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Novel Pilot Development of a Closed-Loop Sustainable System Between Biogas Renewable Energy, Distilling, and Aquaculture by Vermiculture of Stillage Wastes

Samuel C. Kessler
University of Louisville, sckess01@louisville.edu

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Cover Page Footnote

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FULL LENGTH MANUSCRIPT

Novel Pilot Development of a Closed-Loop Sustainable System Between Biogas Renewable Energy, Distilling, and Aquaculture of *Oreochromis niloticus* with Vermiculture of *Hermetia illucens*

AUTHOR: Samuel Kessler[1,8]

ACKNOWLEDGED CONTRIBUTORS: Vikas Kumar[2,3], Waldemar Rossi Jr.[2], Jagannadah Satyvolu[4], Habte Michael Habte Tsion[2], Richard Kessler[5], Keith Phillips[6], John McClean[7]

[1]University of Louisville, Dept. Geography & Geoscience, Environmental Analysis, Louisville KY, 40208
[2]Kentucky State University, Aquaculture Research Center, Frankfort, KY, 40601
[3]University of Idaho, Hagerman Fish Culture Experiment Station, Hagerman, ID, 83332
[4]University of Louisville, CONN Center for Renewable Energy, Biofuels Group, Louisville, KY, 40208
[5]Campbellsville University, Division of Natural Science, Environmental Science, Campbellsville, KY, 42718
[6]Western Kentucky University, Department of Biology, Bowling Green, KY, 42101
[7]MAC Farms Inc. Anaerobic Digestion Services, Campbellsville KY, 42718
[8]Gatton Academy of Science and Mathematics, Research Internship Grant Program, Western Kentucky University, Bowling Green, KY, 42101

ABSTRACT

This study provides a mixed-methods approach in analyzing a potential closed-loop system between renewable biogas production from anaerobic digestion, vermiculture production, aquaculture production, and organic wastes with a particular focus on stillage wastes. Knowledge gaps to implementing this system were qualitatively identified as disconnect between digestate management of biodigestion and aquaculture production. This gap was addressed by conducting a vermiculture rearing trial utilizing a digestate-infused substrate (along with an organic sorbent and stillage wastes) for Black Soldier Fly Larvae (BSFL, *Hermetia illucens*). Those larvae were then analyzed for nutritional content, to consider in comparison to other *Hermetia illucens* used in commercial BSFL products for aquaculture feed production. In a separate aquaculture production trial, said BSFL ingredients were formulated into two different aquaculture feeds to assess a comparison with commercial aquaculture feed for Nile Tilapia (*Oreochromus niloticus)* which is one of the most widely cultured species. Results provide that BSFL feed is readily accepted by Tilapia, and that BSFL reared on the digestate infused substrate are of comparable proximate nutritional content. This study verifies the efficacy of a directional link between anaerobic biodigestion, vermiculture, and aquaculture and, therefore, holds great potential to implement a more closed-loop sustainable system with the primary outputs of food products and renewable biogas energy from anaerobic biodigestion in such a manner that may reduce methane emissions from component practices of the more closed-loop model. The conclusion of this pilot scale work urges large-scale implementation, which necessitates collaboration between scientific and industrial communities along with support from policymakers and legislative bodies to incentivize synergetic behavior across multiple sectors and components of the system.

KEYWORDS: Stillage, Biogas, Aquaculture, BSFL, Nile Tilapia, Vermiculture, Agriculture, Sustainable Model, Closed Loop, Methane, Bioengineering, Environmental Sustainability

INTRODUCTION

This paper provides an analysis on the viability of implementing a more closed-loop sustainable system between stillage and food wastes, renewable energy from biogas via anaerobic digestion of these wastes, vermiculture production, and aquaculture production. This is considered via a mixed-methods approach involving qualitative systematic analyses and quantitative conclusions from a scientific pilot study demonstrating a critical connection between anaerobic digestion, vermiculture, and aquaculture. In combination, evidence is provided supporting the efficacy to develop a closed-loop sustainable system by linking the management of stillage and other organic wastes via anaerobic biodigestion, vermiculture production including the residues of biogas production, and subsequent aquaculture production from larvae which may be reared on digestate-infused substrate. Aquaculture wastes may then be returned to biodigestion. A conceptual model for this sustainable system is provided in Figure 1.

A thorough explanation of components, uses, products, and interactions of the proposed system is provided with Figure 1. External inputs into the system may occur at each point in the above cycle, whether they be added substrate stabilization in vermiculture production, natural or introduced feed additives in aquaculture production, additional nutrients and light source in crop production, wastes apart from stillage such as non-aquaculture-based food wastes, or other biochemical inputs in the control of anaerobic digestion. These are the points at which the system cannot be considered "closed," however, these inputs are classified as external to the proposed "closed-loop" depicted in the conceptual model.

The interconnected components of this system model may be assessed. Beginning with aquaculture, the industry has a need for sustainable feed sources due to a reliance on

