Analyzing Nutrient Mass Balance Transfer and Recycling Yields of Black Soldier Fly Larvae, *Hermetia illucens*, Anaerobic Digestion, and Compost Methods Using Food Waste as A Primary Substrate.

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Report Summary

The purpose of this project was to formulate a reliable model system that can provide alternative biomass reduction and nutrient recycling methods while simultaneously generating economic growth and profit in this field of work. This project reflects work completed under a Divert Nova Scotia Grant and has been and continues to be carried out between Dalhousie University and the Verschuren Centre at Cape Breton University with the help of industry partners as a part of an MSc degree. This program was comprised of a total of four courses, lab work, and a final thesis to be presented at the end of the 2018-2019 year.

Progress to date on the multiple aspects of nutrient disbursement through the three systems has attempted to compare three bioconversion systems (black soldier fly larvae feeding, anaerobic digestion, and compost). Nutrient mass balance transfer has been measured in terms of the by-products from each of the systems and results are being compared between different substrates in each of the systems. These results along with future results will be used in a model system formulated to demonstrate the most efficient nutrient mass balance transfer and recycling capabilities for each of the three systems based on substrate type. One of the key challenges is to be able to compare systems on an equivalent unit basis e.g. nutrient value for energy production, vs protein production. This model can then be used by a community, industry, or municipality to maximize nutrient value through diversion and thereby economic return of any organic waste stream. Organic waste to landfill will not only be reduced as a result of this model but will also allow for maximal value to be achieved for specific source product types and optimal nutrient recovery depending on desired value proposition.

Introduction

Roughly one third or 1.3 billion tons of food meant for human consumption is being wasted every year (Godfray *et al.,* 2010; Gustavsson *et al.,* 2011). Food and agricultural waste biomass provide a unique opportunity as a potential renewable food and energy source. If the food cycles can be closed, the nutrient value that would otherwise be lost in this waste may be restored and put to beneficial use. Three systems that have differing capacity to perform this bioconversion are: the rearing of black soldier fly larvae, *Hermetia illucens,* anaerobic digestion, and composting. Comparatively to what degree these bioconversions work for differing feed stock has not been quantified.

Most of us are familiar with composting as it has been utilized for many years. Anaerobic digestion is a technology that has seen a resurgence over the last 20 years and it is used in large processing plants, WWT, and farm applications around the world. The black soldier fly larvae have just recently begun to show their potential as a biomass conversion organism and their full potential has yet to be discovered. Nutrient conversion in each of these three systems has been studied to some degree before, but never have they been directly compared in terms of biomass conversion per specific substrates.

In other words, which waste types will generate the most efficient and optimum bioconversion in each of these three systems? By conducting research using five common waste substrates on each of these systems, an accurate model can be produced so that maximum bioconversion options and rates can be achieved. This would ensure biomass reduction, along with nutrient extraction, reformation and reuse. This research will allow mass balance conversion comparisons to be done efficiently and reliable between the three very different systems with different waste substrates. A model system can be formulated and utilized by companies, establishments, and municipalities as a profitable and reliable method of recycling nutrients.

Black Soldier Fly

Description

Hermetia illucens, the black solider fly (BSF) is a commonly occurring, fly species native to tropical and warm temperate regions across the globe (Sheppard *et al.,*2002). In North America, it can be found living in the South Eastern United States (Newton *et al.,* 2005). The adult fly is typically half an inch in length, has a dark colored head, thorax, abdomen, and legs while its tarsi are typically a pale white in color. These distinct physical features allow for simplified identification of the BSF in comparison to other fly species. Adult flies typically live for 5-8 days, in which it has time to breed and lay eggs if female. Each female BSF will lay about five hundred eggs, typically near but not on a source of organic waste or feed. Each egg is typically one millimeter in length and is a white cream colour (Tomberlin and Sheppard, 2001).

Perhaps the much more interesting stage of this species is the larval stage. Black soldier fly larvae (BSFL) have a particular yet flexible lifecycle. After the eggs are laid, they will hatch as BSFL in about 104 hours (four days). The larvae stage of the lifecycle can be broken down into six instars in which they pass through over a course of approximately fourteen days. The larvae are riveted and are a drab white in colour. They become duller in colour as they pass through instars and come closer to the pupae stage of the lifecycle. The larvae contain a feeding mouth part at one end of the body and digestive and extraction systems at the other end (Newton *et al.,* 2005).

