Anaerobic digestion of black solider fly larvae (BSFL) biomass as part of an integrated biorefinery

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ABSTRACT

Black soldier fly larvae (BSFL) *(Hermetia illucens)* have been used in a variety of applications including composting, animal feed and biodiesel production. This paper presents the results of bio-methane potential (BMP) assays for BSFL biomass according to several hypothetical biorefinery pathways. The mean biomethane potential (BMP) of BSFL was 671 mL CH_4/g VS, while mean BMP of lipid extracted BSFL (LE-BSFL) was 334 mL CH_4/g VS. Mean BMP of adult flies and chitinous cuticle were 570 and 343 mL CH_4/g VS, respectively. The impact of BSFL diet on bio-methane potential and the biomethane potential of BSFL residue was also investigated. This study suggests that anaerobic digestion of BSFL has potential as part of an integrated energy and waste management solution.

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1. Introduction

Anaerobic digestion (AD) has gained popularity as an effective way to treat organic materials generated in agricultural, municipal and industrial processes, while producing low-carbon energy and reducing greenhouse gas emissions. Early implementation of AD systems have generally focused on treating animal manures or biowastes, however a desire to increase energy production and economic viability has led to digestion or co-digestion of energy crops such as maize, beets, grass silage, wholecrop cereals and algae (e.g., [1]). There is also significant current interest in AD as a key enabling technology for landfill diversion of food waste.

The black solider fly is indigenous to the southern United States and is now distributed to warm temperate regions [2]. The life cycle of the BSFL is divided into four phases with an estimated lifespan of 44 days, depending on the environmental conditions (i.e., temperature, moisture, and air supply) (Fig. 1; [3]). Mature flies have no functioning mouth-parts and are not associated with transmission of diseases or considered pests to humans or pets [4]. Black soldier fly larvae (BSFL) have been used in backyard and farm composting systems for decades, mainly to treat animal manures [5,6]. However, more recently, BSFL have been investigated in a variety of additional beneficial such as food, feed, chemicals, enzymes and bioactive compounds [7] and biodiesel production [8,9]. To the best of our knowledge, there is limited prior research that has considered black solider fly larvae as value-added feedstock for anaerobic digestion to generate biogas.

The capability to process putrescent wastes gives BSFL distinct advantages over traditional composting and vemicomposting, which cannot generally accept meat and post-consumer wastes [5]. BSFL have the ability to rapidly consume large amounts of a wide variety of organic wastes, including animal manure, fecal sludge, meat and kitchen waste [10]. In addition to being robust decomposers, BSFL contain significant amounts of protein and lipids and have been investigated as a dietary supplement to feed chickens, swine and fish [11,12]. The high lipid content of BSFL has also been investigated for its potential to create renewable biodiesel fuel [8,9]. The cuticle (or larval skin) is composed of chitin, a long chain polymer of N-acetyl glucosamine, which has been estimated to comprise approximately 10% of the total BSFL dry biomass [3,13]. Chitin has proved versatile for several medical, industrial and biotechnological purposes [14].

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Fig. 1. Life cycle of the black solider fly.

Unlike energy crops, BSFL would not compete with food production in agricultural land usage, nor pose the associated environmental concerns such as soil erosion and pesticide leakage to surface and ground water [43]. Furthermore, BSFL have a high per-acre productivity, fast growth rate, and can be grown on non-arable land areas. These qualities are similar to algae production without the drawback that harvesting and valorization of algae biomass require significant water use and high capital and operation costs, making it economically challenging [15]. Moreover, production of energy crops and algae depends on the photosynthetic fixation of CO_2 and the limiting factor in such biological energy processes is the conversion efficiency in the range of 1–3% of incident solar energy [16].

Like energy crops, BSFL have not been previously digested and thus may offer higher bio-methane potential than manures or biowastes. Additionally, the characteristics of BSFL biomass indicate that it could be a promising candidate for deployment in an integrated biorefinery, akin to a petroleum refinery that converts various feedstocks using different conversion processes to produce multiple co-products including fuel, heat, electrical power and value-added products such as fertilizer. A biorefinery concept developed around anaerobic digestion could be suitable to treat various organic wastes and convert them to multiple products, thus enhancing the economic viability of the integrated system. In a system using BSFL as a feedstock, the BSFL could reduce the weight of other organic wastes and convert them into more concentrated forms as an initial conversion step, or as a pretreatment process prior to anaerobic digestion to enhance the digestion process. BSFL biomass can also be used directly as animal feed or as feedstock to produce biofuel products (biodiesel and biogas), with their residue serving as a valuable feedstock for anaerobic digestion process. Therefore, the objective of the research described herein was to investigate the potential of BSFL as a feedstock for anaerobic digestion, including integration of anaerobic digestion into several different BSFL biorefinery options. Specifically, we investigated the following pathways: (1) direct anaerobic digestion of food waste; (2) anaerobic digestion of BSFL fed on food waste along with residual food waste; and (3) BSFL fed on food waste used to produce biodiesel and digestion of the residual lipid-extracted BSFL combined with residual food

waste. Data are also reported on the bio-methane of BSF cuticle and adult black soldier flies, and characterization data is provided on all these substrates.

