Evaluating Anaerobic Digestion as Means to Produce Energy in Algae Farming and Integrated Wastewater Treatment Systems

A Thesis

Presented to

the faculty of the School of Engineering and Applied Science

University of Virginia

in partial fulfillment

of the requirements for the degree

Master of Science

by

Shanshan Peng

May

2014

APPROVAL SHEET

The thesis

is submitted in partial fulfillment of the requirements

for the degree of

Master of Science

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May

2014

Abstract

The production of algae-derived energy via anaerobic digestion has been a topic of growing interest. In the past few years, there have been many studies investigating the digestibility and methane yield of various algae substrates; however, these have not yet been integrated into life cycle assessment (LCA)-based evaluation of hypothetical algae-to-energy systems. This study seeks to revise previous LCA assessments of energy return on investment (EROI) for two energy-producing algae systems: 1) "pure" digestion at large-scale algae farms, and 2) co-digestion of algae and wastewater biosolids and municipal wastewater treatment plants (WWTPs). This is achieved via literature review of recent studies, followed by experimental measurement of digestion parameters for pure cultures of *Scenedesmus dimorphus* and mixed WWTP-grown algae. Resulting estimates for methane yield are then incorporated into LCA frameworks for both systems of interest. Results for the "pure" algae digestion evaluation indicate that revised digestion parameters increase the estimate of EROI from 1.75 to 3.29. Similarly, incorporation of algae co-digestion at a WWTP increases the EROI of a WWTP from 0.30 (without algae cultivation) to 0.35 (with algae cultivation). Together, these results underscore the potential for large-scale algae-based energy production.

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1. Introduction

There is growing interest in algae-derived bioenergy sources, in part because algae do not compete with food crops for arable land or fresh water consumption, and they also produce more biomass per area than terrestrial crops (Chisti, 2007). Biofuels produced from algae strains possessing high lipid contents are considered promising alternatives to fossil fuels; however, the energy intensity of drying the algae biomass, extracting the lipids, and converting them into fuels are significant obstacles to implementation of large-scale liquid fuel production from microalgae (Singh et al., 2012). These difficulties could potentially be overcome by using anaerobic digestion of algae biomass to produce methane, which can then be converted into electricity. This is a theoretically feasible means of producing energy from algae (Sialve et al., 2009).

The efficiency and favorability of anaerobic digestion for production of energy from algae has been evaluated via both life cycle assessment (LCA) and experimental studies. Clarens et al. (2011) evaluated production of energy sources for transportation via four different conversion approaches and concluded anaerobic digestion pathway has a higher EROI (energy return on investment) than biodiesel production coupled with anaerobic digestion of nonlipid residues, but that digestion is not as efficient as direct combustion. A key conclusion of Clarens (2011) was that the *algae digestibility* and *methane yield* parameters should be studied in more depth; based on both the lack of reliable data at the time their study was conducted, and sensitivity analyses that indicated that these models were extremely impactful on the model's overall output. In a related, later study, results from a life cycle costing analysis utilizing the same underlying model structure reaffirmed the importance of anaerobic digestion parameters on overall EROI and economic favorability (Resurreccion et al., 2012). Figure 1 depicts some of the sensitivity analysis results of the life cycle costing model, using a so-called tornado plot modified from Resurreccion et al. (2012). From this figure, the two model parameters which are most impactful on the overall profitability of a hypothetical algae-to-energy system are "digestion efficiency" and "methane production efficiency".

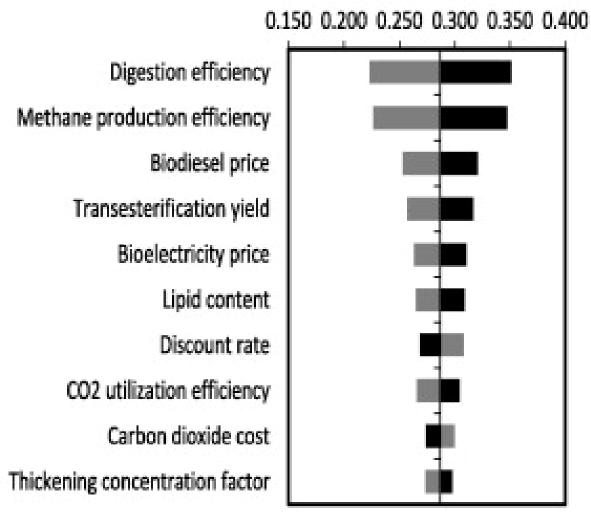

Figure 1. A tornado plots shows the sensitivity of model output (here *profitability index - PI)*, where PI values greater than 1 are indicative of economic viability) **to individual model inputs** (at left). The center line reflects baseline PI of the hypothetical open pond algae system when all model inputs are set to their average value. The width of each bar reflects the increase (right) or decrease (left) in PI associated with a 10% increase (black) or decrease (gray) in each individual input. (Modified from Resurreccion et al, 2012).

The study of anaerobic digestion as means to approach energy recovery from algae dates back to the 1950s (Golueke et al., 1957). These efforts peaked in the 1980s, as a consequence of the first oil crisis (Marzano, et al.,1982; Samson and LeDuy, 1982; Varel et al., 1987); however, as of 2009, there were still very few studies that laid out the types of information required for systematic LCA-based evaluation of proposed algae-to-energy systems. As noted above, this was a significant limitation of Clarens et al (2011) and related studies. Yet, since 2010, many new studies have emerged on the topic methane production during digestion of various microalgae strains (Frigon et al., 2013; Mussgnug et al., 2010), macroalgae strains (Costa et al., 2012) and mixed algae communities (Alzate et al., 2012).

These studies have revealed the effects of substrate composition, operational conditions, and inhibiting factors. For example, it has been demonstrated that co-digestion of algae biomass with other substrates may increase the anaerobic digestibility of algae by improving the carbon-to-nitrogen (C/N) ratio of the digested mixture. Typical C/N for algae biomass is less than 10 (Parkin and Owen, 1986). Use of a carbon-rich co-digestate, such as olive mill solid waste (Fernandez-Rodriguez et al., 2014), corn straw (Zhong et al., 2012), or paper (Yen and Brune et al., 2007), can increase this value to 20-30, which significantly increases methane yield. It is presumed that the increase in C/N ratio improves digestion performance by diluting potentially toxic ammonia. Other research on algae anaerobic digestion has characterized the effectiveness of various pretreatments, including thermal treatment, biological treatment and ultrasonic treatment (Alzate., 2012 ; Conzalez-Fernandes et al., 2013; Keymer et al., 2013). To date, this new information has not been well integrated in previous algae LCA models.

Apart from the progress in algae digestion research, there has also been some growing emphasis on characterizing possible synergies between algae cultivation and traditional domestic wastewater treatment. This is of interest because it has been demonstrated that the use of recycled wastewater effluent as algae growth medium significantly reduces the upstream energy burden associated with nitrogen (N) and phosphorus (P) fertilizers required for algae cultivation (Clarens et al., 2010). For example, wastewater irrigation of an open pond algae system producing electricity via anaerobic digestion of algae biomass yields EROI = 1.72 (Clarens et al., 2011), which is much higher than the EROI of the same system without use of recycled CO2, N, and P (1.06). Wastewater treatment plants (WWTPs) currently account for about 3% of electricity consumption in the US, and many are currently searching for ways to improve their energy performance (McCarty et al., 2011). Therefore, co-digesting algae biomass and wastewater biosolids in existing WWTP digesters could be a viable option for deployment of algae-to-energy systems in the near-term (Menger-Krug, et al., 2012). Before this can happen, there needs to be better understanding of algae digestion parameters, especially those pertaining to possible synergies (e.g., C/N ratio) for the specific case of co-digesting algae and WWTP sludge.