The first five instars occur during the feeding segment of the BSFL lifecycle and the sixth instar simply refers to the pre-pupae stage of development. Over the course of two weeks, the larvae will feed on any decaying matter that is available and will grow to be anywhere between ¼ to ¾ of an inch. After feeding for the span of fourteen days, the larvae will slow their feeding rates until they completely stop eating entirely. By this time the larvae will be much larger and plumper than when they first emerged from their egg capsules. They will also be a charcoal grey to black colour. The pre-pupae tend to crawl away from any remaining organic or decaying matter source before the pupation stage begins. The pre-pupal stage will typically last three to five days. After this, they become pupae.

The pupae stage is the stage just before metamorphosis occurs. During this stage, the pupae stop moving entirely and will use their stored energy gained over the two-week feeding period to promote metamorphosis to the adult fly stage. In total, the BSFL lifecycle is about twenty-one days under favourable conditions (feed, temperature, and humidity). However, under unfavourable conditions, there has been evidence that the BSFL can extend their lifecycles up to months longer than the typical twenty-one-day larval cycle (Tomberlin *et al.,* 2009). This is extremely beneficial when growing larvae in the winter when temperature levels and food sources are much lower than for standard growth.

Nutrient Mass Balance Conversion

Thanks to the highly efficient feed conversion ration of the BSFL, feeding them food waste products will result in mature larvae rich in crude protein, crude fat, and oils (Diener *et al.,* 2009), typically 40% CP, 40% Fat. Additional nutrient value can be found in the remaining frass after the feeding timeframe including nitrogen, carbon, phosphorus, potassium and others.

Applications in Agriculture

Once a BSFL population has been established, this system is quite standard to maintain. Mature larvae and frass can be harvested for their feed-replacement and fertilizer markets respectively.

Black soldier fly larvae can serve as a feed alternative or additive for species such as hogs, poultry, aquaculture and companion animal and the frass material can serve as fertilizer to aid plant and crop growth in the field, greenhouse or nursery. Having a BSFL production systems will therefore present potential savings in animal feed, plant production and reduce costs of waste transportation along with potential environmental implications through greenhouse gas reduction by reducing food waste biomass buildups.

Anaerobic Digestion

Description

Anaerobic digestion is a naturally occurring process in which organic material (food waste, animal manure, yard clippings, sewage waste, etc.) undergoes a series of steps under specific conditions that enhance microbial digestion into two very intriguing by products. Each distinct step of anaerobic digestion is governed by a specific class of microorganism (McInerney and Bryant, 1981). Anaerobic digesters designs can be classified based on temperature (mesophilic or thermophilic) and flow mechanism. Key control parameters are; the lack of oxygen in the system, the temperature of the system at each specific step, and the pH of the system. Retention time (HRT), mixing techniques, carbon nitrogen ratio (C:N), organic loading rate, total solids, and volatile solids are additional input factors affecting anaerobic digestion.

The first step in the process of anaerobic digestion is hydrolysis. During this step, larger polymers in the organic waste such as proteins, starches, and fats are broken down by acidogenic bacteria. This process generates smaller polymers such as sugars, amino acids, and long chain fatty acids, which can then be further reduced. The hydrolysis step is typically the rate determining step of anaerobic digestion, especially with substrates that have high organic content (Bajpai, 2017). Acidogenesis is the next step in anaerobic digestion. During this step acid-forming bacteria convert the organic substrate into a series of organic acids which are then further transformed into primarily acetic acid. The further conversion of organic acid products is described as the third step of anaerobic digestion, acetogenesis. This step is carried out by acetogenic bacteria and is sometimes included in the acidogenesis step. The main products of this step are hydrogen and acetic acid. Both are substrates which methanogens can use to generate methane. Methanogenesis is the final and most critical step of anaerobic digestion. This is when the biomethane is produced. Methanogens will produce methane in two different ways, either by cleavage, where two acetic acid molecules are cleaved to form methane and carbon dioxide or by the reduction of carbon dioxide with hydrogen (Lettinga, 1995).

The temperature is important because once again, microorganisms thrive under different conditions. Mesophilic bacteria prefer temperatures between 30° and 40°, while thermophilic bacteria are most active at a higher range of 50°C to 70°C. Anaerobic digestion can occur at either or both temperature ranges (in separate tanks) depending on system. Both temperature ranges have their benefits and associated costs (Ziembinska *et al.,* 2014). The optimum temperature may depend on the type of substrate that is being used in the digester. The pH level for anaerobic digestion is typically optimal above 7 and below 8. A neutral pH will ensure acidogenesis is not inhibited along with nor be harmful to the methanogen population in the digester (Steyer *et al.,* 2002).

