co-digestion with sewage sludge on biogas and methane production kinetic parameters.

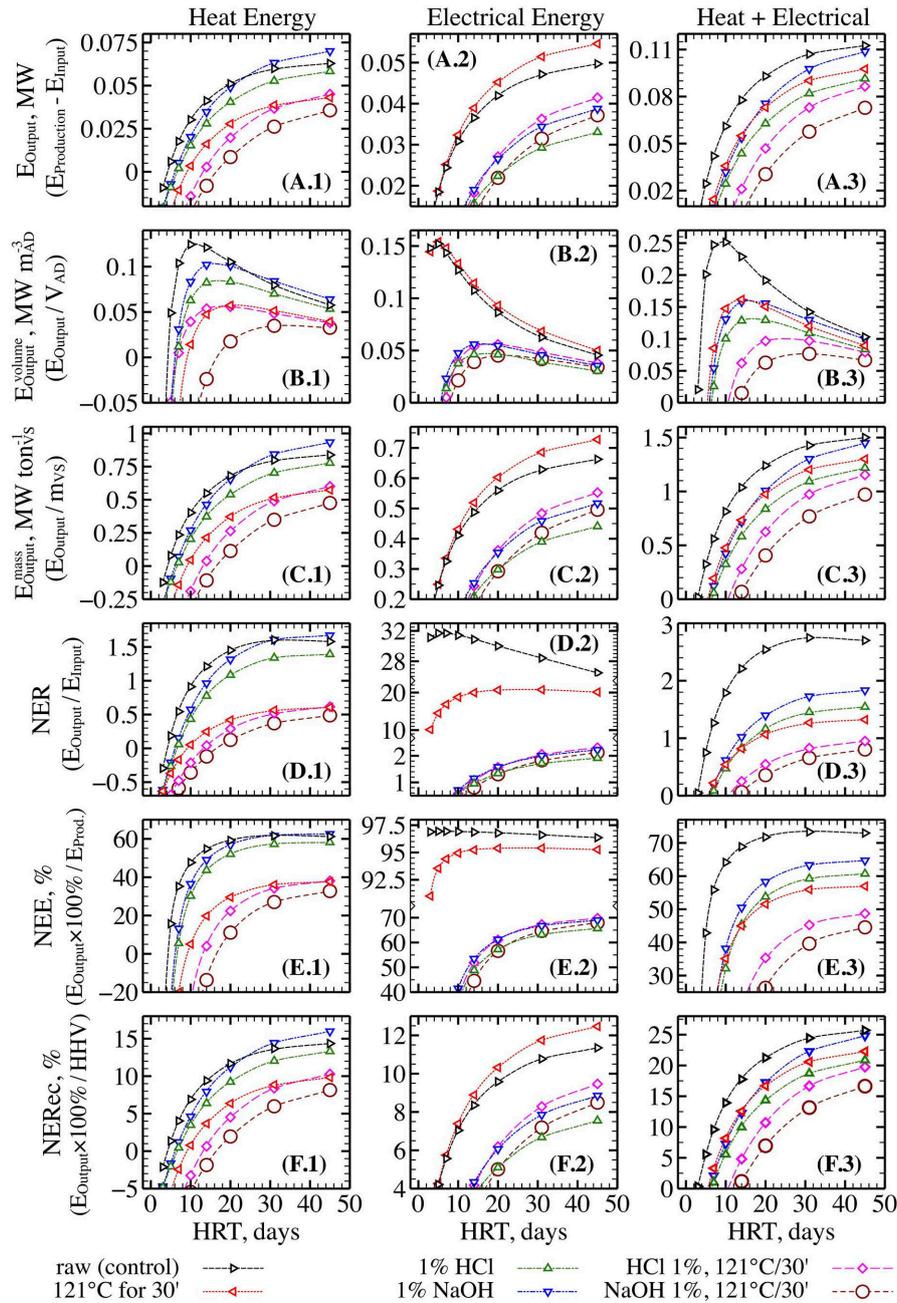
Sample	1 <sup>st</sup> -order rate model	Pseudo-parallel 1 <sup>st</sup> -order rate model			Modified Gompertz model		Transference function model	
	k, d <sup>-1</sup>	k <sub>1</sub> , d <sup>-1</sup>	k <sub>2</sub> , d <sup>-1</sup>	P <sub>biodeg</sub>	k, mL gVS <sup>-1</sup> d <sup>-1</sup>	λ, d	k, mL gVS <sup>-1</sup> d <sup>-1</sup>	λ, d
<b>BIOGAS PRODUCTION</b>								
A100:S0	0.106	0.102	0.0	0.55	37.2	0.16	56.6	0.33
A90:S10	0.097	0.092	0.0	0.60	37.3	0.12	57.1	0.37
A80:S20	0.101	0.096	0.0	0.62	40.0	0.13	61.3	0.36
A65:S35	0.116	0.115	0.0	0.58	44.8	0.00	68.7	0.23
A50:S50	0.121	0.123	0.0	0.59	48.9	0.00	74.4	0.17
A35:S65	0.133	0.134	0.0	0.57	53.7	0.05	81.6	0.17
A20:S80	0.137	0.141	0.0	0.57	56.5	0.00	86.2	0.14
A10:S90	0.141	0.143	0.0	0.56	58.2	0.11	87.7	0.16
A0:S100	0.148	0.147	0.0	0.53	60.4	0.33	88.3	0.19
<b>METHANE PRODUCTION</b>								
A100:S0	0.098	0.091	0.0	0.61	23.9	1.05	32.7	0.60
A90:S10	0.093	0.085	0.0	0.65	24.3	1.06	33.6	0.65
A80:S20	0.097	0.090	0.0	0.66	25.8	0.77	36.8	0.55
A65:S35	0.112	0.111	0.0	0.62	28.2	0.25	42.0	0.31
A50:S50	0.120	0.122	0.0	0.62	30.7	0.00	47.0	0.19
A35:S65	0.135	0.139	0.0	0.60	35.0	0.00	53.6	0.15
A20:S80	0.139	0.146	0.0	0.59	37.2	0.00	57.0	0.13
A10:S90	0.145	0.149	0.0	0.58	38.8	0.06	59.0	0.14
A0:S100	0.155	0.155	0.0	0.57	41.5	0.17	62.6	0.14