Specific research questions addressed were:

- What is the bio-methane potential of BSFL as a feedstock for anaerobic digestion?
- What is the potential for anaerobic digestion in an integrated BSFL biorefinery?
- Can BSFL be used as a pre-treatment to increase bio-methane potential of food waste?
- Does diet have a significant impact on bio-methane potential of BSFL?
- How do BSFL compare to other common anaerobic digestion feedstocks?

2. Materials and methods

2.1. Black soldier fly larvae (BSFL) cultivation

The BSFL used in the study were purchased approximately eight days after being laid as eggs (Biogrubs, California) [45]. Two batches of approximately 1600 BSFL (15 mg/larvae) were inoculated into different feed samples to determine how the nutritional composition of BSFL would be effected by diet. Larvae were held in separate feeding containers in laboratory incubators at room temperature (20–23 °C) for 30 days. When the larvae reached prepupae stage they were harvested.

The first batch of BSFL was fed on 960 g of commercial chicken feed (CF) (Manna Pro, non-medicated starter) combined with vegetable oil (Wegmans brand) totaling 500 g and water totaling 900 g to soften the texture and encourage consumption throughout the cultivation period. Another batch of BSFL was grown on 1000 g of food waste (FM) obtained from the source-separated waste collection bins of the Grace Watson Dining Hall at Rochester Institute of Technology (RIT). FW consisted of fruit and vegetable peelings and seeds, and waste left on plates returned to the dish room. Table 1 shows the nutritional composition of these two feed materials. Harvested BSFL were washed with distilled water to remove residue. The larvae were inactivated at 105 °C for 10 min and stored at 4 °C until used in the experiments described below.

2.2. BMP substrate preparation

Ten different samples were prepared in this study as feedstock for bio-methane potential assays:

Whole body WB BSFL(FW): Intact BSFL (i.e., not chopped and ground) grown on food waste.

Black soldier fly larvae (BSFL-FW) & (BSFL-CF): Batches were prepared of BSFL grown on both food waste (FW) and chicken feed (CF). Mature BSFL were chopped and ground with a porcelain mortar and pestle to obtain a homogeneous particle size which was then passed through a sieve to achieve a particle size distribution between 90 and 250 μm.

Table 1
Composition of chicken feed and food waste destined for BSFL feed.

	Chicken feed (CF)	Food waste (FW)
Crude protein (%dw)	18.0	10.8
Crude fat (%dw)	2.5	13.0
Carbohydrates (%dw)	NM	72.4

Note: NM (not measured), dw (dry weight).

Lipid-extracted black solider fly larvae (LE-BSFL-FW) & (LE-BSFL-CF): After deactivation, both BSFL grown on food waste and chicken feed were dried approximately 12 h at 70 °C until a constant weight was achieved. Dried larvae were ground with a porcelain mortar and pestle to produce a powder which was then passed through a sieve to achieve a particle size distribution between 90 and 250 μm. The ground dried larvae were placed into a filter bag and crude fat was extracted using the ANKOM XT10 (Macedon, NY) extractor per modified AOAC 920.39 [17]. The filter bags were placed in the extractor at 90 °C for 1 h. Triglyceride compounds were extracted using a reflux condenser with petroleum ether (350 mL) under high temperature and pressure. The lipid extracted black soldier fly larvae (LE-BSFL) were dried at 105 °C for 30 min to remove petroleum ether.

Flies (F): Files that had died naturally were carefully removed from the breeding cage and stored at 4 °C until use.

Cuticle (C): Larval cuticle was obtained by manually squeezing out the biomass of the inactivated larvae. The cuticle was dried at 60 °C for 12 h, and ground with a porcelain mortar and pestle to reduce particle size.

Food waste (FW): Approximately 3 kg of food waste (FM) was collected, mixed and prepared by grinding in a VitaMix® blender (1825 Professional Series 750) to reduce particle size to less than 2 mm and produce a homogenous slurry. FW was stored at 4 °C until use.

Residue (R): Residue was collected from the experimental containers after the BSFL were fed until maturity. Residue includes unconsumed food and excretory products. This material was ground in a VitaMix® blender (1825 Professional Series 750) to reduce particle size to less than 2 mm and produce a homogenous slurry. Samples were stored at 4 °C until used.

Manure (M): Dairy manure slurry was collected from a local dairy farm in western New York State that uses a scrape manure collection system.