The objective of this study was to improve the accuracy of LCA-based evaluation of two hypothetical algae-to-energy systems incorporating anaerobic digestion: 1) “pure digestion” systems at algae farms; and, 2) synergistic “co-digestion” systems at wastewater treatment plants, where algae is cultivated in treated wastewater effluents and then digested together with wastewater biosolids. This increase in accuracy is achieved using both literature survey and bench-scale experiments to collect updated digestion parameters, which are then incorporated into existing algae LCA models.

2. Materials and Methods

2.1. Algae cultivation and harvesting

Pure cultures of *Scenedesmus dimorphus* were pre-incubated aseptically in capped tubes containing 10 mL of protease-peptone medium (PPM) for 5 d and then transferred into sealed 200-mL flasks containing 50mL PPM for another 5 d. These were incubated in a shaker subjected to 12 h of illumination (125w 6500K fluorescent light) and 12 h of darkness per day. Growing cultures were then cultivated in 1-L flasks containing 600 mL of modified Bold 3 (MBN3) medium and were swirled on the shaker subjected to the light-dark sequencing of 20 h illumination and 4 h darkness. These reactors were continuously aerated with filtered air. When the absorbance (OD600) of the culture reached a plateau (approximately 8-14 d), 50 mL of the algae inoculum was inoculated into a new flask and the remaining algae biomass was settled by gravity. Concentrated algae were easily separated from the supernatant and then used as substrates for anaerobic digestion experiments. All algae biomass was stored at 4 ° C for up to 1 week before use in digestion experiments.

Figure 2. Laboratory cultivation of pure *Scenedesmus dimorphus.*

Natural-grown algae were collected from a secondary clarifier at the Moore's Creek WWTP (Charlottesville, VA, USA). Microscopic analysis of these samples showed a mixture of unicellular and filamentous algae strains, including some *Scenedesmus sp.* The raw algae slurry was stored at 4° C for up to 1 week before use in digestion experiments. In order to increase uniformity, the slurry was slightly homogenized via mixing in a kitchen blender for 5 s before being fed into digesters.

2.2 Anaerobic digestion experiments and operational conditions

Anaerobic co-digestion of the algae biomass with WWTP biosolids was tested in 500mL glass serum bottles with a working volume of 450mL. The reactors were constantly stirred by magnets at the speed of 200 rpm and maintained in a covered water bath to maintain a temperature of 35 °C and also reduce the potential for photosynthesis. The biogas produced during digestion was filtered through a packed syringe filled with crystalline potassium hydroxide (KOH), to remove carbon dioxide. It was assumed that the remaining volume comprised only methane (CH_4), which was quantified over time using a PF-8000 Aerobic/Anaerobic Respirometer System (RAS, Fayetteville, AR).

Figure 3. PF-8000 Aerobic/Anaerobic Respirometer System, for simulation of algae anaerobic digestion and co-digestion with WWTP biosolids.

Primary sludge, secondary sludge and anaerobic seed samples were collected regularly from the Moore's Creek WWTP. Primary sludge and secondary sludge were mixed 1:1 by volume and stored at 4 °C before use. Initially, each reactor was filled with 450 mL of anaerobic sludge and flushed with nitrogen for 2 min to create an anaerobic environment. Once the microbes were acclimated and steady daily gas production was confirmed, the digesters were fed every 2 days. Four different feed compositions were tested in duplicate: 100% algae, 50% algae + 25% sludge 25% algae + 75% sludge, and 0% algae. These compositions account for mass ratios on a VSS (volatile suspended solids) basis. pH was controlled at the range of 6.7-7.3 by adding 2ml of 0.5 N $NaNO_3$ solution to reactors every 2 days. Samples of effluents were collected from each reactor, after steady gas production had been achieved for at least one solids retention time (SRT, 15 d).

In the first experiment, lab-grown pure culture *Scenedesmus dimorphus* was fed into the digesters at an organic loading rate (OLR) of 0.5gVSS $L^{-1}d^{-1}$. Solids retention time was 15 d. These parameters were set based on the limited amount of algae biomass that could be grown per time under lab conditions. In the second experiment, harvested wastewater-grown algae was fed into the digesters at the same OLR and SRT so that the pure culture results could be compared with the "real-world" results.

2.3 Analytical methods

Total solids (TS) and volatile solids (VS) and also total suspended solids (TSS) and total volatile solids (VSS) of pre- and post-digestion samples were measured according to APHA Standard Methods. (APHA, 1998) The mass of soluble solids was confirmed to be negligible in pre-assays and assumed to be zero in this study. pH was directly measured from liquid samples with a digital pH meter (Fisher Scientific, Pitsburgh, PA).

2.4 LCA Modeling

A life cycle assessment (LCA)-based modeling framework for the "pure digestion" case was adapted from an existing model correspond to "Case 4A" of Clarens et al. (2011). Digestion inputs were revised based on literature reports and experimental results. The modeling framework for "co-digestion" of algae and WWTP sludge was constructed based on a survey of energy consumption at typical municipal WWTPs (Menendez, 2010). These data were used to compute a baseline EROI for a WWTP treating 10 MGD (million gallons per day) of domestic wastewater, without algae cultivation and digestion. The baseline value was then modified to account for additional energy consumption and production associated with algae cultivation and conversion processes, based on Resurreccion et al (2012) and digestion data from this thesis.

3. Results and Discussion

3.1 Evaluation of bioenergy production via anaerobic digestion of pure algae

3.1.1 A summary of methane yields from algae based on published literature

The theoretical methane potential of organic matter with known stoichiometry ($C_aH_bO_cN_d$) can be calculated by a formula adapted from the Buswell equation. (Symons and Buswell, 1993). This estimate is referred to as the specific or stoichiometric methane potential (SMP) per gram of solids (VS) destroyed, and its value is equal to $\frac{(4a+b-2c-3d)}{8(12a+b+16c+14d)}V_M$, where V_M is the normal molar volume of methane. Given the typical formula of proteins ($C_6H_{13.1}O_1N_{0.6}$), lipids ($C_{57}H_{104}O_6$) and carbohydrates ($(C_6H_{10}O_5)_n$), Sialve et al. (2009) estimated the SMP of several algae stains (Table 3.1). The SMP of individual lipids, proteins and carbohydrates are 1.014, 0.851, and 0.415 L/g respectively, so strains with higher lipids generally have higher methane potential as well. Heaven et al. 2011) calculated the empirical formulas for algal protein based on weighted average amino acid composition and concluded that different algae have similar empirical formulas ($C_{1.9}H_{3.8}O_1N_{0.5}$) and C/N ratios for proteins. Also, he commented that the protein formula used by Sialve et al. was not representative, which resulted in the overestimation of methane yields (Table 3.1). Revised SMP values vary from 471 to 579mL/g VS reduced, and these values are proportional to lipids content.