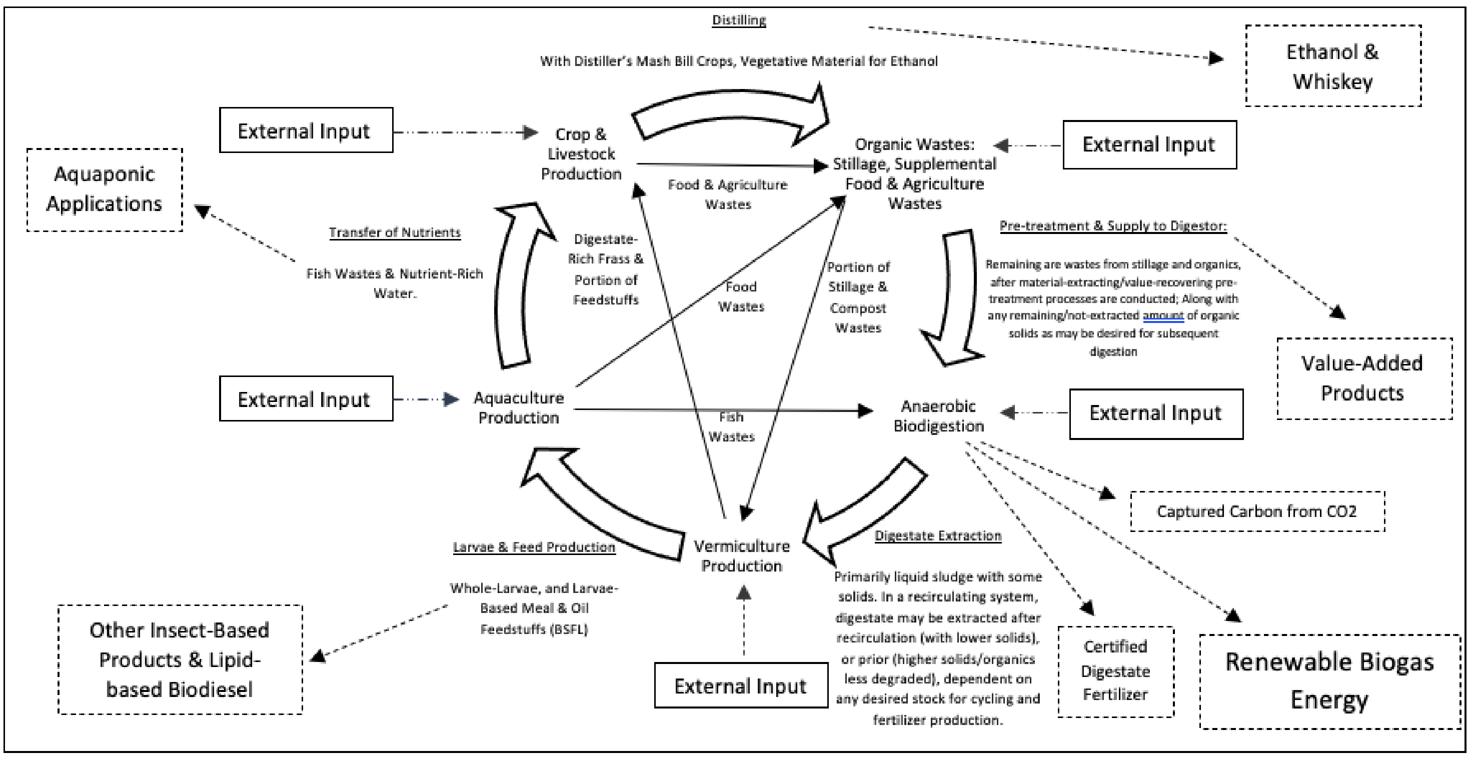

Figure 1. *Model of Closed-Loop cycle between anaerobic digestion/renewable biogas, vermiculture, aquaculture, and distilling.*

*__Key:__ Labels between arrows represent components. Arrows represent actions between components. Dashed boxes represent system output products and/or activities. Solid boxes represent potential external inputs to the system where desired.

***Explanation**:. For the purposes of the figure, "Mash Bill" refers to grains found in the production of any particular whiskey, including corn, rye, wheat, and malted barley. "Distillate Stillage" refers to any form of stillage waste from the distilling process of alcohol production. "Food wastes", stemming from aquaculture production, refer to any wastes associated with human consumption of fish products; "Food & Agriculture Waste" refers to any organic wastes from food production/consumption or agricultural activity, from crops/crop-residues, or wastes of crop-based products. The label beginning with "Portion of Stillage…" refers to a percent diversion of distillate and solid food wastes for use in vermiculture as necessary to establish substrate volume detailed herein. The label beginning with "remaining wastes" refers to the remaining wastes after extraction of valued materials as a process of pretreatment before biodigestion, along with any remaining solids as necessary for the digestion process and desired future digestate. "Frass" is the resulting product from a substrate which has been fed to larvae for vermiculture, which is often dry and may be used as soil additive, fertilizer, or as a potential feed component. Such pretreatment extraction of valuable materials may include protein extraction, fiber extraction, sugar extraction, or other, by physical and/or chemical means. Extracted valuable products from these wastes are considered "value added products" in the Figure. "Digestate" is the waste byproduct/co-product remaining after anaerobic digestion, containing any indigested material, having reduced organic content from feedstock. It has low solids content and is often used as a fertilizer. Digestate may be dewatered for recovery of solids as soil additive. Depending on the digestion process employed, digestate may contain high ammonium, salinity, phosphate, high chemical oxygen demand, and/or remaining active enzymes and bacteria. Digestate volume and bacteria are often recycled within the system to maintain digestion activity, however, after a degree of cumulation digestate-based volume must be removed from the system to allow continued intake of feedstock volume & to occur at the front end of the digestion system. An especially relevant digestate waste is certified digestate (American Biogas Council, 2019). In any instance, "External Input" refers to optional input from external wastes streams relevant to the component or component process or biochemical/biophysical regulation of said process. Note that the author solely claims rights of intellectual property for the system diagram, though not necessarily individual components.

menhaden-derived ingredients which are often considered a keystone species to many fisheries. This has led to the suggestion that insect-based protein and fat sources be widely implemented, including sustainable use of insects reared on waste products and common waste streams (Shumo, 2019). In the European Union, certain larvae have been observed to not bioaccumulate contaminants above stringently legislated safety limits (Lievens, 2020). It has been further established that aquaculture wastes are a potential source of methane where aquaculture sludge even from brackish sources may be used as a potential fuel source to anaerobic biodigestion (Mirzoyan, 2008). Aquaculture effluent and other fish wastes may also be irrigated and used as fertilizer to improve yields, including when applied to crops such as wheat, barley, corn, and rye (Snow 2008; Tung, 2021; Lund, 2014) which are also common components of distillers' mash-build. The subsequent byproduct waste of stillage from processes such as bourbon production is also known to have high organic load and subsequently high biological & chemical oxygen demand (near the 6700 mg/L range, as provided to the author from a confidential source, using analysis method SM 5520 D 2011). Both stillage and food wastes have been identified for sustainable implementation in anaerobic biodigestion (Nasr, 2012 , Slorach, 2019). From these wastes, anaerobic biodigestion may then be used as a sustainable method of renewable energy production with combined heat & power from captured biogas having significant net carbon reduction from the prevention of natural methane emissions (He, 2018). Following the biodigestion process, a digestate byproduct is produced with very low total solids content. In some cases, the "loop" is then broken where this byproduct is treated as waste or discharged to sewer systems.