2.3. Substrate characterization

The substrates were characterized to determine total dry solids (TS) and volatile solids (VS) according to the APHA Standard Methods 2540B and 2540E [18] which involves gravimetric moisture determination at 105 °C and ignition of the dried sample at 550 °C. The pH was measured using a Mettler Toledo meter at room temperature (22 ± 1 °C) calibrated with buffers at pH = 4.0, 7.0, and 10.0.

Crude protein was calculated from Kjeldahl nitrogen (TKN) content per modified AOAC Method 984.13 [19] by a third-party lab (Counterparts Chemistry, Rochester, NY). Crude protein was calculated by multiplying measured TKN by a factor of 6.25 (assuming 16% N). However, as noted by Diener et al. [2] and Yang et al. [20]; this can result in an overestimation of protein content due to nitrogen contained in the chitinous cuticle. Therefore, a corrected crude protein content of BSFL was also reported whereby the N content associated with the chitin of BSFL was subtracted prior to multiplying by the protein conversion factor of 6.25.

Crude fat was measured by extraction with solvent per modified AOAC 991.36 [21]. Crude carbohydrates were calculated by subtracting the crude fat, crude protein, moisture, ash and chitin content from the total dry mass of the sample per modified AOAC Method 986.25 [22] and as described in Yang et al. [20] as follows:

$$\text{Carbohydrate } \% = 100 - (\text{moisture} + \text{ash} + \text{crude fat} + \text{crude protein} + \text{chitin}) \times 100 \% \quad (1)$$

Food waste (FW) and residue (R) were also analyzed for hemicellulose, cellulose and lignin by an external lab (Dairy One, Ithaca, NY) based on the ANKOM Technology Method 5 for acid detergent fiber in feeds, Method 9 for acid detergent lignin per modified AOAC 973.18 (1977) and method 6 for neutral detergent fiber per modified the methods of Van Vuuren et al. [23] using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, NY, USA). In this study, the chitin content was not measured and the chitinous fraction of larvae was adopted from Diener et al. [2].

2.4. Batch bio-methane potential (BMP) assays

The biomethane potential (BMP) assays conducted were based upon the original protocol described by Owen et al. [24] with modifications based upon other prior studies [25–27]. The inoculum was obtained from the solid-separated effluent of a commercial anaerobic digester co-processing dairy manure and industrial food wastes in a 70:30 proportion and operated at mesophilic temperature (37 °C). Inoculum was degassed at 37 °C for five days to deplete residual biodegradable organic matter. BMP samples were prepared using a 2:1 ratio (gVS inoculum: gVS substrate added). No additional nutrient media were added, as the dairy manure-based inoculum was assumed to contain the appropriate anaerobic microorganisms [28,29].

Batch BMP assays were prepared in triplicate and conducted using the AMPTS II Bioprocess Control system (Lund, Sweden). BMP vessels has volume of 600 mL with working volume of 300 mL. Three blank inoculum samples were prepared and the BMP results of the substrates were obtained by subtracting the average methane production of the blanks. Results were normalized by the mass of volatile solids of substrate added and reported as standard methane yield (mL CH₄/g VS added). Microcrystalline, 20 μm cellulose (SigmaCell type 20) was used as a positive control sample to measure inoculum performance. The pH of each sample was measured after the solution was prepared and prior to the start of the experiment, and ranged from 6.5 to 7.5. After sealing, each sample was flushed with 99.99% purity nitrogen to establish an anaerobic environment at the start of the test. Samples were incubated at 37° (± 1 °C) for 30 days or until the BMP experiments were terminated when daily biogas production during three consecutive days fell below 1% of the cumulative volume of the biogas [30]. The reactor bottles were mixed intermittently using a stirring motor for 10 s every 60 s. Biogas produced was continuously fed through a 3M fixing solution of sodium hydroxide (NaOH) to absorb carbon dioxide (CO_2). An automated data collection system measured bio-methane production via a volumetric flow device and adjusted to standard temperature and pressure (STP). Concentrations of CO_2 and CH_4 were measured between tests to verify the fixing efficiency of the system prior to and after the fixing station, using a gas chromatograph with thermal conductivity detector (TCD) with helium carrier gas and HaysepQ packed column.

2.4.1. Estimation of theoretical bio-methane potential (B_u) and extent of biodegradability (f_d)

Theoretical BMP production (B_u) of all substrates were calculated using Buswell's equation (Eq. (2)). These B_u values were estimated by the nutrient composition of each substrate where, where proteins ($C_5H_7O_2N$), carbohydrates as glucose ($C_6H_{10}O_5$), fat/lipids ($C_{57}H_{104}O_6$) and chitin ($C_8H_{13}O_6N$) were assumed to have biomethane potentials of 496, 415, 1014 and 441 mL CH₄/g VS, respectively. Biomethane potential of chitin was calculated using Buswell's formula according to its chemical composition [31]; see Section S.1 for details. Results were compared to the theoretical BMP production (B_u) estimated based upon the organic fraction composition (OFC) as described by Nielfa et al.