It must be pointed out that the theoretical approach of SMP estimation does not take into account the needs for cell maintenance and anabolism among the methanogenic organisms. As such, the actual measured methane production per unit of algae destroyed is generally less than theoretical potentials. Also, calculation of SMP based on the assumption that all organic matter is completely consumed without producing any inhibiting substances generally also leads to overestimation of SMP. When taking VS removal efficiency into consideration, a much lower yield per unit of algae introduced is likely to be observed from experimental results.

Anaerobic biochemical methane production (BMP) tests, operated over 30 days, have investigated the optimal methane yields of a variety of dominant algae strains. Results show that biogas production is strongly dependent on the algal strain used. Strains having the highest methane yield include *Scenedesmus dimorphus* (397±10 mL/g VS_{in}) *Scenedesmus sp.-AMDD Jul-2011* (410±6 mL/g VS_{in}), *Isochrysis spp.* (408±4mL/g) from Frigo et al. (2013), and *Chlamydomonas reinhardtii (*387.42±5.8) mL/g VS_{in} from Mussgnug et al. (2010). However, there is no correlation between phylogenetic relationship and methane production yield. In the study of Mussgnug et al. (2010), the best and worst substrates were both algae strains from the class *Chlorophyceae.* Considering strains from same genera, six strains of *Chlorella sp.* have been tested in previous studies, and reported yields are from 123 to 361 mL/gVS_{in} (Wang et al., 2013; Frigo et al.,2013). *Scenedesmus dimorphus,* which has a relatively high lipid content (~40%,) has a much higher yield than the closely related species *Scenedesmus obliquus* (see Table 3.1). These indicate that the methane yield is highly dependent on the chemical composition of a specific species.

Comparing available experimental results with calculated theoretical methane potentials (revised), conversion efficiency can be calculated. These values are presented in Table 3.1. These represent the portion of volatile solids converted into methane gas. Most values fall into the range of 50%-70%. *Chlorella vulgaris* and *Scenedesmus obliquus,* two of the most ubiquitous and well-investigated strains, however, achieve rather low conversion efficiency (42% and 32%, respectively) and thus exhibit low yields (240 and 177 mL CH_4 /VS_{in}).

Based on above discussion, the known biochemical composition of an algal strain can be used to predict its methane production potential to some extent. However, the digestibility of algae substrates, described as either VS removal efficiency or conversion efficiency, is also a crucial factor in practice. The effect of operational conditions on digestibility and optimal parameters for algal substrates are discussed in the following paragraphs.

Table 3.1 Methane production potential and conversion efficiency of some algae strains

Algae Strains	Proteins (%TS)[a]	Carbs. (%TS)[a]	Lipids (%TS)[a]	CH_4 potential (mL /VS_R)[b]	Revised potential (mL/VS_R)[c]	Measured yield (mL/ VS_{IN})	Conversion efficiency (%)[k]
Chlamydomonas rheinhardii	48	17	21	690	579	387.42[d]	67
Chlorella pyrenoidosa	57	26	2	800	450	264.71[j]	59
Chlorella vulgaris	51-58	12-17	14-22	630-790	544-569	195.64-361[e,f]	34-66
Dunaliella salina	57	32	6	680	471	323.2 [d]	68
Euglena gracilis	39-61	14-18	14-20	530-800	555-558	324.95 [d]	58-59
Scenedesmus dimorphus	8-18	21-52	16-40	-	-	397 [e]	-
Scenedesmus obliquus	50-56	10-17	12-14	590-690	531-536	177.94-240 [d]	33-45
Spirulina maxima	60-71	13-16	6-7	630-740	483-484	260-350 [g,h]	53-72
Spirulina platensis	46-63	8-14	4-9	470-690	481-500	355[i]	71-74

[a] Beck, 1994; TS= Total suspended solids
[b] Sialve et al., 2009
c Heaven et al., 2011
[d-j] Mussgnug et al., 2010 ; Frigo et al., 2013; Ras et al., 2011; Samson and LeDuy,1982; Samson and LeDuy, 1986; EL-Mashad, 2013; Prajapati et al., 2014a
[k] Conversion efficiency = Measured yield/Revised theoretical yield

3.1.2 Solids retention time (SRT)

The Solids Retention Time (SRT) is the average time that the digestible biomass spends in the digester. (Metcalf and Eddy, 2003). This parameter is recognized as the most important parameter for designing an anaerobic digester, because it sets up the ecology of the slow-growing anaerobic organisms that perform the digestion reaction. In a study of anaerobic digestion of sewage sludge (McCarty, 1974), increasing SRT increases both methane production per mass VS destroyed and also the VS removal efficiency. Thus, higher methane yields can be obtained when feeding same amount of VS at higher SRT. In conventional digesters and single-stage, high-rate digesters, hydraulic retention time (HRT) is equivalent to SRT (Parkin and Owen, 1987). These two terms will be used interchangeably throughout this thesis.

Figure 3.1 summarizes results from three separate studies in which algae biomass were anaerobically digested at various HRT duration (Ras et al., 2011;Samson&LeDuy, 1986;Varel et al., 1987; Golueke et al., 1957). All reactors were operated semi-continuously. The types of algae used include *Chlorella sp., Scenedesmus sp.* mixture, and *Spirulina maxima.* Notably, each dataset appears to take the same general form, whereby there is a dramatic increase in methane yield with increased HRT up to roughly 15 HRT, and thereafter, there is a plateau for HRT greater than 20-30 d HRT. When increasing HRT from 16 d to 28 d, the methane yield of *Chlorella vulgaris* increases significantly, from 147 to 240 mL CH4/g VS_{IN} (Ras et al., 2011). These values are consistent with results from batch BMP tests. Prajapati et al (2014b) reports that the cumulative biogas production from *Chlorella vulgaris* (comprising ~53% methane) production increases from 250mL/g VS_{IN} at HRT = 16 d to 360 mL/g VS_{IN} at HRT = 28 d, indicating that experimental results are consistent for both continuous reactors and batch reactors. In batch BMP tests operated over longer durations, it has been demonstrated that the majority of methane is produced within the first 30 d (Prajapati et al., 2014a, Mussgnug et al., 2010, Frigon et al, 2013).