### Effect of Co-digestion With Sewage Sludge on Energy Balance

The energy balance modeled for an AD system processing filamentous algal biomass co-digested with sewage sludge is shown in **Figure 11**. The algal biomass, as a sole substrate, was found to be less advantageous than sewage sludge (SS) alone. Sludge is substantially richer energetically than algae, with theoretical and observed methane yields (~30% higher, **Table 1**). As a consequence, the total energy and volume-specific/mass-specific energy outputs from SS as a single-substrate were nearly 1.5- to 2-fold greater than from algae as a single-substrate (**Figures 11A–C**). This finding is true even though the same amount of biomass was fed into the AD system in terms of volatile solids (VS). Co-digestion of algal biomass with sewage sludge boosted the total energy output from 0.11 MW (algae only) up to 1.7 MW at algae-to-SS ratios of 10% to 90%. However, in this case, the fixed amount of algal biomass was complemented with an increasing amount of sludge in order to raise the sludge fraction in the mix from 0 to 90%. Therefore, the gain in energy output was mostly due to the addition of more substrate into the digester. In contrast, the volume-specific energy output reached 0.53 MW m<sup>-3</sup> when SS was 80% of the mix and remained nearly constant when the sludge fraction was increased to 90% (0.54 MW m<sup>-3</sup>) and 100% SS (0.56 MW m<sup>-3</sup>, **Figure 11B**). Moreover, the mass-specific energy outputs at 80 and 90% SS were slightly higher than the output from digestion of 100% SS (**Figure 11C**). The favorable effects of co-digestion were even more prominent on NER and NEE, with highest values

of 4.3 and 81%, respectively, observed at algae-to-sludge ratios of 20%:80% and 10%:90% (**Figures 11D,E**). At these ratios, the NERec reached ~39%, which compares favorably to digesting SS as a single-substrate (33%, **Figure 11F**).