[32] and Raposo et al. [33] and modified to account for chitin as follows:

$$Bu = 0.415(\% \ Carbohydrates/VS)$$
$$+ 0.496(\% \ Crude \ Proteins/VS)$$
$$+ 1.014(\% \ Crude \ Fat/VS) + 0.441(\% \ Chitin/VS) \quad (2)$$

The extent of biodegradability (f_d) was computed as the ratio of observed bio-methane potential (B_o) to theoretical bio-methane potential (B_u) on a VS basis (i.e., mL CH$_4$/g VS added):

$$f_d = \frac{Bo}{Bu} \qquad [3]$$

3. Results and discussion

3.1. Substrate characterization

The measured characteristics of the samples are summarized in Table 2. BSFL fed on both diets (food waste and chicken feed) contained about 65% moisture, with high crude fat and protein contents compared to their diets. BSFL grown on fresh food waste (FM) had crude protein content of 51.3% of TS, while the BSFL grown on chicken feed (BSFL-CF) had 38.2% of TS. However, adjusting the crude protein for N found in chitin, which misrepresents actual protein availability, resulted in 47.5% protein in BSFL (FW) (Table S.1) and 34.4% protein in BSFL (CF) based on a chitinous fraction of BSFL of 8.72% (DW) [2]. Lipid content from BSFL (FW) and BSFL (CF) was 38.5%TS and 41.6%TS, respectively (Table S.2). These values were similar to those reported in the literature by Diener et al. [2] (also see Table S.3). The crude protein content of adult flies (F) exceeded values for BSFL, however these values were not adjusted for cuticle protein content as the percent cuticle in mature flies could not be found in the literature. Thus we can assume that the reported value is slightly overstated.

The composition of food waste (FW) and residue (R) remained relatively constant for most nutrients, with a few notable exceptions (Table 3). Nitrogen content (TKN) of residue was determined to be nearly twice that of raw food waste. This may be due to the digestive enzymes released from the salivary gland and gut of the larvae while feeding on food waste [34] which increases N-mineralization, elevating the concentration of ammonia (NH$_4^+$) in the food waste residue [35]. It also may be due to chitin discarded by the pupae after each instar (i.e., growth stage of the larvae) that

may be contained within the residue. Higher lipid content and slightly higher cellulose and lignin concentrations were also observed in the DR which was expected because BSFL prefer non-fibrous foods. However, Li et al. [36] reported that BSFL degraded and consumed lignocellulose in dairy manure and thus enhanced accessibility of enzymes. A decrease of 13% in total solids content of food waste in the current study was also observed, which was attributed to evaporation.

3.2. Bio-methane potential

Measured and theoretical biomethane potential (BMP) results are presented in Table 4. The biomethane potential of cellulose controls showed good agreement with expected results measuring 322 (σ = 11) mL CH$_4$/g VS (n = 3). The measured bio-methane potential of 238 (σ = 19) mL CH$_4$/g VS (n = 3) for dairy manure also agreed well with previous studies [27,29]. BSFL showed similar B_o for both feed regimes, with BSFL (FW) yielding 675 (σ = 118) mL CH$_4$/g VS (n = 9) and BSFL (CF) resulting in 661(σ = 29) ml CH$_4$/g VS (n = 3), and thus BSFL diet did not appear to result in a statistically significant difference in mean biogas production. It is also notable that the standard deviation of BSFL (FW) was large relative to other tested substrates. We believe this may have resulted from larval development and consumption rates that were highly dependent on the local composition of the food waste and the specific local environmental conditions. The nutrient value and chemical composition of larvae may have therefore varied significantly during various life stages of the black soldier fly, and this variation was reflected in the resulting biomethane potential measurements. The standard deviation of BMP measurements from BSFL fed on chicken feed (BSFL(CF)) was substantially smaller, but in this case the feed material was produced by a manufacturing process and therefore much more homogeneous.

BMP experiments with Residue (R) produced 502 (σ = 9) mL CH$_4$/g VS (n = 3) while fresh food waste yielded 449 (σ = 53) mL CH$_4$/g VS (n = 3). The difference is not statistically significant and could be attributed to heterogeneity of the food waste. Before ingestion and during digestion, BSFL produce and discharge digestive enzymes, which promote the conversion of food waste biomass into a more soluble and liquefied form. BSFL also have the ability to degrade the cellulose, hemicellulose and lignin contents in food waste and modify the structure of fiber content. BSFL activity in the food waste leads to deposition of nutrients in the residue (uneaten food + excretory products) that are more readily available for bacteria during the anaerobic digestion process

Table 2
Characterization of all substrates. FM: fresh matter, TS: total solids, VS: volatile solids.