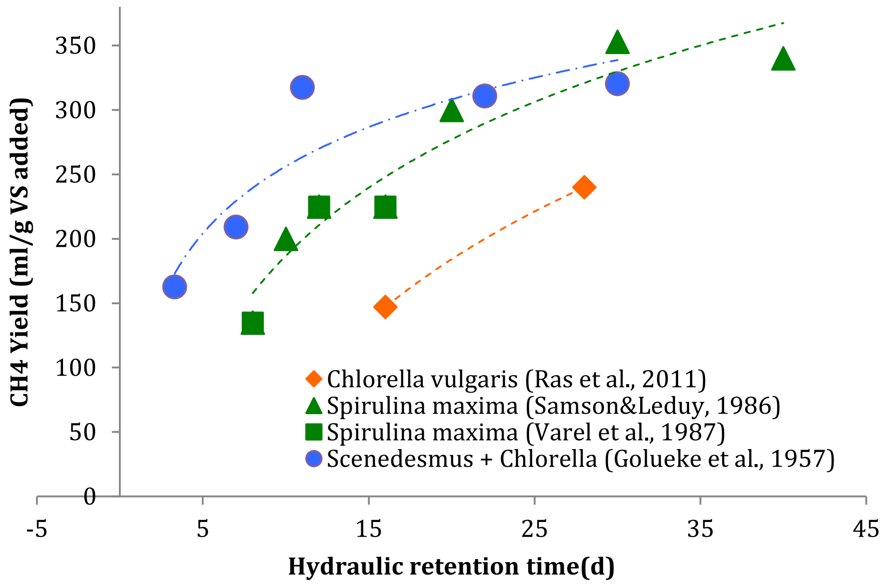

Figure 3.1 Methane yields as a function of HRT in studies examining multiple HRT values under the same conditions.

In practice, shorter HRT tends to correspond to higher organic loading rate, because more material is loaded into the digester in a shorted period of time. Therefore, shorter HRTs are helpful for reducing digester volume (Park and li, 2012). However, HRT must be greater than 12 days to avoid "washout" of the relatively slow-growing methanogens (Gerardi, 2013). For digestion systems in which algae is the sole digestible substrate, many researchers suggest that an extended HRT be used, because algal cell wall are more resistant to digestibility than wastewater biosolids, resulting in low digestibility at short HRTs (Wang et al., 2013). Given these constraints, an optimum HRT for a pure algae digestion system should exist when above which little added benefit in methane production can be attained.

3.1.3 Organic Loading Rate

Organic loading rate (OLR) is the amount of organic matter flowing into the digester per time, usually expressed as gVS/L/d or kg VS/m^3/d. The typical values of OLR for conventional digesters are between 0.5-3 g VS/L/d (Gerardi, 2003). Increasing OLR can be an approach to increase the methane yield production per time or per mass introduced (Yen and Brune, 2007); however, feeding a digester at an OLR above the acceptable range can result in limited digestibility and accumulation of digestion inhibitors. Studies on how OLR affects anaerobic digestion of algae have reported system failures due to overloading (Samson and LeDudy, 1986; Cecchi et al., 1996; Park and Li, 2012). Additional work suggest that OLR appears to be a sensitive parameter governing digester performance at elevated ("thermophilic") temperatures, but that increasing OLR above the recommended range cannot be offset by use of extended HRT (Varel et al., 1988; Samson and LeDuy,1986).

The OLR is closely associated with the growth rate of algae and other operational parameters such as HRT and total digester volume. In general, an extended HRT coupled with a relatively lower OLR (<2 g/L/d) gives optimal methane yield.

3.1.4 Temperature

Temperature is another key operational factor for anaerobic digesters, since it affects the physical and physico-chemical properties of compounds present in the digester and also the rates of biochemical processes carried about by the digesting microorganisms (Boe, 2006). Two temperature conditions have been explored in previously published studies; namely mesophilic conditions (28-38 ºC) and thermophilic conditions (50-55 ºC). According to Varel et al (1988), thermophilic conditions increase the initial rate of CH_4 production but do not significantly increase the total yield.

3.1.5 Summary and integrating revised digestion data into algae LCA models

Table 3.2 summarizes previously published literature pertaining to each of the key digestion parameters referenced in the preceding paragraphs.

Table 3.2-1. A summary of digestion studies evaluating digestion parameters of interest.(Published before 2010)

Reference	Algae types	CH_4 yield (mL/g VS)	Reduction (% VS)	CH_4 (%)	Temp. (°C)	HRT (d)	OLR (g VS/L/d)
Yen and Brune, 2007[a]	*Secenedesmus and Chlorella*	90-143.25	-	68.1-71.4	35	10	2 to 6
Sanchez Travieso, 1993 [a]	*Chlorella vulgaris*	720-855[c]	-	67.8-76.1	28-31	64	-
Chen, 1987[b]	*Algae mixture*	420	-	72	35	28	1
	Spirulina	310-320	-	-	35	28	0.91
	Dunaliella	440-450	-	-	35	28	0.91
Samson and LeDuy, 1986 [a]	*Spirulina maxima*	37-353	8-48.3	46-76	15-52	5-40	*0.5-10*[d]
Samson and LeDuy, 1982 [a]	*Spirulina maxima*	260	65.80	68-72	35	33	0.97
Asinari Di san Marzano et al., 1982 [b]	*Tetraselmis* (fresh)	310	-	72-74	35	14	2
	Tetraselmis (dry)	260	-	72-74	35	14	2
	Tetraselmis (dry) + NaCl 35g/L	250	-	72-74	35	14	2
Golueke et al.,1957[a b]	*Algae mixture Scenedesmus and Chlorella*	*170-320*	36.4-60	62-64	35-50	3-30	1.44-2.89

a References for the anaerobic digestion model in Clarens et al., 2001
b Sialve et al., 2009
c Estimated from data give in CH_4 g COD/L when COD/VS=1.5
d Estimated from initial loading So=20-100VS/L, OLR(gVS/L/d)=$\frac{S(g\ VS/L)}{HRT(d)}$

Table 3.2-2. A summary of digestion studies evaluating digestion parameters of interest.(Published since 2010)

Reference	Algae types	CH_4 yield (mL/g VS)	Reduction (% VS)	Temp. (°C)	HRT (d)	OLR (g VS/L/d)
Prajapati et al., 2014a	*Chroococcus spp.*	271.68	-	35-37	30	-
Prajapati et al., 2014b	*Chlorella pyrenoidosa*	264.71	51	35-37	30	-
	Chlorella minutissima	166.12	39	35-37	30	-
	Chlorella Culgaris	195.64	45	35-37	30	-
Wang et al., 2013	*Chlorella sp.*	123	59	37	45	-
Zhong et al., 2012	*Microcystis(>99%)*	201	41.26	35	30	0.67
Gonalez-Fernandez et al., 2011	*C. vulgaris & S. obliquus*	160.62	18a	35	40	-
Monique Ras et al., 2011	*Chlorella vulgaris*	147	33	35	16	1
	Chlorella vulgaris	240	51	35	28	1
Philip Keymer et al., 2013	*Scenedesmus sp*	180	29a		35	-
Alzate et al., 2012	*Acutodesmus obliquus(58%), Oocystis sp (36%)*	395	54-70		35	-
	Acutodesmus obliquus and Oocystis sp.	188	22-33		35	-
Mussgnug et al., 2010	*Arthrospira platensis (P/S)*	293.41	-	38	32	-
	Chlamydomonas reinhardtii (E/F)	387.42	-	38	32	-
	Chlorella kessleri (E/F)	217.75	-	38	32	-
	Dunaliella salina (E/S)	323.2	-	38	32	-
	Euglena gracilis (E/F)	324.95	-	38	32	-
	Scenedesmus obliquus (E/F)	177.94	-	38	32	-

a In units of % COD removed

To reiterate from Section 1, previous life cycle assessment (LCA) studies of hypothetical algae-to-energy systems have shown that the energy sustainability of these systems is very sensitive to input parameters pertaining to anaerobic digestion (e.g., Clarens et al., 2011). In particular, the EROI (energy return on investment) metric is highly sensitive to estimation of methane yield. Given the scarcity of good algae digestion data that was available (table3.2-1) when early algae LCA studies were published, it is desirable to reevaluate the conclusions of those analyses in light of the large volume of algae digestion data that has been recently published (table3.2-1).