The data presented in **Figures 10, 11** underscore the importance of energy balance analysis when comparing approaches for biomass pretreatment and co-digestion. A similar conclusion on the significance of energetic and economic evaluation of pretreatment technologies was reported for lignocellulosic biomass (Croce et al., 2016). While pretreatment methods enhanced ultimate methane yields by 5–27% (**Figure 4**), they had negative effects on AD system energy balance parameters. Such unexpected results may be explained in part by the following factors. First, the application of thermal pretreatment and chemicals, such as NaOH and HCl, substantially increase the energy input required for operation of the AD system. These energy requirements are the result of a direct demand for heat energy and for indirect energy required for the production of the pretreatment reagents. Both NaOH and HCl are typically synthesized industrially using energy-intensive brine electrolysis (Worrell et al., 2000; Scheme, 2010). Second, reagents, such as sodium, can cause inhibition of methanogenesis and extend the lag-phase in methane production (**Figure 3**). Finally, the methane yield from untreated algal biomass (300–340 mL gVS<sup>-1</sup>) observed in this study is higher than the yields reported for wastewater-grown algal biomass (130–250 mL gVS<sup>-1</sup>) in other studies (Kinnunen et al., 2014; Passos et al., 2014; Wang and Park, 2015; Bohutskyi et al., 2016a). Pretreatment of



**FIGURE 10 |** Effect of different types of pretreatment on AD system energy balance parameters including energy output (A), reactor volume-specific energy output (B), feedstock mass-specific energy output (C), Net Energy Ratio (D), Net Energy Efficiency (E), and Net Energy Recovery (F) (heat energy left panels, electrical energy central panels, and combined heat and electrical energy right panels).

poorly biodegradable algal biomass typically leads to a 60–70% gain in methane production (Passos et al., 2013, 2014, 2015) and, therefore, may result in larger relative improvements in energy balance parameters. On the other hand, the examination of the energy balance demonstrates how relatively small synergistic effects observed for methane yield as the result of co-digestion (Figure 7) are translated into relatively substantial advancement of the AD system energy efficiency parameters.

Counterintuitively, the addition of less energy-dense algal biomass to sewage sludge did not compromise energy production and other energy balance parameters, rather it led to enhanced Net Energy Ratio, Efficiency, and Recovery in a scaled AD system. These findings highlight that co-digestion may be a more advantageous approach than chemical, thermal or thermochemical pretreatments for improving AD system energy balance when processing relatively biodegradable biomass.