Substrates	%TS/FM	%VS/TS	%VS/FM	Composition of fresh matter (FM)[a]					Composition of solids (TS)[a]			
				% moisture/FM	% ash/FM	% lipid/FM	% protein/FM	% carbo-hydrate/FM	% ash/TS	% lipid/TS	% protein/TS	% carbo-hydrate/TS
WB BSFL (FW)	41.1	94.7	39.0	58.9	NM	NM	NM	NM	NM	NM	NM	NM
BSFL (FW)	35.4	95.8	33.9	64.6	1.5	13.6	17.8	2.4	4.3	38.5	51.3	5.9
BSFL (CF)	34.4	91.9	31.6	65.6	2.7	14.3	13.9	3.5	7.8	41.6	38.2	12.4
LE-BSFL (FW)[b]	96.1	99.2	95.3	64.6	1.1	NM	17.8	2.4	4.3	NM	51.4	11.1
LE-BSFL (CF)[b]	95.7	87.6	83.8	65.6	2.7	NM	13.9	3.5	7.8	NM	38.2	12.4
Flies	51.7	95.3	49.3	48.3	2.4	15.7	29.3	4.3	4.7	30.3	56.7	8.3
Cuticle	43.8	84.7	37.1	56.2	6.7	NM	37.1	NM	15.3	NM	84.7	NM
Food waste	25.5	96.1	24.5	74.5	1.0	3.3	2.8	18.5	3.9	12.9	10.8	72.4
Residue	22.6	93.3	21.1	77.4	1.5	4.7	4.4	12.0	6.7	20.8	19.4	53.1
Manure	7.4	85.9	6.3	92.6	1.1	NM	NM	NM	14.5	NM	NM	NM

Note: NM (not measured). All samples were measured in triplicate.
[a] Rounding error may lead to nutrients not summing to 100% total solids. WB-BSFL (FW): Whole body Black solider fly grown on food waste, BSFL (FW): BSFL grown on food waste, BSFL (CF): BSFL grown on chicken feed, LE-BSFL (FW): Lipids extracted BSFL grown on food waste, LE-BSFL (CF): Lipids extracted BSFL fed with chicken feed.
[b] 38.5% and 41.6% of crude lipid was extracted from BSFL(FW) and BSFL(CF). The samples were dried at 105 °C prior to the lipid extraction and the moisture content of two samples before preceding the extraction was 3.9% and 4.3% respectively. The percent of volatile solids per gram of fresh lipid extracted BSFL (%VS/FM) without the drying process is 35 (%). Protein calculation is based on total nitrogen x 6.25 for all the substrates.

Table 3
Characteristic of food waste and residue.

Characteristic	Fresh food waste (FW)	Residue (R)	Difference (R − FW)
pH	7.9	8.1	+0.2
Dry matter (%wt)	25.5	22.6	- 2.9
Moisture content (%)	74.5	77.4	+2.9
Nitrogen, TKN (g/kg TS)	17.2	31	+13.8
Lipid (%dw)	13.0	20.8	+7.8
Hemicellulose (%dw)	6.9	7.1	+0.2
Cellulose (%dw)	3.6	5.5	+1.9
Lignin (%dw)	1.4	2.3	+0.9

*Note: dw (dry weight), TS (total solids).

Table 4
Measured bio-methane potential (B0) compared with theoretical bio-methane yield (Bu) calculated using Buswell's equation.

Substrate	Measured BMP (B_0, ml CH4/g VS) Average (S.D.)	Theoretical BMP (B_u, ml CH4/g VS)	Extent of bio-degradation ($f_d = B_0/B_u$)
WB-BSFL (FW)	108 (65)	698	0.15
BSFL (FW) (n = 9)	675 (118)	698	0.97
LE-BSFL (FW)	363 (32)	472	0.77
BSFL (CF)	661 (29)	720	0.92
LE-BSFL (CF)	306 (23)	442	0.69
Food waste	449 (53)	505	0.89
Residue	502 (9)	566	0.89
Cuticle	343 (7)	373[a]	0.92
Flies	570 (51)	653	0.87

*Samples were tested in triplicate (n = 3) unless otherwise noted.

[a] Theoretical BMP of chitin is 441 mL CH4/g VS. Measured ash percent per total solids of cuticle is 15.4 (%), therefore absolute theoretical BMP produced from cuticle is 373 mL CH4/g VS.

without the requirement of chemical, mechanical or thermal pre-treatments [11,37]. Therefore, the potential of BSFL as a pre-treatment for AD should be further investigated.