This study focuses on updating and revising EROI estimates originally put forth by Clarens et al (2011) and later revised by Resurreccion et al (2012). The model framework accounts for cultivation of freshwater algae species in an open pond configuration. The yield is roughly 11 $g/m^2/d$. It is assumed that recycled, treated wastewater is used to irrigate the algae ponds in order to reduce the amounts of nitrogen and phosphorus fertilizer that must be consumed. The algae is harvested from the pond via gravity settling and then digested in bulk in a conventional anaerobic digester. The resulting methane is converted into electricity. For the revised analysis, all inputs related to algae cultivation, dewatering, and methane combustion remained unchanged. In contrast, digestion parameters were updated to reflect emerging data. Table 3.3 presents original and revised values of pertinent digestion parameters.

Table 3.3. A comparison of digestion conditions and parameters, as used in a previously published LCA study by Clarens et al (2011) or updated (revised) in this thesis.

Digestion Parameter	Used in Clarens et al. (2011)	Revised LCA Model
HRT(d)	5-64	28-33
Temperature	15-52	30-38
OLR (gVS/L/d)	0.5-10	0.5-2
Methane yield (mL/gVS_{IN})	167.00	265.15
VS removal efficiency (%)	54.00	47.38

Using data from Table 3.3, it is possible to compute a revised value for the amount of methane generated per ha in a typical algae farm. This number can then be used to estimate what amount of electricity (energy) to be produced via methane combustion. This quantity is the numerator for computing EROI of a hypothetical algae-to-energy system. Table 3.4 summarizes this calculation, based on parameters taken from Clarens et al (2011) and Resurreccion et al (2012). Table 3.4 also summarizes parameters and intermediate values required for computation of the EROI denominator, which accounts for energy consumption in the hypothetical algae-to-energy system. These values were adapted directly from Resurrection et al (2012). Combining these two quantities gives a revised estimate of EROI for hypothetical large-scale algae farming systems.

Table 3.4. Input parameters and selected output from the original Clarens et al (2011) model and a revised LCA-based analysis of hypothetical algae farming systems.

Parameter	**Used in Clarens et al. (2011)**	**Used in revised LCA**
Algae yield (Mg/ha/y)	91.2	91.2
Algae VS content (g VS/g TS)	0.86	0.86
Methane yield (mL/g algae VS)	169.65	265.15
VS removal efficiency (%)	54.00	47.38
Methane production (Mg/ha/y)	14449	22546
Methane energy content (MJ/Mg)	50	50
Turbine efficiency	0.54	0.54
Electricity production (MJ/ha/y)	391,316	610,614
Total energy consumption (MJ/ha/y)	585,862	605,180
EROI (E_{OUT}/E_{IN})	1.75	3.29

Table 3.4 reveals that use of recently published algae digestion parameters dramatically increases the estimate or EROI for hypothetical algae-to-energy systems incorporating anaerobic digestion of the bulk algae biomass; from 1.75 to 3.29. This change arises from increasing the methane yield parameter by roughly 60%. In Clarens et al (2011), methane production was computed as the production of VS reduction (%) times SMP VS destroyed. These parameters were both assigned to triangular distributions. In the revised model, the multiplication was no longer necessary, since most recently published data reports methane yield per g VS fed into the digester. Future work will focus on incorporating uncertainty into the revised LCA model estimates, using the same Monte Carlo approach that was originally used in Clarens et al (2011). For now, the values in Table 3.4 reflect output based on "likeliest" values of each input (i.e., averages).

Another reason for the change in EROI is better focus on what digestion operating conditions are important. In the Clarens analysis, lack of data made it impossible to choose digestion yields that actually matched the set of operating conditions that would be used to optimize methane yield from algae. In contrast, the information presented in Sections 3.1.2-3.1.5 was used to ensure that the digestion parameters have values that reflect the operating conditions that would most likely be used. These conditions are summarized in Table 3.3-2. Evidently, these conditions are very promising for delivering excellent EROI performance in hypothetical algae-to-energy systems.

3.2 Evaluation Anaerobic co-digestion of algae and municipal sewage sludge

The recalcitrance of algal cell walls and ammonia toxicity caused by algae's imbalanced C/N ratio and are two commonly cited causes of low methane yields from algae compared to other digestible materials (Sivale, et al., 2009; Mussgnug et al., 2010). Algal cell walls generally contain 70% cellulose (on dry weight basis), which tends to be resistant to digestion (Baldan et al., 2001). As such, suitable pre-treatment methods can increase methane yield by disrupting the algal cell wall (Keymer, et al., 2013). Examples of pre-treatments that have been proved to increase algae digestibility and methane yield include thermal pretreatment (Gonzalez-Fernandez et al., 2013; Mendez et al. 2013) and high-pressure thermal hydrolysis (Keymer, et al., 2013). However, the energy inputs required for these treatments are so high as to make both practically unreasonable.

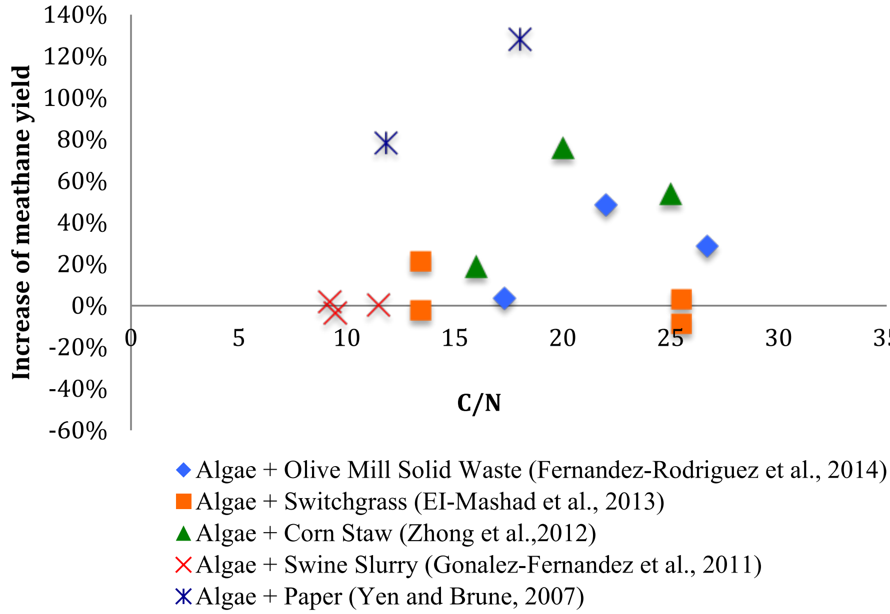

Figure 3.2. Increase of methane yield for mixtures of algae biomass and digestible organic materials exhibiting a range of C/N ratios. The increase of yield is calculated from experimental measurements verses calculated values. $Y_{CALC} = Y_c \times C_c + Y_A \times C_A$, where Y_A and Y_c are methane yields for algae and co-digestate substrates, respectively,

Another possible option for increasing the methane yield from algae biomass is co-digesting it with other substrates. This is evident from Figure 3.2, which shows methane yield increases for algae biomass mixed with various quantities of carbon-rich organic materials to achieve proper C/N ratios in the algae/sludge mixture. A proper C/N ratio is desired for healthy methanogenic communities; to avoid the accumulation of ammonia or fatty acids, which tend to occur at overly low and high C/N ratios, respectively (Zhong et al., 2012) As is shown in figure 3.2, the optimal C/N ratios for co-digesting mixture is at the range of 18-25. In addition to increasing the C/N ratios, co-digestion may also increase digestibility of algae by increasing the diversity and quantity of enzymes in digester. For example, addition of paper to algae improves the cellulose activity in digester as well as causes a positive impact on methane production efficiency (Yen and Brune, 2007).