Digestate has been identified as a soil additive and alternative to mineral fertilizer (Panuccio, 2018), however, permitting is required in some instances or otherwise further processing to extract the small amount of total solids from the digestate or to dewater the digestate, which would also require energy input. Where stillage is a bottlenecking byproduct waste to the distilling process—meaning that without the removal of stillage the distilling process is halted—digestate may be considered a bottlenecking byproduct of anaerobic biodigestion. Treatment is often costly and can entail implementation of a wastewater treatment plant in some cases (Vutai, 2016). These conditions suggest that digestate waste management by lower cost means, such as vermiculture, may be favorable to form a sustainable system. The remaining "link" in the sustainable system lies in determining whether a vermiculture substrate may be infused with digestate to successfully rear larvae of adequate nutritional quality, comparable in performance to standard aquaculture feeds. An additional observation of value is whether the remaining frass content may be observed to have high enough total solids for digestate containment and be of sufficient quality as fertilizer.

To answer this question, and substantiate the concept of this sustainable system, this study designed a vermiculture rearing trial utilizing a digestate-infused substrate with Black Soldier Fly Larvae (BSFL, *Hermetia illucens*). This was conducted such that trial BSFL would be analyzed for proximate nutritional content, to be compared to the standard nutritional value of BSFL used in commercial processing into oil and meal products at industrial scale. Separately, commercial BSFL meal and oil products were used to formulate feeds in an aquaculture trial designed to assess a comparison with standard commercial aquaculture feed. An analysis of the results from both trials would then identify any potential for a directional link between anaerobic biodigestion, vermiculture, and aquaculture – where demonstrated potential would suggest the viability of implementing a more closed-loop sustainable system. As shown in Figure 1, along with many added benefits from processes in the system the primary outputs of said system would include food products and renewable biogas energy produced from methane-reducing anaerobic biodigestion of system wastes.

BACKGROUND & QUALITATIVE ANALYSIS

Feasible Environmental Impact & Economics for Anaerobic Digestion of Stillage Waste

In recent years the United States Environmental Protection Agency has reported that methane, one of the most potent greenhouse gasses, accounts for approximately 10% of annual greenhouse gas emissions from human activity. Much of this emission originates from the decomposition and use of organic wastes. Methane emissions come primarily from enteric fermentation (the decomposition of material in animal digestive systems) an abundance of which occurs in the agriculture & food production sector, where food waste and loss are responsible for between 8 to 10% volume of total GHG gas emissions (Shukla et. al, 2019). Even where carbon capture does not occur, unlike some forms of bioenergy (i.e. gasification, which varies more based on feedstock and feed rate), anaerobic digestion significantly offsets the total GHG effect in the exchange of emitted gas from methane (which may be up to 80 times the warming power of carbon dioxide in during the first 20 years after emission) to less potent carbon dioxide which may also be sequestered (Zaks, 2011). In general the majority of methane emissions are tied to either some form of non-renewable energy or biological decomposition in the environment whether by digestion or decay. Implementing anaerobic digestion systems promises the capability of capturing methane from digesting organic wastes and, as a result, producing renewable biogas. This process includes agricultural, industrial, and food wastes that can be diverted from landfills. Wastes are often consistently available in cities where urbanization has brought increased density of solid waste, in addition to waste from consolidated industrial areas. In any instance, logistical and cost-based challenges exist in managing organic wastes.

Given that the key restricting factor of practicing anaerobic digestion is achieving desired economic benefits (Chen et al, 2017) local economic drivers, external to the biodigestion process itself, tied to certain waste streams can increase the economic & environmental benefits of biodigestion. This can be observed when considering anaerobic digestion as a cheaper waste management alternative for wastes as opposed to strictly a means of renewable energy production. For example, in several areas of the United States such as Kentucky and Tennessee, stillage wastes from the distilling industry are a waste management challenge across rural and urban areas. Stillage is a waste product from distillery production which poses an environmental concern due to low pH, high chemical and biological oxygen demand, and high organic load. It is also classified as an odor pollutant, due to gaseous emissions which can occur in aerobic conditions and is regulated as an industrial pollutant (Kentucky Energy and Environment Cabinet, 2017). Anaerobic digestion has been identified as a comparatively very effective method of stillage waste treatment (Mikucka & Zielińska, 2020). The cost of anaerobic digestion from a third-party wastes manager can be far cheaper than the costs of most typical management including stillage discharge, lagoon management/aerobic digestion, or production of grains from running expensive drier systems – as well as having the added benefit eliminating and capturing emissions for energy production. Analysis conducted by the University of Louisville CONN Center for Renewable Energy provided confidentially to the author have also suggested that the operating cost of energy-producing anaerobic digestion (even where operated by a third-party wastes manager) is a significant margin of cost cheaper than typical fees for stillage discharge to sewer managers, or using the same volume of stillage for the production of grains from running expensive drier systems (Figure 2). These economic benefits align with the environmental benefit of eliminating and capturing emissions for energy production (Cavinato, 2010). Involving stillage wastes (or for that matter, other wastes that fall under similar economic and environmental contexts) in sustainable system design allows a unique economic basis for companies, federal or provincial government, or city-based entities to make investments in anaerobic digestion to produce renewable energy. This is especially relevant