Lipid-extracted BSFL fed on food waste (LE-BSLF-FW) produced more methane (363 (σ = 32) mL CH4) than the equivalent chicken feed-fed biomass (LE-BSLF-CF) (306 (σ = 23) mL CH4). This may be because FW is more readily digestible than the grains in chicken feed. Whole body black soldier fly larvae (WB-BSFL) showed the lowest B_o of 108 (σ = 65) mL CH4/g VS, with a slow decay rate and poor biogas production throughout the test period of 30 days. It was observed that the structure of cuticle was not easily degraded by microbes, which prevented them from accessing nutrients within. Therefore, it is suggested that BSFL should be ground to reduce the particle size and increase the surface area available for microbial activity. BSFL release their cuticle into the residue when they reach the prepupae stage, and again when the larvae turn into flies. Hence, bio-methane potential of the chitinous cuticle was measured resulting in B_o of 343 (σ = 7) mL CH4/g VS (n = 3) Mature black soldier flies, which live only 8–9 days and do not consume anything other than water, were also tested and yielded BMP of 570 (σ = 51) mL CH4/g VS (n = 3). Fig. 2 summarizes the measured bio-methane potentials of the various substrates studied.

3.3. Comparison to theoretical BMP

The extent of bio-degradation (f_d) was calculated via Eq. (3) and compares the observed bio-methane potential (B_0) to the theoretical bio-methane potential (B_u). (Table 4). B_u provides the maximum biomethane potential yield, which is expected to be higher than Bo because some of the available nutrients are not accessible for the anaerobic bacterial leading to incomplete digestion. BSFL (FW) and BSFL (CF) produced f_d values higher than 90% and thus were highly degradable. Degradability values are affected by the sample preparation variability and/or uncertainty in determining the lipid and protein content of substrates which are key variables in the theoretical calculation. Degradation for lipid-extracted BSFL fed on food waste (FW) and chicken feed (CF) were 77% and 69%, respectively.

Food waste and residue had the same biodegradability value of 89%. Cuticle displayed high biodegradability (90%) when compared to the theoretical value for chitin, however the unknown amount of biomass (protein, lipids or carbohydrates) remaining in the cuticle sample could have contributed to an inflated degradation estimate. Adult flies also resulted in bio-degradability of >85%.

3.4. BSFL as anaerobic digestion feedstock

Because of the current interest in anaerobic digestion (AD) as an organic waste management and renewable energy production technology, it is also instructive to compare the results presented herein for BSFL biomass to other potential AD feedstocks. The measured mean biomethane potential (B_o) of BSFL (CF) was 661 mL CH4/g VS and for BSFL (FW) 675 mL CH4/g VS. These values are higher than many common AD feedstocks, including energy crops, algae and manures (see Table S.4). Moreover, it takes 14–28 days to harvest BSFL biomass depending on the feeding and environmental condition [2], which is substantially shorter than most dedicated energy crops (e.g., 157 days for maize) [2,38,44]. also reported that under favorable conditions, one square meter could yield approximately 145 g of dry prepupal biomass per day (252 g/m^2/day; wet weight) when fed with 4.6 kg of food waste per square meter per day. To compare potential areal methane production values, it is reasonable to envision a scaled-up version of a BSFL- or algae-based AD system using an area for feedstock production that is on the order of 100 m by 100 m. Using a hectare as the area basis, methane production potential of food waste alone and BSFL alone were calculated to be approximately 1,800,000 m^3 CH4/hectare and 340,000 m^3 CH4/hectare, respectively (see S.2 and S.3 for calculations). Alternatively, methane production from a BSFL composting system, in which BSFL reduces approximately 19% of the mass of food waste and leaves 81% of residue, produced approximately 1,874,168 m^3 CH4 per hectare per year (see S.4 for calculations). The latter value includes the combined methane production of BSFL and residue, and demonstrates that the mixed substrates may provide higher methane production than the individual substrates processed separately.
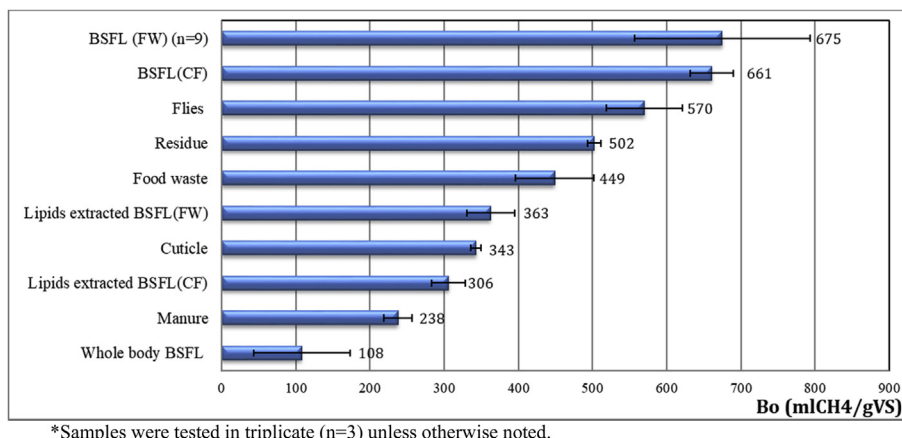
*Samples were tested in triplicate (n=3) unless otherwise noted.