One obvious choice of digestion co-substrate is so-called “sludge” from municipal wastewater treatment plants, as large amount of sludge is produced and digested in WWTPs daily. The C/N ratio of municipal sludge varies widely, from 5-16, depending on influent wastewater characteristics and the ratio of primary to secondary sludge. There are several key synergies between algae farming and municipal wastewater treatment, whereby, it makes sense to consider collocating both operations in the same facility. These synergies include: 1) significant energy savings for both the WWTP and the algae farm when nutrients in the WWTP effluent are “recycled” back into the algae cultivation pond for use as fertilizer, instead of being subjected to intensive nutrient removal processes; and 2) the presence of digesters at most municipal WWTPs and a growing emphasis on making WWTPs energy “self-sufficient” by maximizing the amount of material that can be digested into methane to produce electricity. Given these factors, it is worthwhile to evaluate the energy balance of hypothetical systems integrating municipal wastewater treatment and algae farming. But first it is necessary to investigate the performance of methane gas production during co-digestion of algae and WWTP sludge.

3.2.1 Measuring methane yield from co-digestion of pure and mixed algae cultures with WWTP sludge

A series of respirometry experiments were performed to quantify specific methane yield (SMP) (in units of L CH4/g VS_{IN}) for various mixtures of algae and WWTP sludge. Two types of algae were used: lab-grown, pure cultures of *S. dimorphus* and also natural-grown (i.e., "wild") algae collected periodically from clarifiers at the Moore's Creek WWTP. All WWTP primary and secondary sludge samples, as well as the anaerobic digester seed organisms (methanogens), were also collected from the Moore's Creek WWTP. Table 3.5 summarizes the characteristics of the substrates and anaerobic seed used in the digestion experiments. Primary and secondary sludge were mixed at equal volumes for all experiments.

Table 3.5. Characteristics of substrates and inocula used in the digestion experiments. Average values for n = 3 samples of sludge or seed and n=8 samples of algae are presented. Error bars represent 1 standard deviation. Seed 1 and seed 2 are the inoculums used in "WWTP algae" experiment and "pure algae" experiment respectively.

	Primary sludge	**Secondary sludge**	**Anaerobic seed (1)**	**Anaerobic seed (2)**	**WWTP Algae**	***S. dimorphus***
TSS (g/L)	8.76±0.79	24.12±1.44	24.17±0.41	20.59±0.46	16.82±4.73	12.24±4.17
VSS (g/L)	6.65±0.21	19.06±0.76	14.19±0.90	13.92±0.09	8.94±2.37	11.32±3.86
VSS/TSS	0.76±0.04	0.79±0.02	0.67±0.03	0.68±0.01	0.53±0.01	0.93±0.01

The objective of each digestion experiment was to measure SMP (methane yield) for a different ratio of algae mixed with WWTP sludge. For applicability, all reactors in each experiment were operated for 1-2 HRTs before methane yield measurements were recorded. This allowed the reactors to come to steady-state, neutralizing lag phase or acclimation effects caused by changes in feed, initial seed inoculum, operating conditions, etc. Additionally, a normalized methane yield was computed for each algae/sludge mixture in order to facilitate comparisons among data from different experiments. This was done by dividing the methane yield from an algae/sludge mixture by the methane yield of the 100% sludge sample for the same experiment. Again, this was meant to neutralize possible impacts of different starting inoculum, feed compositions, operating conditions, etc. for the various digestion experiments. Table 3.6 summarizes experimental results from the algae/sludge co-digestion studies.

Table 3.6. Experimental data obtained through co-digesting mixed municipal sludge with pure culture algae or WWTP algae. Upper results are for experiments with pure algae (*Scenedesmus dimorphus*), lower results are for experiments with mixed algae collected from the Moore's Creek WWTP. Error bars refer to 1 standard deviation.

	100% Sludge	**25% Algae + 75% Sludge**	**50% Algae + 50% Sludge**	***100% S. dimorphus***
CH_4 yield (mL/g VS_{in})	405 ± 12	392 ± 14	332 ± 4	267 ± 1
VS removal rate (%)	49 ± 3	47 ± 1	45 ± 2	39 ± 2
Normalized CH_4 yield	1	0.97	0.82	0.66

	100% Sludge	**25% Algae 75% + Sludge**	**50% Algae + 50% Sludge**	**100% WWTP Algae**
CH_4 yield (mL/g VS_{in})	520 ± 6	509 ± 18	442 ± 17	355 ± 16
VS removal rate (%)	35 ± 1	33 ± 2	31 ± 1	31 ± 2
Normalized CH_4 yield	1	0.98	0.85	0.68

There are several interesting observations arising from Table 3.6, pertaining to how digestion parameters for the various mixtures compare to one another and previously published work. First, it is interesting that the SMP values for both experiments decrease with increasing algae content in the algae/sludge mixtures. For both sets of experiments, with pure (lab-grown) algae and natural (WWTP-grown) mixed communities, the percent decrease in SMP for 100% sludge versus 25% algae (75% sludge) is not significant, about 2-3% on average. It is possible that adding a small amount of additional algae may not remarkably influence the overall conditions of anaerobic digestion. In contrast, the percent decrease in SMP for 100% sludge versus 100% algae is 32-34% on average. This is a significant decrease. In practice, it is not expected that algae would comprise up to or more than 50% of total mass therefore, it may be valuable for future studies to focus more on methane yield for algae/sludge mixtures on the range 0-50% algae. Based on the composition of typical domestic wastewater, principally the ratio of organic material (which gives rise to biosolids) and nutrients (which give rise to algae), it is estimate that algae may make up 5-20% of the algae/sludge mixture to be digested at typical municipal WWTPs.

Second, the 100% *S. dimorphus* sample appears to produce 25% less methane than the 100% WWTP algae sample. This is interesting, and it could suggest that the higher diversity of the mixed WWTP sample makes for better variety of digestible substrates and thus greater ecological diversity among the methanogens carrying out the digestion reactors. However, comparing the normalized SMP values across both experiments, it is evident that the first set of experimental conditions was better for producing higher methane yields. This makes it is impossible to draw a definitive conclusion about whether the mixed WWTP algae is more or less digestible than the pure algae. One previously published study on *S. dimorphus* reported a methane yield of 397 ± 1 mL/g $VS_{in.}$ (Frigo et al, 2013). This is nearly 50% greater than the value measured in this study. The VS removal rate from the previous study was also substantially larger: 49 ± 3% versus 39 ± 2% from Table 3.6. These differences could be because the HRT used in this study (15 days) was shorter than the 35-50 days HRT (incubation time) used by Frigo et al (2013).