Retention time, or the total time it takes for completion of the anaerobic digestion process is typically 25-30 days for a mesophilic digester and about half that time for a thermophilic digester. Mixing of the organic substrate is important to maximize contact between microbes and substrate and nutrients available. An optimum carbon nitrogen ratio of 20-30 (C:N) is necessary for optimum biogas production, which can often be reached by mixing of substrates that have a high carbon nitrogen ratio with others of lower carbon nitrogen ration in the digester. An example of this would be food waste (higher C:N ratio) mixed with animal manure (lower C:N ratio). Organic loading rate is also very important because each anaerobic digester has a loading rate limit which if surpassed can cause inhibition of the entire process. This limit is typically expressed in terms of volatile solids (VS) or chemical oxygen demand (COD) per cubic meter. Total solids in the substrate is important as this determines the volume available in the digester and will affect how much water is needed to run the system (Steyer *et al.,* 2002).

Nutrient Mass Balance Conversion

Food waste sent through an anaerobic digestion process would produce a renewable energy source in the form of biomethane gas. The amount of biogas produced can be manipulated based on the ratio of food waste and inoculum used in the digester. The anaerobic digestion process will also produce a valuable but high volume, nutrient rich liquid digestate..

Applications in Agriculture

Anaerobic digestion provides a method to appropriately control and recycle specific liquid animal manures. By blending animal manure and slurries with other bioproducts, produces more biomethane, a renewable, clean energy source that can be used directly or indirectly through CHP units . The leftover digestate can be added to soil as a slow release fertilizer to aid with plant and crop growth (Holm-Nielson *et al.,* 2009).

Compost

Description

Composting, a more familiar and traditional process is a method in which large amounts of food waste can be reduced to a format for reuse. This process, like anaerobic digestion allows for the breakdown of organic matter primarily by microorganisms. However, unlike anaerobic digestion, composting is an aerobic process, involving facultative aerobic microorganisms.

For successful composting it is recommended for the material to have approximately a starting 30:1 carbon nitrogen ratio so that organic material can be optimally broken down into a soil-like substance by the respective microorganisms and macroorganisms (Ghazifard *et al.,* 2001). The microorganisms include some bacteria and fungi species, within which, aerobic bacteria species are the most influential to the composting process. There are three main types of aerobic bacteria species that aid in composting. Psychrophilic bacteria work at the lowest temperature range and generate the least amount of heat. However, they do release a substantial amount of heat for the next type of bacteria to begin breaking down the material which are mesophilic bacteria. The mesophilic bacteria begin rapidly breaking down easily degradable compounds which simultaneously produces additional heat. These rising temperatures (approximately 50°C - 70°C) allow for thermophilic bacteria to thrive in the compost. These bacteria are responsible for breaking down proteins, fats, and complex carbohydrates such as cellulose (Ghazifard *et al.,* 2001). Actinomycetes aid in breakdown of material that is more resistant to the other types of bacteria such as cellulose, starches, and proteins (Wang *et al.,* 2014). The microorganisms therefore modify the chemistry of the compost material. As the microbes use up oxygen during the composting process carbon dioxide and heat is given off as by-products and after approximately 30 days a young, nutrient rich, humus-like compost remains.

Nutrient Mass Balance Conversion

Composting food waste substrates will generate a soft nutrient rich humus containing available nutrients, particularly nitrogen, phosphorus, and potassium, all essential to plant and crop growth. The compost will also contain other macro and micronutrients such as calcium, magnesium, sulfur, manganese, iron, and zinc.

Applications in Agriculture

The compost process will promote improved availability of important macro and micronutrients. The nutrient value of the compost can be utilized to aid plant and crop growth making it a great alternative or additive to any conventional fertilizer products (Martinez-Blanco *et al.,* 2013). Composting is also a relatively low tech and inexpensive process with substantial amount of upside for those individuals or companies working in the agricultural industry. Primarily the benefits will be as a fertilizer, but composting may also reduce costs for conventional fertilizers, reduce waste transport costs and food waste biomass..

Hypothesis

It is hypothesized that the characteristics of each biological system will be a valuable tool to differentiate desired methods for the bioconversion of food waste types fed to each of the black soldier fly larvae, anaerobic digester, and composter allowing for a model system to be formulated.

Objectives

Overall Objective:

The objective of this project was to formulate a working model demonstrating the most efficient nutrient mass balance transfer capabilities of black soldier fly larvae, anaerobic digestion, and compost systems based on substrate type.