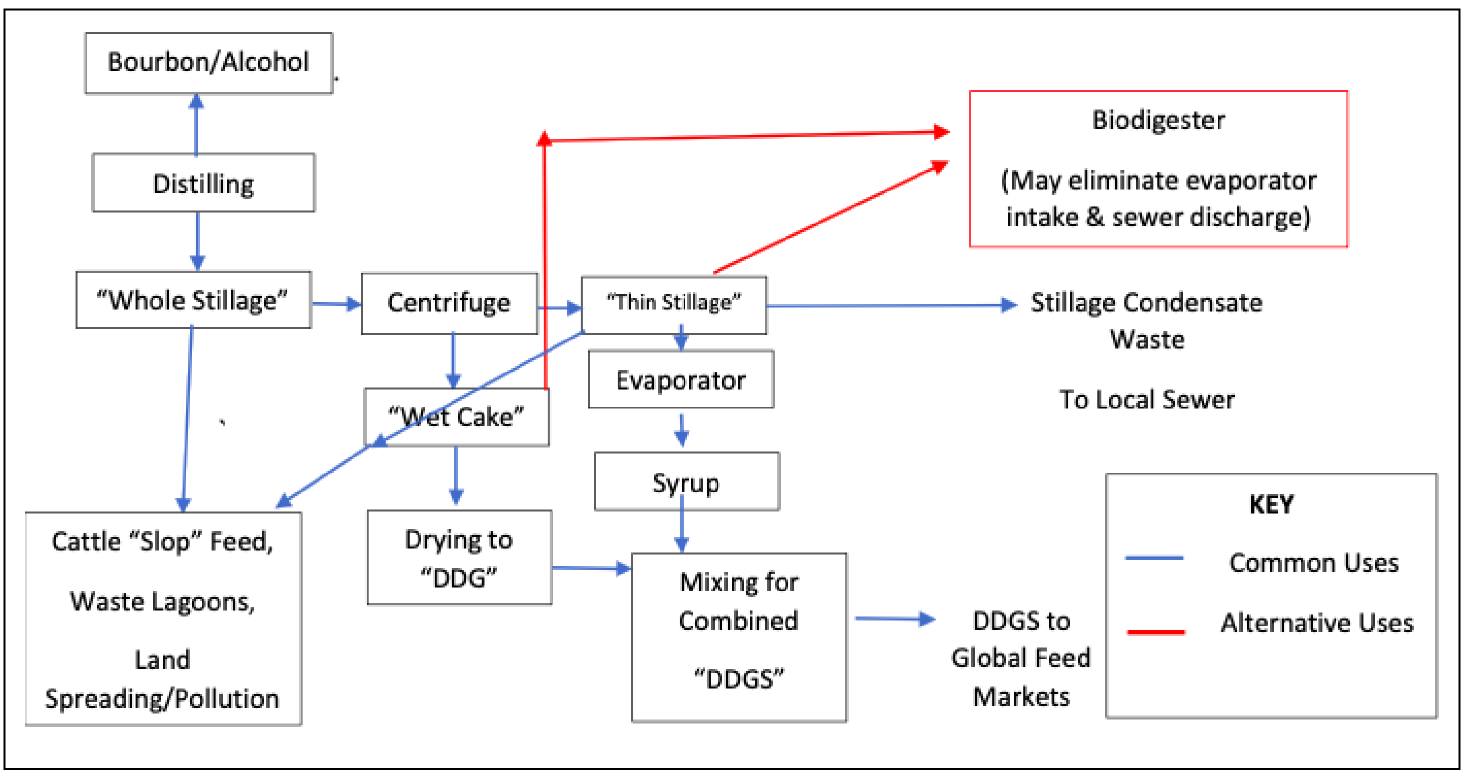

Figure 2. *Model of Stillage Management from Bourbon & Alcohol Production.* Wet Cake – Subsequent solids from centrifuge, Thin Stillage- Separated liquids after centrifuge, Syrup- Condensed product after evaporation of thin stillage, DDG – Distillers Dried Grains, DDGS – Distillers Dried Grains with Solubles, Cattle "Slop" Feed – Combination of thin stillage and wet cake

where groups must decide under mandates how to pursue renewable energy benchmarks or a cost-effective alternatives to fossil fuels.

In instances involving organic wastes like stillage into a sustainable system with biodigestion, a unique economic basis exists for companies, government, or city-based investments in anaerobic digestion to produce renewable energy in deciding whether to pursue renewable energy benchmarks or a cost-effective alternative to fossil fuels. This is partly due to the recent boom in the bourbon industry specifically in areas like the Commonwealth of Kentucky which has driven economic growth (University of Louisville Urban Studies Institute, 2017), and likewise stillage wastes. Where anaerobic digestion is implemented with stillage, the digestor captures methane which would otherwise be emitted over a relatively short amount of time compared to other wastes– whether by decomposition or aerobic digestion of stillage waste, or by the enteric fermentation of cattle by digestion of direct stillage feed or stillage-based grain products. Other common and sometimes illicit practices include chemically induced aerobic digestion in waste lagoons (where illicit discharge may occur), cattle feeding which may occur in excess, or landspreading (Personal Communication, Director of Compliance Assistance, Kentucky Energy & Environment Cabinet). Case studies have shown that the use of anaerobic digestion to take various waste streams for renewable gas production can be considered a strongly sustainable solution (Meester et al, 2012). It may better preserve natural environmental capital by avoiding pollution through waste diversion and elimination. This process also “upcycles” value from wastes by recovery of biogas, along with the potential for low-nitrogen biofertilizer from remaining residue wastes. It may also lower the costs of urban waste management since lowering the total organic level of a waste material through anaerobic digestion then lowers the cost of discharge of remaining residues, a byproduct referred to as digestate.

Under present economic conditions, the cost for discharge still represents significant cost to urban and rural distilleries either from sewage discharge or stillage-based feed production. The other option where stillage is not discharged is the thinning and drying of stillage, which includes operation of evaporator and dryer systems (Figure 2) to produce a feed product of dried distillers’ grains with solubles (DDGS). Distillery-based estimates of methane emissions from the thinning and drying processes are not readily available, however, significant energy demand exists for this process- a cost which serves to shrink any margins of profit on the sale of DDGS. Other “shrinking” forces include those from global trade, DDGS is often sent to markets in China and throughout Asia. It has been an economic target of the PRC from recent trade wars with the United States (Kentucky Department of Agriculture, Senior Trade Advisor, Personal Communication, 2018). Also for consideration is additional greenhouse gas emissions from the shipping process. In some cases where DDGS production would a be more economically attractive option than paying discharge costs, urban distilleries do not have the space required to install the capital necessary to produce DDGS. Small and craft distilleries often lack adequate funding to do so. With these drivers, the primary one being the high cost of stillage management especially in urban areas, an urban to rural exchange of the waste can often occur. This creates an economic opportunity for rural entities to remove stillage from distilleries in more urban areas. This trend can then, unfortunately, significantly raise the potential for illicit landspreading.