Fig. 2. Summary of the observed specific bio-methane yield per unit mass (B0) for the substrates tested (ml CH4/g VS added). Error bars represent the standard deviation of B0 for each substrate.

Reported algal productivity ranges between 13 and 40 g/m^2/day [39]. The growing rate of larvae is much faster (145 g/m^2/day [2]); than that reported for algae, thus requiring only 0.27 m^2 to produce the same amount of biomass per day. BSFL show comparatively high biomass yield and bio-methane production per hectare due to their fast growth rate and high bio-methane potential. The standard methane yield of BSFL (mL CH$_4$/g VS) is 1.5–2 times higher than the best performing energy crops in use today, generally ranging from 375 to 450 mL CH$_4$/g VS, and about 2 times higher than the selected algae in terms of same land foot print area needed for feedstock production. Another system-level factor to consider which has not been taken into account in these calculations is the hydraulic retention time. Energy crops typically are anaerobically digested 50–150 days [40] and the rention time for algae ranges between 15 and 28 days [41], whereas food waste and BSFL may have retention times on the order of 28 days. Further research is needed to understand the various resource and infrastructure assets required to make large-scale BSFL farming economically viable.

3.5. Integrated BSFL biorefinery for biogas and biodiesel

A biorefinery is a renewable analog to a petroleum refinery, in which all system outputs are utilized in some manner, with minimal or zero waste. Drawbacks of crop-based biogas and bio-diesel production are high feedstock cost, and competition with food resources and land use. Larvae have high lipid content between about 20% and 40%, which is comparable to other biodiesel feedstocks (i.e. soybean, rapeseed oil, sunflower oil, algae). Thus, crude lipids extracted from BSFL fed with solid organic wastes could be a non-food crop feedstock for biodiesel production as well as. Using the well-known reaction for biodiesel production via transesterification, wherein fatty acids can be derived from oil extracted BSFL, Li et al. [36] reported that most of the fuel properties of biodiesel produced from BSFL were comparable to rapeseed oil-based biodiesel. In our experiments, 25 g of larval oil were extracted from 184 g of larvae biomass (FM), or 35.4% DW, which is consistent with other studies (see Table S.5). Li et al. [42] and Zheng et al. [8] reported that biodiesel yield converted from larvae oil was approximately 93% by mass. Therefore we assume that the observed oil extracted from the BSFL biomass could produce standard quality biodiesel at a conservative conversion rate of 93%.

Because BSFL biomass contains primary feedstock constituents for both biomethane and biodiesel production, various hypothetical bio-refinery scenarios can be constructed and analyzed. The mass flows and energy outputs of three biorefinery options based upon the conversion of 1 kg of food waste (FM) and measurements from this study are illustrated in Fig. 3.
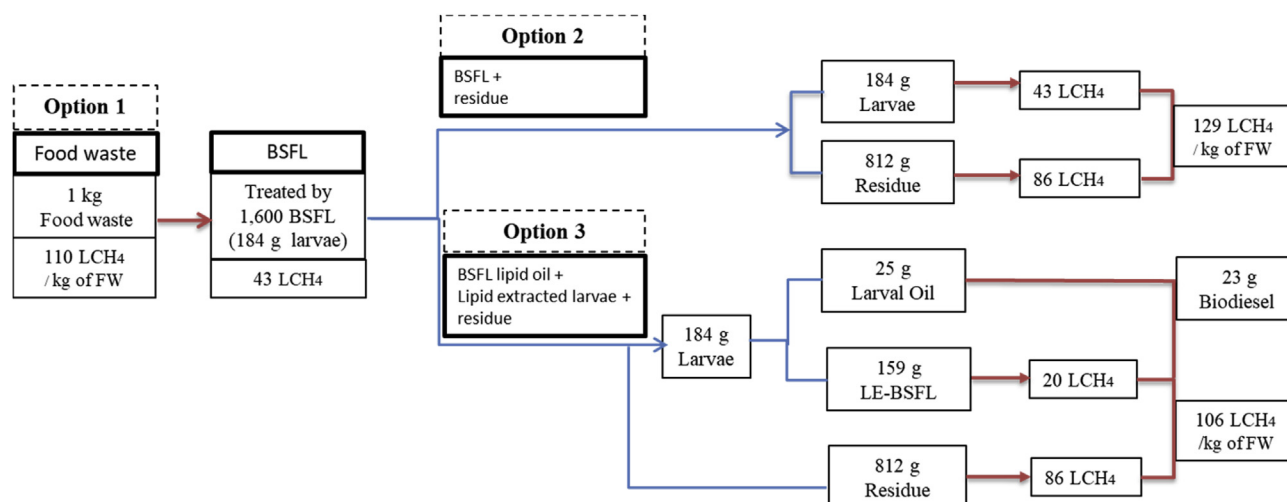