Specific Objectives:

1. Determine the ability of black soldier fly larvae to reduce waste biomass and recycle nutrients by evaluating nutrient mass balance values in dried larvae in terms of protein, lipids, and oil content after being fed five specific waste-based diets.
2. Determine the ability of anaerobic digestion to reduce waste biomass and recycle nutrients by evaluating the nutrient mass balance transfer from five food waste substrates to biomethane gas quantities and digestate nutrient profile and fertilizer capability.
3. Determine the ability of a compost system to reduce waste biomass and recycle nutrients by evaluating the nutrient mass balance transfer from five food waste substrates to nutrient value in the compost.
4. Compare systems in a model approach relating initial substrate attributes to best end use

Approach/Strategy

For each of the three systems (Black soldier fly larvae, anaerobic digestion, and compost), trials have been running using different substrates through each system and evaluating the nutrient mass balance conversion in terms of bio-products produced from each system. The black soldier fly portion of the project ran over a summer period utilizing two fabricated facilities. One for feeding trials and the other intended for breeding purposes. The feeding facility was built sitting on a tabletop as a simple rectangular box with two hatch-operating doors on the anterior surface. The uppermost and posterior surface gaps were filled using six-millimeter-thick vapour barrier plastic, while the two side surfaces were draped with fiberglass insect mesh. Inside the facility, there were three strips of heat tape installed on the flooring. Each strip of heat tape was accompanied with a temperature controller thermostat (Model: STC-1000) to control the internal temperature of the facility. There was no supplementary lighting or humidity sources installed on this feeding facility, as it was housed in a high temperature room. Further black soldier fly larvae trials will be repeated and continued in the summer months of 2018, utilizing changes in lighting for breeding cycle enhancement. Nutrient value will be ascribed to the larval value in feed for poultry, and frass nutrient value relative to crop requirements.

The anaerobic digestion trials, also on-going for optimization, are carried out utilizing the laboratory biomethane system AMPTS II LIGHT. This system can be broken into three units of equipment: An incubation unit, where the substrates are mixed with an inoculum source and the three steps of anaerobic digestion take place, a Carbon Dioxide absorbing unit, where all carbon dioxide gas is trapped, and a gas volume-measuring unit, where total biomethane gas levels are measure using a water displacement method. Each unit was connected to the next unit via Tygon® plastic tubing and the entire three-unit system was connected to a computer with the AMPTS II LIGHT database installation used to collect and display the data of the running experiment. The anaerobic digestion trials will continue throughout the coming summer months to gather sufficient data for modelling, as each substrate batch requires a 30 day HRT. Relative value will be attributed to the biogas value and the value of digestate in terms of plant growth requirements.

The compost work comprises utilization of a standard control base blend of balanced C:N basal substrate in a controlled batch system, with addition of each of the five feedstocks and will be continued in the summer months to gather sufficient data on cured compost nutrient composition and value. Composting capacity will be tracked daily for pH, gas production and temperature. Nutrient balance is determined on the basis of lab analyzed composition of input ingredients and output finished compost. Relative value is assessed relative to specific crop demand and match of nutrients.

Experiment Work

Black Soldier Fly Larvae:

In this portion of the project nutrient mass balance transfer was assessed in terms of protein, lipid, and oil contents of dried larvae after being fed a substrate specific diet for a period of approximately two week batches. These are considered the value addition components, since there is potential market price points attributable to the oil and protein meal. Process optimizations focused on the feeding patterns of this organism and the variables needed for optimum survival and food consumption. The black soldier fly larvae are unique in the sense that they have a highly efficient food conversion ratio. This means that a significant amount of the input material supplied is consumed and is retained in their body through bioconversions to larval oil and protein in the body, which can be further extracted for sale. Several trials were conducted with the larvae testing different feed sources including vegetable mixes, fruit mixes, potato skins, and dried mink compost. Some of the results are described in Figure 1 and Table 1 below. Value attributes from the meal are valued at approximately $1000-1500/t and the frass component can be utilized for potting mix with similar value to worm castings.