Several observations may be drawn that suggest stillage wastes (whole stillage, wet cake, thin stillage) are an important waste stream of focus for biodigestion not only for the economic reasons previously discussed. One key opportunity is in improving the sustainability of the distilling industry by replacing current management systems with high energy demand often powered by fossil fuels, and potentially use biogas from stillage to provide heating necessary to the distilling process (Eskicioglu et al., 2011, Ziganshin et al., 2011). The use of stillage waste as a source of renewable biomethane through anaerobic biodigestion has the potential to reduce GHG emissions where implemented through renewable biogas. Incentives exist for the biodigestion of stillage as it is an improved avenue of waste management that saves costs, produces renewable energy, and in preventing pollution and methane emission, improves environmental quality.

Eliminating the necessity for this process, provides significant incentives for distilleries to finance the infrastructure necessary to directly link stillage waste with the digestor system (e.g. piping, trucking, etc.). Other technological advancements, patented by the University of Louisville, allow for added revenue in repurposing solid wastes from stillage into other products, while using remaining high-organic content reduced liquid in biodigestion. The operating costs of digestor systems and transmission of waste to a digestor could be absorbed by revenue and savings. The biodigestion portion of the sustainable system has been proven through technological development and is feasible. Due to otherwise high discharge costs and high operating costs with DDGS development, a unique situation creates a financing opportunity for the development of biodigesters and logistics of waste transportation, whereafter biodigesters may then accept other wastes from other sources such as food wastes.

Following biodigestion and production of renewable energy from produced methane, the by-product waste from biodigestion referred to as "digestate" is drastically reduced in total organic load from the digestion process (and often total solids content, the degree of which depends on the design of the digestion system). This reduction in organic load often suggests a reduction in total costs of digestate discharge or management—"lower" cost being relative to the higher cost of managing non-digested organic wastes by potentially more costly means or means other than anaerobic digestion such as direct sewer discharge. This comparatively lower cost poses economic motivation to provide wastes to anaerobic digestion systems, however, the remaining lower organic content of digestate waste (or otherwise some form of wastewater) must still exit the digestion system. This cost barrier can often be absorbed by waste providers who pay a fee equivalent to digestate management costs, however, it is nonetheless an economic barrier to overcome (but more-so in areas where economic drivers behind wastes like stillage are not available). Typical management in urban areas may include discharge to sewer systems, while management in areas with more space may include de-watering systems for fertilizer production, lagoon management, or use of a wastewater treatment plant. In general, these methods still pose a cost and do not pose a continuance of upcycling, which occurs at the front-end of digestion by converting wastes to wattage.

Emerging Link between BSFL Vermiculture & Sustainable Aquaculture

Commercial-scale vermiculture suggests promise for low-cost organic reduction and potential for a more closed-loop connection. This may occur as larvae upcycle recovered nutrients into feed production, which may then fuel aquaculture production. Aquaculture may then resupply wastes to anaerobic digestion systems. Insect proteins have been identified as a new source for animal feed (Stamer, 2015). The black soldier fly's larvae (*Hermetia illucens*) (BSFL) is a non-pest species generally researched as waste management technology and alternative protein feed-source for poultry. Thus, it may serve as a connection between food production in the aquaculture industry and waste management. BSFL digestion of organic matter has been shown to reduce discarded waste up to 50% with subsequent harvests of the larvae producing a feedstuff with protein and lipid content averaging 42% and 35% respectively, while nutrition content depends on rearing substrate (Sheppard et al. 1994; Newton et al. 2005). The BSFL may be considered an optimum candidate for aquafeed replacement (Stamer et. al, 2014). The BSFL are of sustainability interest, as the nutritional profile generally offers an acceptable composition of essential amino acids and long-chain omega-3 fatty acids for waste-management while providing the potential to reduce GHG emissions as a low-tech waste reduction solution. The BSFL allow the value of waste to be upcycled, in the form of BSFL feed products high in fat and protein.

Studies have suggested that the BSFL fish feeds may partially or fully replace fish meal (FM) in the diets of channel catfish (*Ictalurus punctatus*), African catfish (*Clarias gariepinus*), flathead catfish (*Pylodictis olivaris*), yellow catfish (*Pelteobagrus fulvidraco*), blue tilapia (*Oreochromis aureus*), rainbow trout (*Oncorhynchus mykiss*), and Atlantic salmon (*Salmo salar*) (Bondari and Sheppard, 1987; Newton et al., 2005; Adeniyi and Folorunsho, 2015; Xiao et al., 2018; Lock et al., 2014). One study with turbot (*Scophthalmus maximus*) attributed findings of lower growth rate and feed efficiency to potential intolerance of even low inclusions of chitin (Kroeckel et al., 2012), though other studies have not explicitly found such effects.

The global aquaculture industry now provides at least 50% of all consumable fish (FAO, 2016), however, high quality and sustainable protein sources must be identified or developed to support continued industry growth. Meanwhile, as global food consumption increases, approximately one-third of food is wasted or lost with negative environmental impacts including an estimated annual greenhouse gas (GHG; CO_2 and CH_4) emission of 3.3 billion metric tons of CO_2 equivalents (Gustavsson et al., 2011; Surendra et al., 2016).

To further demonstrate the applicability of BSFL feedstuffs, this study sought to determine nutritional applicability of BSFL meal and oil products derived from larvae reared on dried distillers' grain with solubles (DDGS) and other food wastes in the diet of Nile Tilapia (*Oreochromis niloticus*). Nile Tilapia is regarded as the most common species to commercial aquaculture.

To better illustrate the connection between food production and waste-management, a postliminary larval rearing trial was conducted to assess proximate nutrition composition of larvae reared on a substrate infused with liquid biodigestate, a byproduct of anaerobic biodigestion and methane sequestration. The methods and quantitative analyses of these trials are outlined below.