Third, it is interesting to see how the measured methane yields from algae/sludge mixtures compare to what would be expected on the basis of calculated linear summation. The calculated yield (Y_{CALC}) is given by the following equation: $Y_{CALC} = Y_S \times C_S + Y_A \times C_A$, where Y_S and Y_A are methane yields for sludge and algae, respectively, and C_S and C_A are fractions of sludge and algae, respectively. Measured values of methane yield that are greater than Y_{CALC} would indicate that there is some synergy during digestion of algae and sludge; whereas, measured values less than Y_{CALC} would suggest that inhibition is occurring. Figure 3.4 summarizes the comparison of measured and calculated yields for the pure algae and mixed algae experiments, based on measured sludge methane yields (dashed lines) and algae methane yields (solid lines). When algae content increases up to 50%, the calculated yield becomes very close to the measured values, showing that linear summation can be a convenient way to estimate the methane yield of the mixture when the algae fraction of the mixture is very small (~0%) or very large (≥50%). However, for both sets of experiments, there is a clear synergy occurring when algae comprises roughly 25% of the total VS. In this region, which is closest to the expected range for typical integrated algae systems, estimation of methane yield should account for this increase compared to the yield calculated based on linear summation.

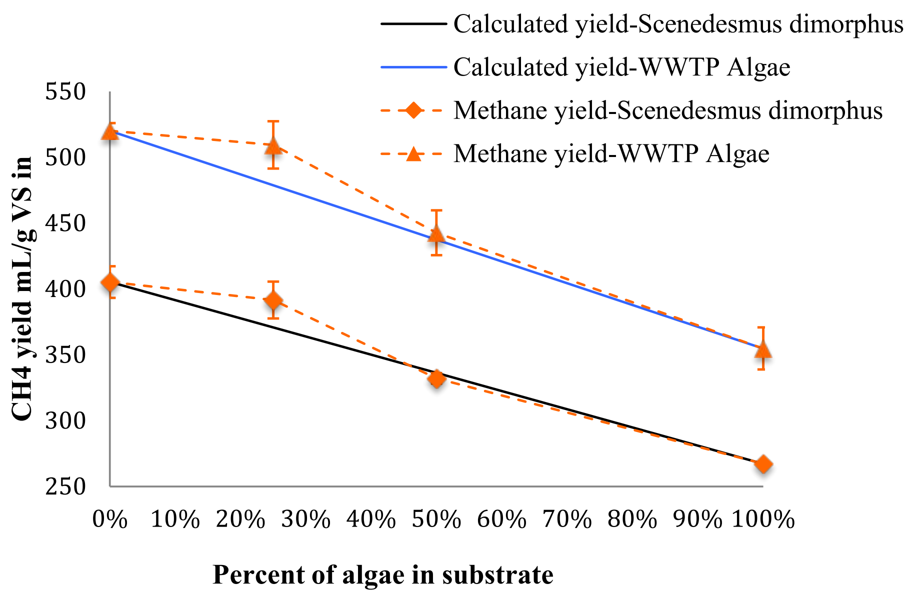

Figure 3.4. Comparison of measure and calculated methane yield values for mixtures of algae and WWTP sludge.

Finally, the normalized methane yields are remarkably consistent across studies, despite the significant differences in algae composition. Normalized yields are roughly 97-98% for 25% algae, 82-85% for 50% algae, and 66-68% for 75% algae. The consistency in normalized yield makes this a useful parameter for making comparisons among previously published results, even if they were collected using different operational conditions. Figure 3.3 shows the relationship between normalized methane yield and algae fraction in digested mixtures of algae and WWTP sludge, for three previously published studies and the experimental results in Table 3.6. From this figure, there is a significant negative correlation between percent of algae and normalized methane yield, showing the methane yield of a mixture decreases when algae portion increases. This correlation is fairly linear, as shown by the trend line and high R^2 value (67%). It must be pointed out that lower methane yield with additional algae mass is not necessarily results in lower total energy output, as the increased total digestible materials counteracts the decreased yield per gram of biomass.

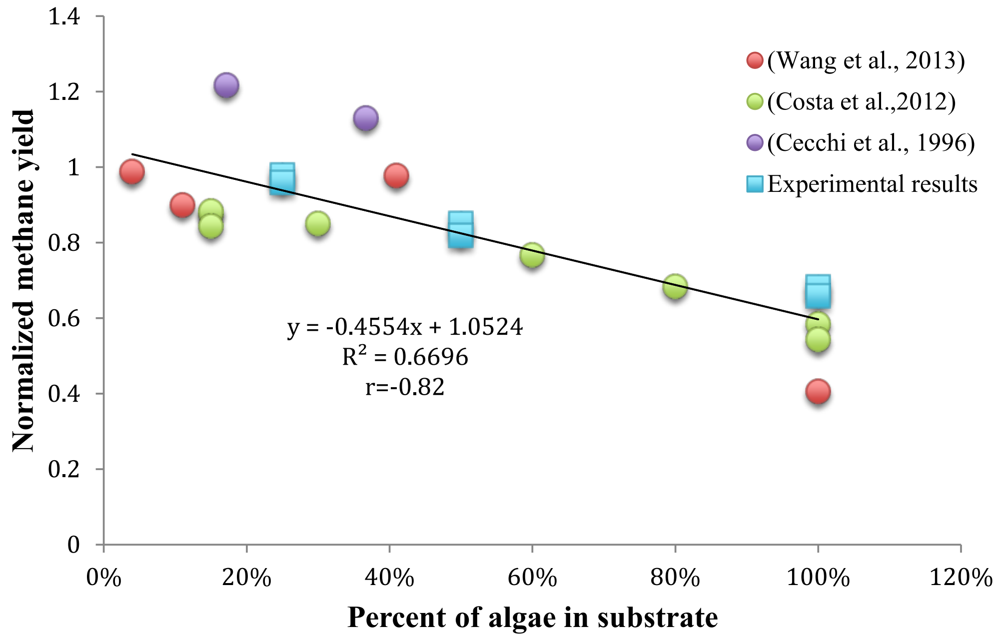

Figure 3.3. Normalized methane yields as a function of algae fraction in algae/sludge mixtures.

3.2.2 Summary and integrating revised digestion data into algae LCA models

Table 3.6 provides a summary of co-digestion parameters for mixtures of algae and WWTP sludge, from recently published studies (shown in Figure 3.3) and this thesis. Similar to the pure algae digestion results summarized in Section 3.1.5, these data are useful for life cycle assessment (LCA)-based evaluation of hypothetical algae-to-energy systems.

Table 3.6. Co-digestion parameters for mixtures of algae and WWTP sludge.