Anaerobic Digestion:

For anaerobic digestion nutrient mass balance transfer from organic waste is evaluated primarily as biomethane gas quantities, however the production of biogas from the digester does not lead to marked nutrient reduction from the original input substrates, the remainder remaining in the liquid digestate. Digestate has considerable liquid fertilizer potential though the land base required for distribution requires further study. Land base and crop uptake may be limited and therefore further nutrient extraction and valorization are being explored for this system. Biogas is one of the main by-products of anaerobic digestion which is a multi step process in which organic matter is broken down at a specific temperature range under anaerobic conditions by select groups of microorganisms. This process is beneficial because it can reduce organic waste biomass along with providing a renewable energy source. Trials running the anaerobic digestion trails with vegetable waste diet as well as with the other substrates were run for a period of 30 days retention time at a temperature of 38°C. An example of the bio-methane production from the thirty-day trial can be seen for the experimental group (vegetable) and control groups (inoculum and cellulose) in Figure 3 below. In order to be utilized the biogas produced from the large-scale digester must be scrubbed (CO_2 removal, S, etc) for access to natural gas pipelines, or can be run through CHP for recovery of heat and gas. According to Heritage Gas, natural gas feed in rates in the Province are in the range of 499 Gigajoules (GJ) per year to over 50,000 GJ per year, and Natural gas prices range $3-8. Having another source of renewable natural gas would be exceptionally valuable and profitable. A transportation cost is associated with the use of digestate for fertilizer and this will be calculated on the assumption of a radius of agricultural land under typical crop production.

Compost:

Compost nutrient transfer will be evaluated in terms of beneficial plant growth and nutrients in the compost itself. In other words, how well suited will a compost comprised of any of the five substrate types be as a fertilizer replacement or supplement for optimum plant or crop growth. All the necessary materials and methods have been acquired and mapped. The composting system is in a contained and controlled environment (greenhouse) where temperature and humidity are closely controlled and monitored daily, along with temperature and humidity of the compost. The 15 compost bins setup for the five substrate types (3 replicates) are all run together to remove environmental variance. A constant amount of substrate in terms of biomass (fixed C:N) added to each of the 15 compost bins was mixed with fixed proportion of the respective five feedstocks and monitored (turned and watered) daily over a period of 30 days, and temperature, moisture and ammonia monitored. The final carbon nitrogen ratio for each bin is calculated and analysed, as it has been shown that an optimum carbon nitrogen ratio for composting is 30:1. After the composting process is complete, each individual compost is analyzed for essential nutrients that would be needed for optimal plant and crop growth and compared to nutrient value of the substrate input, and relative fertilizer value for crop growth (on N,P,K basis). The respective mass balance for specific nutrients and their value as crop products are then compared for both substrate source and against the other two systmes.

Results

Black Soldier fly exhibited a propensity to utilize a wide range of substrates, however bio-products generated are much reduced (Figure 1) with less viable feedstocks, vegetable waste being the optimum.

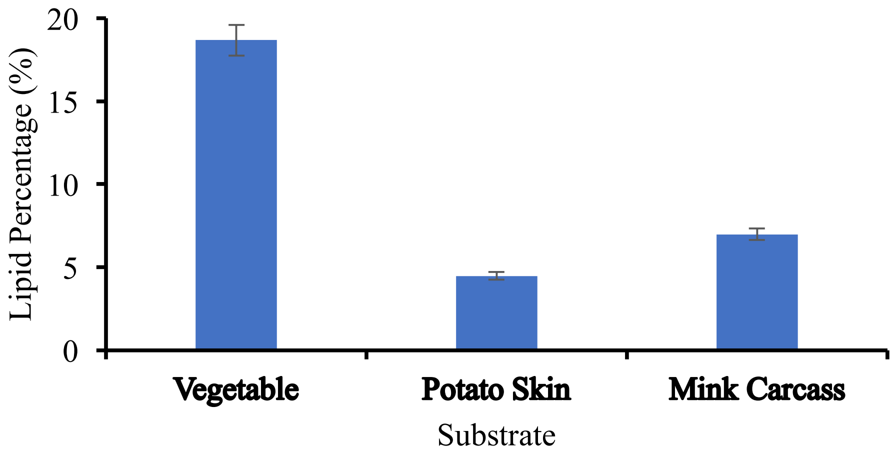

Figure 1. Average lipid content of dried black soldier fly larvae fed vegetable, potato skin, and mink carcass-based diets over a fourteen-day period.

Table 1. Average black soldier fly larvae counts and weights pre and post-feeding of vegetable, potato skin, and mink-carcass based diets.