Ingredients (g/100g of dry matter)	Diet		
	Control	BSFL Meal	BSFL Meal + Oil
Menhaden fishmeal	8.0	0.0	0.0
BSFL meal	0.0	40.0	40.0
Soybean meal	42.1	13.4	13.4
Soy protein concentrate	3.0	3.0	3.0
Wheat gluten	3.0	3.0	3.0
Wheat flour-70% starch	32.9	34.0	34.0
CMC	1.5	1.5	1.5
Menhaden oil	1.5	1.5	0.0
Soybean oil	4.8	0.0	0.0
BSFL oil	0.0	0.0	1.5
[a]Vitamin premix	0.6	0.6	0.6
Stay C 35%	0.3	0.3	0.3
Choline chloride	0.2	0.2	0.2
[b]F1-Mineral premix	0.4	0.4	0.4
Dicalcium phosphate	1.7	2.0	2.0
DL-Methionine	0.2	0.3	0.3
Proximate composition (g/100g of dry matter)			
Crude protein	33.0	32.7	32.9
Moisture	10.6	10.3	10.4
Extractable oil	9.7	8.8	9.4
Total oil	10.5	9.7	10.2
Fiber	3.7	3.8	4.4
Ash	8.3	8.3	8.3

Table 1. *Ingredient and proximate composition (g/kg) of the experimental diets*. [a]Provides per kg of diet: retinyl acetate (vitamin A), 3000 IU; cholecalciferol (vitamin D), 2400 IU; all-rac-α-tocopheryl acetate (vitamin E), 60 IU; menadione sodium bisulfite (vitamin K), 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid, vitamin C), 120 mg; cyanocobalamin (vitamin B12), 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine, HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg. [b]Provides per kg of diet: sodium chloride (NaCL, 3%Na, 61%CL), 3077 mg; ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20% Fe), 65 mg; manganese sulfate (MnSO4, 36% Mn), 89 mg; zinc sulfate (ZnSO4·7H2O, 40% Zn), 150 mg; copper sulfate (CuSO4.5H2O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica, 1000 mg).

METHODS AND QUANTITATIVE ANALYSIS

Tilapia Diet Formulation

A feeding trial was conducted over a period of eight weeks using nine tanks within a recirculating aquaculture system (RAS). BSFL meal and oil was varied among three formulated diets and was derived from BSFL reared on DDGS. A control diet (Control) served to replicate a commercial standard containing 0% BSFL composition. The first test diet (BSFLM) was composed of 40% BSFL meal as partial replacement of conventional soybean meal and total replacement of sardine fishmeal, with total removal of soybean oil. Menhaden oil was used as an added lipid source. The second test diet (BSLFM+O) was also composed of 40 % BSFL, using BSFL meal and oil as a partial replacement of conventional soybean meal and total replacement of both sardine fishmeal and menhaden oil, as well as soybean oil. To maintain isonitrogenous and isocaloric content of diets 2 and 3 by their BSFL replacement of ingredients from diet 1, dicalcium phosphate was increased by 17.6% to account for replacement of marine ingredients, DL-methionine levels were increased by 5% to retain omega-3 fatty-acid balance due to total soy protein replacement, with wheat flour also increased by .03% to maintain carbohydrates. All formulations retained a composition of 35% crude protein (CP), 8% lipids, and estimated digestible energy (DE) of 3.3 kcal/g (Table 1). To prepare all diets, dry ingredients were mixed for 20 minutes using a Hobart mixer (A200, Hobart, Troy, OH, USA). Oil sources were added slowly to the dry mixture and mixed for 5 minutes. Water was added to obtain 30% moisture, and the ingredients were mixed for an additional 10 minutes. Diets were passed through a grinder with a 2-mm die, then air dried for 24 hours. Diet strands were pelletized, with proximate composition of formulated aquafeed diets verified by NIR spectroscopy by using a microphazir ag-

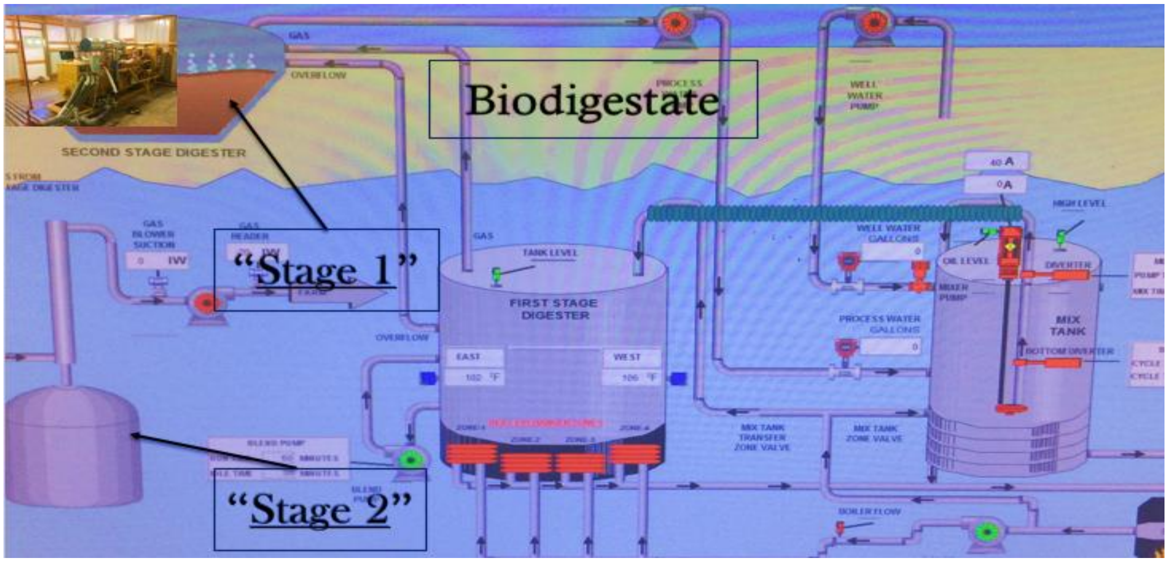

Figure 3. *Photograph of control-panel schematic of two-stage biodigestion of stillage & food waste, designed for rural/agricultural land use.* Multiple stages of digestate labeled. Provided from personal communication with commercial biogas industry participant.

analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Recirculating Aquaculture System & Water Quality Maintenance