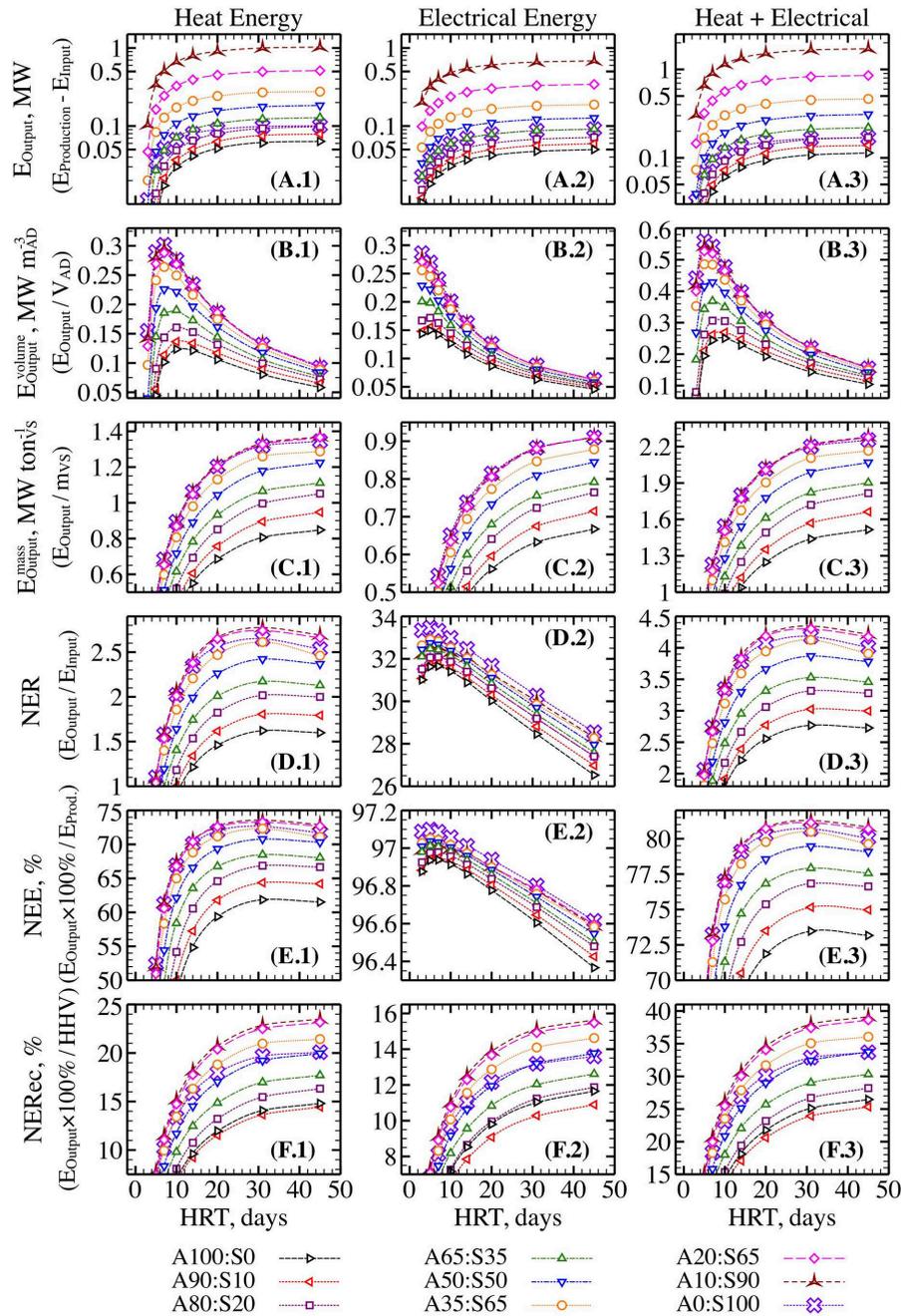


FIGURE 11 | (A–F) Effect of algae to sewage sludge co-digestion ratio on AD system energy balance.

### CONCLUSIONS

Natural polycultures of benthic algae can be utilized to remove wastewater nutrients and their resulting biomass can serve as an affordable feedstock for bioenergy. While biomass pretreatment methods and co-digestion are often suggested as an effective means for improving algal biodegradation for enhanced biomethane production, they should be applied with caution

due to their high requirement for additional energy inputs. The current study demonstrates the importance of evaluating the energetic ramifications of pretreatment biomass prior to anaerobic digestion. The results show that in some cases thermal, chemical, and thermochemical pretreatments may have either negative or no effects on energy balance despite enhancing the ultimate methane production from algal biomass. In contrast, co-digestion of algal biomass with sewage sludge (or other

waste biomass) merits additional attention as a potentially viable method for improving the methane yields from algal biomass anaerobic digestion. Synergistic effects from co-digestion on methane production can lead to relatively small improvements in ultimate methane production but still result in substantially improved energy balance parameters when modeled for full-scale anaerobic digestion systems.

## AUTHOR CONTRIBUTIONS

PB and TK obtained funding, developed the concepts, and designed the experiments. TK, DP, MP, ML, and LR collected and assembled the data. PB, TK, and AK analyzed and interpreted the data. PB and TK drafted and revised the article to improve its important intellectual content.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenrg.2019.00047/full#supplementary-material>

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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