Fig. 3. Flow diagram of integrated biorefinery options based on converting BSFL biomass to biogas and biodiesel.

Table 5
Material inputs and energy outputs of integrated biorefinery options. FM: fresh matter & FW: food waste.

Option	Substrate	%VS/FM	Mass (kg FM)	Estimated BMP yield (m³ CH₄/kg VS) & Biodiesel yield (kg)	Energy yield (MJ)	Total energy output (MJ/kg FW)
1	FW	24.52	1.00	0.45	4.22	4.2
2	BSFL(FW)	33.94	0.184	0.68	1.59	4.9
	R	21.11	0.812	0.50	3.30	
3	Larval Oil	–	0.025	0.023	0.77	4.9
	LE_BSFL (FW)	35.0	0.159	0.36	0.87	
	R	21.11	0.812	0.51	3.30	

Lower heating value (methane) = 38.5 MJ/m³; NCSU Cooperative Extension, Conversion factors for bioenergy "http://www.ces.ncsu.edu/forestry/biomass.html". Lower heating value (biodiesel) = 37.52 MJ/kg.

- **Option 1: Direct anaerobic digestion of FW:** 1 kg of food waste (245 gVS) digested via anaerobic digestion process produces 110 L CH₄, based on measured BMP of 449 mL CH₄/g VS.
- **Option 2: Pretreatment of FW by BSFL followed by AD of BSFL and residue (R):** About 1600, 5-day old larvae (15 mg/larva) were inoculated into 1 kg of FW. 188 g of FW were consumed after 30 days (reduction of 19% FM by mass). 184 g of BSFL (62 gVS) were produced and 812 g of residue remained (171 gVS). 184 g of BSFL and 812 g of residue were treated in an anaerobic digestion process and produced 43 L CH₄ and 86 LCH₄ respectively. A total of 129 L CH₄ was obtained from option 2.
- **Option 3: BSFL fed on FW harvested for biodiesel production and remaining residuals (LE-BSFL and DR) anaerobically digested for biomethane:** Similar to Option 2, 1 kg of food waste was converted by BSFL to produce larval biomass and 25 g of larval oil was extracted from 184 g of larvae. Approximately 23 g of biodiesel could be obtained from larvae oil with 93% biodiesel conversion rate [8]. 159 g of fresh LE-BSFL was obtained after the lipid extraction that produces 20 LCH₄ via anaerobic digestion. A total of 106 LCH₄ would be produced from the combination of lipid-extracted BSFL (LE-BSFL) biomass and residue (R).

Material inputs, energy outputs, BMPs of each substrate and biofuel yields for these three biorefinery systems are summarized in Table 5. Option 1 (direct food waste digestion) as the reference scenario produced 4.2 MJ from 1 kg fresh food waste and its energy output was obtained solely from bio-methane production. Option 2 provided bio-methane from both BSFL and residue, and yielded an energy output of 4.9 MJ (17% higher than direct food waste digestion). Option 3 produced the same total energy output as Option 2 through biodiesel and bio-methane production. However, the potential advantage of Option 3 is that biodiesel can be used in the transportation sector and generally commands a higher market value on a per MJ basis. In any case, the added capital and operation and maintenance (O&M) cost of BSFL growth and harvesting would need to be rigorously evaluated against the projected benefits of greater biomethane production, or combined biomethane/biodiesel production. Also, for deployment in a biorefinery application, other potential value-added uses of the BSFL biomass should be considered, such as animal feed, with the residue still used as anaerobic digestion feedstock as described above.

4. Conclusions

Bio-methane potential of BSFL is 1.5–2 times higher than other representative feedstocks, including energy crops and algae. LE-BSFL (FW) yielded 363 mL CH₄/g VS with 0.12 g biodiesel/g dry BSFL. The larvae diet did not significantly affect the anaerobic digestion output. BMP of residue left by larvae was 502 mL CH₄/g VS, higher than that of untreated food waste, which indicates BSFL pretreatment has the potential to increase biogas production. This study has demonstrated that BSFL can be a viable part of integrated biorefinery systems. E-supplementary data can be found in the online version of the paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.renene.2018.04.093.

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