Reference	Substrate	CH_4 Yield (ml/g VS_{IN})	Normalized yield (% Sludge yield)[b]	VS removal (%)
Wang et al., 2013	*Chlorella sp.*	123	-	59
	100% WAS[a]	302	-	52
	Algae (4%) +WAS	299	0.99	54.5
	Algae (11%) +WAS	272	0.90	55.5
	Algae (41%) +WAS	296	0.98	57.5
Costa et al., 2012	*Ulva spp.*	196	-	48[c]
	100% sludge	335	-	46[c]
	Algae (15%) +sludge	296	0.88	44[c]
	Algae (30%) +sludge	285	0.85	45[c]
	Algae (60%) +sludge	257	0.77	37[c]
	Algae (80%) +sludge	229	0.68	47[c]
Cecchi et al., 1996	100% sludge	147		28.7
	Algae (17%) +sludge	180	1.22	27
	Algae (38%) +sludge	167	1.13	26.1
This thesis (Exp. 1)	100% sludge	520	-	34.67
	Algae biomass	355	-	30.51
	Algae (25%) +sludge	509	0.98	32.71
	Algae (50%) +sludge	442	0.85	31.14
This thesis (Exp.2	100% sludge	405	-	42.93
	S.dimorphus	267	-	32.59
	Algae (25%) +sludge	392	0.97	38.45
	Algae (50%) +sludge	332	0.82	40.69

[a] WAS is "waste activated sludge".

[b] Normalized yield refers to yield from the algae/sludge reactor as divided by the yield from the 100% sludge reactor in the same experiment.

[c] In units of % COD removed

LCA has been applied to a hypothetical 10-MGD municipal WWTP to evaluate its EROI with and without integration of algae cultivation. The baseline scenario for this WWTP is based on a WWTP energy audit in Menendez (2010), and it assumes that the plant is operating primary clarification, secondary aeration with advanced nitrogen removal, secondary clarification, sand filtration, chlorine disinfection. The energy consumption for this "typical" plant is 17,860 kWh/d (64,483 MJ/d). The energy production from methane gas produced via anaerobic digestion of primary and secondary sludge is calculated based on the estimation of daily sludge output and typical parameters for energy conversion. Primary sludge production is computed based on mass balance of TSS in the influent and effluent of primary clarifier. Secondary sludge production is calculated based on heterotrophic biomass growth, cell debris production, and non-biodegradable VSS in primary effluent. (Metcalf and Eddy, 2003). All parameters including raw influent TSS (245mg/L), influent BOD (250mg/L), activated sludge yield (0.6gVSS/g BOD), and cell decay rate (0.1 d^{-1}) are based on typical values (Metcalf and Eddy, 2003). Finally, multiplying total sludge yield by the likeliest methane yield (0.28 m^3/kg VSS in) gives the methane production for the WWTP: 1,877 m^3/d. Applying typical capture and conversion efficiencies (WEF, 2009), the corresponding energy production for the integrated WWTP + algae farming system is roughly 19, 310 MJ/d. The resulting EROI is 0.3.

A co-digestion scenario can be evaluated for the same WWTP by adding terms for energy production and consumption associated with algae operations to the EROI numerator and denominator, respectively. For this scenario, it is assumed that an algae cultivation pond is placed the downstream of the secondary clarifier. Assuming that the residence time of the algae pond would be roughly 2 d (Clarens et al., 2010). For the assumed flow rate (10 MGD) and this residence time, the area of the pond required to handle all of the WWTP's effluent would be 14.83 ha. Multiplying this by an assumed algae yield of 40.3 Mg/ha-yr (Clarens et al., 2010), 597.7 Mg of algae VSS would be produced at the WWTP annually (156 kg/d). Based on Clarens et al. (2010), the amount of energy consumed for pumping and mixing this algae pond would be roughly 809 MJ/d.

This is the additional amount of energy the WWTP would have to expend to implement on-site algae cultivation.

Anaerobic digestion of the additional algae biomass would also result in increased energy production. This can be computed using the regression equation presented in Figure 3.3, assuming that the methane yield for 100% sludge is 0.28 m^3/kg VS. It also assumed that the digestible mixture will comprise roughly 19% algae + 81% sludge (on VS basis), based on the algae yield referenced above and expected sludge production for the hypothetical WWTP. Applying the regression equation for these conditions, gives a normalized methane yield of roughly 96% for the algae/sludge mixture. Multiplying this value by the sludge methane yield, the methane yield for the algae/sludge mixture is approximately 0.27 m^3/kg VS. This parameter can be combined with total amount of algae/sludge mixture produced per day and the estimated value of energy consumption for algae operations to give an EROI value for the entire integrated system. This EROI calculated is 0.35, which is higher than the baseline WWTP EROI (0.30). This indicates that addition of algae farming to WWTP operations would reduce the energy intensity of municipal wastewater treatment.

Table 3.7 Important parameters and energy balance for WWTP and WWTP integrating algae calculation.

	WWTP	WWTP + Algae Pond
Total Electricity Consumption (MJ/d)	**64,483**	**65,292**
WWTP baseline (m^3/kgVS)	64,483	64,483
Algae cultivation-pumping/mixing (MJ/d)	/	809
Total Electricity Production (MJ/d)	**19,310**	**23,071**
Total VSS (Mg/d)	6.52	8.08
Methane yield (m^3/kgVSS)	0.28	0.28
Normalized yield	1.00	0.96
VSS reduction rate (%)	58	58
Low heating value (MJ/m^3)	35.8	35.8
Methane capture efficiency (%)	90	90
Efficiency of electricity production (%)	33	33
EROI	**0.30**	**0.35**

With the algae pond in place, the WWTP may be able to rely on algae-mediated nitrogen removal to help them meet their discharge standards. Accounting for possible reductions in WWTP energy consumption that could arise from leaving higher nutrient concentrations in the secondary effluent. Thus, it is assumed that less energy is required for extensive N and P removal, which accounts for electricity savings of 563MJ/d and 7MJ/d, respectively (Clarens 2010). The calculations summarized in Table 3.7 correspond to so-called “biological nutrient removal” or “advanced nutrient control” wastewater treatment (Menendez, 2010; Claren et al., 2010), which produces very low concentrations of nitrogen and phosphorus in the effluent. It is expected that greater energy savings, and thus larger increases in EROI, would be observed for WWTPs producing larger effluent nutrient concentrations; i.e., "conventional activated sludge works" without advanced nutrient removal. This will be explored in figure work.

4 Conclusions

Recent studies of anaerobic digestion of algae biomass, alone and together with WWTP sludge as a co-substrate, have enabled better characterization of what operating parameters lead to best methane yield. Extended HRT (> 30 days) and lower organic loading rate (OLR) tend to improve methane yield from algae. For systems digesting just algae biomass, methane yield is roughly 265 mL/g VS_{IN}.

For systems co-digesting algae biomass with WWTP sludge, the normalized methane yield of the algae/sludge mixture tends to decrease linearly with increasing algae content. This relationship has been fit to a linear model, so that methane yield can be predicted as a function of sludge methane yield and fraction of algae in the digestion mixture. Integrating revised digestion parameters into an LCA-based modeling framework demonstrates that algae digestion is a promising means of making renewable energy.

For the case of the algae farm, updating algae digestion parameters dramatically increases EROI from 1.8 to 3.3. For the WWTP case, integration of algae farming increases EROI from 0.30 to 0.35. Future work will focus on incorporating uncertainty into the LCA models, to better understand how distributions of inputs affect the distribution of output EROI.

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