Tilapia were stocked in an indoor recirculating aquaculture system (RAS), comprised of nine racked aquaria (0.16 m^3), a 1000-L mechanical and biological propeller-washed bead filtration system, QL-8 ultraviolet sterilization filter (Lifegard Aquatics, Cerritos, CA), and heated sump with two-stages of pad filtration containing bio-balls for maintaining bacterial growth. Continuous circulation occurred between the sump, bead-filter, and UV-sterilizer. Aeration was provided by a Rotron blower (Ametek, Kent, OH), which supplied oxygen to one 12-inch air diffuser per tank. Prior to this feeding trial, juvenile Nile Tilapia were reared for approximately six weeks using commercial EXTR 400 Rangen feed (40% CP, 14% crude fat) until reaching an average stocking weight of 49 ± 1.5g. Triplicates were randomly selected to form three groups, one group for each feed mix. A total of 90 juvenile Nile tilapia were assessed for body weight and randomly stocked into the tanks in groups of 10, with an average feed-group stock density and total RAS stock density at 3.08 ± 0.2 kg/m^3 and 3.09 ± 0.1 kg/m^3, respectively. Throughout the experiment, fish were fed to satiation. Temperature, pH, salinity, and dissolved oxygen (D.O.) continued to be monitored using a Hydrolab Quanta device (Hydrolab Corporation, Loveland, CO), with average temperature 26 degrees Celsius, pH 7.8, and D.O. 5.05 mg/L. TAN and NO_2-N were monitored with a HACH DR 2800 spectrophotometer and controlled through partial change of water dechlorinated with sodium bicarbonate. Average NO_2-N and TAN were 0.137±.02 mg/L and 0.758±.09 mg/l respectively.

Tilapia Harvest & Analysis

After a period of 8 weeks, all fish were weighed by tank and a random sample of three fish per tank were euthanized, weighed, measured, and dissected to determine intraperitoneal fat (IPF), gut, and liver weight. From this data, production parameters including feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), hepatosomatic index (HSI), and weight gain rate (WGR) were determined. Blood samples from euthanized tilapia were also taken from the tail and analyzed by I-STAT E3+CL and VetScan VS2 comprehensive diagnostic blood analyzer.

Rearing Methods for Separate BSFL Analysis

In comparison to BSFL larvae products used in the aquaculture study, two larvae-rearing trials were conducted using spent DDGS as a supplement combined with anaerobic biodigestate in a liquid form. A commercial biodigestion facility operated by MAC Farms, Inc. which primarily uses stillage for digestion provided sampling points for this study. Biodigestate was captured from a stage of initial methagenesis in a bladder and a later stage post-bladder (referred to hereon as "stage 1" and "stage 2," respectively).

The BSFL rearing trials occurred within a commercially available Bio-Pod Plus system and varied by use of stage 1 and stage 2 biodigestate. With an initial target TS content of the rearing substrate between 30-40% (Dortmans, 2015), the substrate mixture volume for Trial 1 consisted of 0.28 parts peat moss, to 1-part pressed DDGS, to 1-part stage 1 biodigestate. The substrate mixture for Trial 2 consisted of 0.28 parts peat moss, to 1-part pressed DDGS, to 1-part stage 2 biodigestate. A period of 12 hours was allowed for the substrate to settle in the BioPod after formulation, at which point 500 BSFL were introduced in each trial. Initial age of the BSFL was approximately four days at introduction to the substrate in each trial, therefore, rearing time for both trials was determined to be 11 days to satisfy an approximate two-week period prior to the prepupal instar at harvest (Sheppard et al, 2002). The relative mean temperature for Trial 1 was 23 degrees Celsius and 26 degrees Celsius for Trial 2; optimal temperature for BSFL is 27 degrees Celsius (Tomberlin, 2009). Throughout both trials, a filtering pad of the Bio-Pod remained in place on top of the drainage port to retain and absorb moisture and allow for any draining of the system without loss of solids at the end of the trial. As primarily digestate liquids filtered into the catchment area and drainage port, it was manually recycled throughout the system on a once-daily basis for both trials. As expected, there was a visible increase in TS content observed throughout the trials as the absorbed substrate material was consumed by larvae.

BSFL Harvest Methods and Preliminary Data

Larvae were harvested on day 11 at the end of the trial, the substrate-biomass was removed, and contents were sieved to separate larvae manually as opposed to post-migratory harvest. In Trial 1, 324 larvae were manually harvested from the BioPod. For Trial 2, 336 larvae were harvested in the same manner. Collected larvae for both trials were then stored at -20 degrees Celsius, then freeze-dried by a Labconco FreeZone machine (LABCONCO, Kansas City, MO) at minus 84 degrees Celsius and .570 mBar until total moisture was below 10%. Then, 10 samples of independent, random samples of 30 larvae from each trial were collected and ground with a pestle for microphazir analysis where results were then averaged.

RESULTS

Tilapia Production Performance & Blood Parameters

Performance of juvenile Tilapia diets post-harvest are represented in Table 2. Results were compared using

	Diet		
	Control	BSFLM	BSFLM+O
FBW (g)	149.2±8.4	140.6±6.4	135.5±7.1
WG (%)	204.5±15.0	183.1±13.0	173.8±10.0
SGR (%/day)	1.6±0.1	1.5±0.1	1.4±0.1
S (%)	100.0±0.0	96.7±3.3	100.0±0.0
FER	0.9±0.0^{a}	0.6±0.1^{b}	0.6±0.0^{b}
FCR	1.2±0.1^{b}	1.7±0.2^{a}	1.6±0.1^{a}
PER	2.6±0.1^{a}	1.9±0.2^{b}	1.9±0.1^{b}
K (%)	2.1±0.1	2.2±0.0	2.2±0.1
ISI (%)	3.6±0.4	2.7±0.1	3.5±0.4
HSI (%)	3.1±0.3^{a}	1.9±0.1^{b}	2.9±0.4^{ab}

Table 2. *Growth performance and feed utilization of tilapia fed the control and experimental diets for 8 weeks**. *FCR- Feed Conversion Ratio, PER- Protein Efficiency Ratio, SGR- Specific Growth Rate, HIS- Hepatosomatic Index, ISI- Intestinosomatic Index, WFR- Weight Gain Rate, K-Condition Factor.Values are mean ± SE of three replicates per group for FBW, WGR, SGR, Survival, FER, FCR and PER, and nine replicates for K, ISI and HSI. Means in the same row with different superscripts are significantly ($P < 0.05$) different. Data were statistically analyzed using SPSS version 20 (SPSS, Chicago, IL, USA), and then subjected to one-way analysis of variances (ANOVA). Significant differences among the group means were further compared using Duncan's multiple range tests. $P < 0.05$ was statistically significant.