	Vegetable mix	Potato skin	Mink mix
Larvae Count			
Pre-feed	280	280	278
Post-feed	258	257	270
Dried	100	100	100
Larvae Weight (g)			
Pre-feed	15.45	10.36	13.00
Post-feed	40.85	10.14	14.22
To oven	15.15	4.61	6.31
Dry weight	3.48	0.86	1.24
Frass Weight (g)			
Pre-feed	171.75	174.50	174.30
Post-feed	234.15	193.67	280.48

Dry weight	94.48	100.60	188.11
Feedstock			
Dry matter (%)	12.9	15.8	23.1
Total amount fed (g)	611.60	591.40	590.65

*Veg mix= Spinach, lettuce, tomato, carrot, yellow pepper, red pepper, cucumber, and mushroom.

With higher carbon: nitrogen ration, the growth rates of larvae were reduced (Table1), and resultant weight of larval dry matter. Similarly, composition of larvae is less desirable, assuming high oil and protein is both desired. In the model however, a value for each component will be included such that if the relative value of protein meal increases, then ingredient mix can be modified to maximize value generation from this process. It is evident for combination mixes tested in the larval feed system that a more ideal mix can further optimize larvae composition.

Bio-methane production values for each ingredient show a range, relative to volatile solids composition of the product which in turn relates to some extent to chemical composition. These values will also be related in the model to biogas volume and value for each singular substrate. Later runs will look at synergies between substrates, for mixed co-digestion prospects derived from more precise balance of VS and organic content. An example sole substrate BMP is provided in Figure 2.

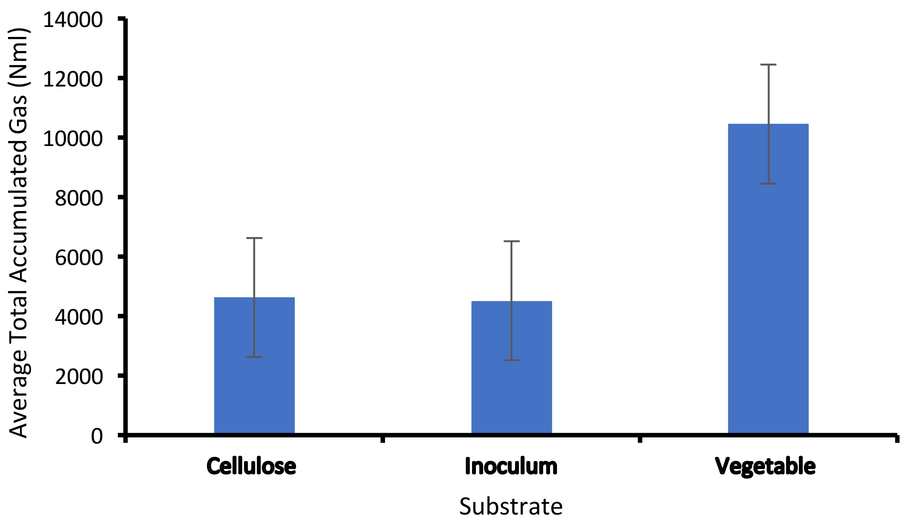

Figure 2. Average total biomethane gas levels recorded after a 30-day anaerobic digestion trial run at a mesophilic temperature of 38°C.

BMP values for ingredients with highest VS and available sugars are highest. For comparative value, the respective methane values will be applied as value proposition initially (without consideration for value of liquid digestate, as this would require subtraction of freight costs which may negate value). These will be estimated as described to valorize the complete process.

It is anticipated that although all chosen substrates will compost well over the reaction time in the vessel, the fertilizer value from each will be directly related to initial C:N in the mix, and N,P,K at start time, assuming minimal losses in the contained system (as compost tea, which are re-introduced). Value creation will be attributed as directly relating to this measured fertilizer value.

Preliminary Conclusions & Further Work Toward Data for Model Creation

Based on results to this point, the vegetable-based mixture diet has shown the most promising results in both the black soldier fly larvae and anaerobic digestion systems. This is to be anticipated based on initial composition of starting material. The vegetable mix has shown the greatest yield in terms of macronutrient value in the dried larvae and produced a substantial amount of biomethane gas in the anaerobic digester. Once sufficient repetitions are run on all three systems, the by-product yield of each system will be further quantified and valorized for comparative assessment through the model to be produced on an equivalency basis. The model will provide relative value comparison on the basis of the overall mass balance through each system multiplied by bio-product value, hence total value creation from each method, and thereby determine the best fit of product to outlet processing method. On preliminary value creation from substrates such as the vegetable material it is becoming evident that this should primarily be directed toward the highest value outlet, that being BSFL production, if available close to source, and secondarily to anaerobic digestion. These options can be provided a ranking in the model outputs to serve as decision tools for municipalities to ascribe diversion cost pay back.