Diet	TP g/dL	Glu mg/dL	ALT U/L	ALP U/L	ALB g/dL	GLOB g/dL	TBIL mg/dL	HB mmol/L	Hct %
1	3.8 ± 0.1	51.3 ± 4.3	56.7 ± 25	38.0 ± 3.5	1.9 ± 0.0	1.9 ± 0.1	0.3 ± 0.0	7.2 ± 0.3	21.3 ± 0.9
2	4.5 ± 0.6	55.3 ± 4.7	63.7 ± 22	38.0 ± 7.8	2.1 ± 0.2	2.4 ± 0.4	0.3 ± 0.1	8.5 ± 0.7	25.0 ± 2.1
3	3.9 ± 0.3	51.7 ± 2.9	40.3 ± 23	41.3 ± 3.8	1.6 ± 0.1	2.2 ± 0.2	0.4 ± 0.0	7.8 ± 0.4	23.0 ± 1.2

Table 3. *Random sample tilapia averages of biochemical and hematological blood data, respectively.* TP, total protein; Glu, glucose; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ALB, albumin; GLOB, globulin; TBIL, total bilirubin; Hb, hemoglobin; Hct, hematocrit. SE calculated by SPSS statistical package (IBM, Chicago).

ANOVA data analysis performed by SPSS statistical package via Duncan's statistical analysis for significance. FCR, FER, and PER were held statistically significant from the Control diet.

The Control diet yielded a significantly higher PER and FCR than other diets, indicating greater feed utilization efficiency. However, the FCR and FER for both Diets 2 and 3 were significantly higher than Diet 1, and Diet 1 HSI was not significantly greater than BSFL+O. Any differences in final body weight (FBW) and specific growth rate (SGR) of BSFL feeds were not statistically different from the control. No significant difference with the control was found among blood-chemical and hematological parameters (Table 3, s 4 & 5) and the mean values are regular.

Hepatic enzyme presence (alanine aminotransferase-ALT, alkaline phosphatase-ALP) is consistent (Figure 4), showing no statistically significant difference, while remaining within a desirable reference range with no detected liver damage or evidence of hemolysis. Meanwhile, ALB and GLOB, and total bilirubin (TBIL) (Figure 5) are deemed acceptable across all diets suggesting consistent immune system functionality with a low level of blood clotting and healthy urinary excretion. Lack of significant difference in these areas among sampled diet groups suggests a general uniformity of immune and digestive health, which is consistent with assessment of glucose (GLU) levels which suggests near uniform stress (Barton, 2002) observable across all diet groups.

BSFL Rearing Trial

For both trials, microphazir analysis showed larvae nutrient content to be similar, with protein content at 44.2 and 44.7%, and extractable oil at 32.8 and 33.5%, respectively (Table 4).

The proximate content demonstrated by this result suggest that larvae reared on either stage of biodigestate meet the proximate nutrition requirements as successfully implemented Nile Tilapia aquafeed reared on mixed stillage. Thus, it is demonstrated that the BSFL can utilize anaerobic digestate in a substrate with mixed stillage as sorbent to a degree qualifying to satisfy Tilapia nutrition.

Concerning hematological parameters, higher hematocrit levels as well as hemoglobin rate—supportive of higher blood protein—are typically good indicators for oxygen transport capacity, which can be impaired by infection causing infected fish to develop an amneic state (Sebastiao et. al, 2011). The Hct and Hb levels in this study indicate organic defense by circulating blood cells was maintained in the RAS environment. Meanwhile, ALB and GLOB, and total bilirubin (TBIL) (Table 3) are uniformally at an acceptable level, suggesting consistant immune system functionality with a low level of blood clotting and healthy urinary excretion.

Nutrient Parameter	Trial 1	Trial 2
	%	%
Protein	44.22	40.74
Moisture	2.10	1.95
Extractable Oil	32.84	33.47
Total Oil	33.36	33.77
Fiber	4.12	4.22
Ash	12.47	14.46
Starch	0.94	2.90

Table 4. *BSFL proximate nutritional content.* Microphazir analysis of BSFL for rearing trail 1 and trial 2, respectively.

Nutrient Analysis	Trial 1	Trial 2
	%	%
Protein	32.54	32.08
Moisture	8.05	1.82
Extractable Oil	3.60	8.88
Total Oil	12.15	9.52
Fiber	4.05	4.48
Ash	8.86	8.45
Starch	27.37	34.84

Table 5. *Post-trial rearing substrate proximate nutritional content.* Microphazir analysis of freeze-dried substrate with stage 1 biodigestate for rearing trial 1 and freeze-dried substrate with stage 2 biodigestate for rearing trial 2, respectively.

DISCUSSION & CONCLUSION

Considering the indicated general level of health, the feeding trial demonstrated the resulting feedstuffs developed from DDGS reared BSFL are suitable feed ingredients for Nile Tilapia. Although BSFL-derived meal and oil evaluated in this study had lower nutritional value relative to FM, SBM, FO, and SBO regarding feed efficiency, diets were readily accepted by the Tilapia and supported similar growth rate and final weight. Any potential decrease in feed efficiency is tolerated, in addition to SGR, which is supported by uniform stress throughout blood parameters. Concerning PER, HSI, and feed efficiency, chitin has been suggested as a reason for inhibiting performance even at 1% diet inclusion, where it has also been suggested that defatting procedures in processing BSFLM may cause integration of lipids into developed chitin structure, affecting their availability for digestion (Rust, 2002; Kroekal et al., 2012). Nonetheless, considering a small degree of difference in amino acid-N and protein-N sources, as well exclusive use of mechanical methods producing the BSFL ingredients in this study, effects of chitin on PER and feed efficiency may be negligible as no significant negative effect on growth parameters was observed.

In the BSFL trial, reared proximate content mirrored that of BSFL used in products for aquaculture feed production, having a typical protein content over 40% and fat/oil over 30%. Thus, larvae reared on anaerobic biodigestate show a sufficient applicability as an ingredient. Additionally, the resulting frass may be an adequate candidate to serve as fertilizer or soil additive or potential supplement to aquaculture feed as has been observed in other studies (Webster et al., 2015), though further frass analysis is needed. Analysis of the post-trial substrate as frass from trials one and two (Table 5) revealed protein content of 32.5 and 32.1%, and total oil 12.15 and 9.12%, respectively.

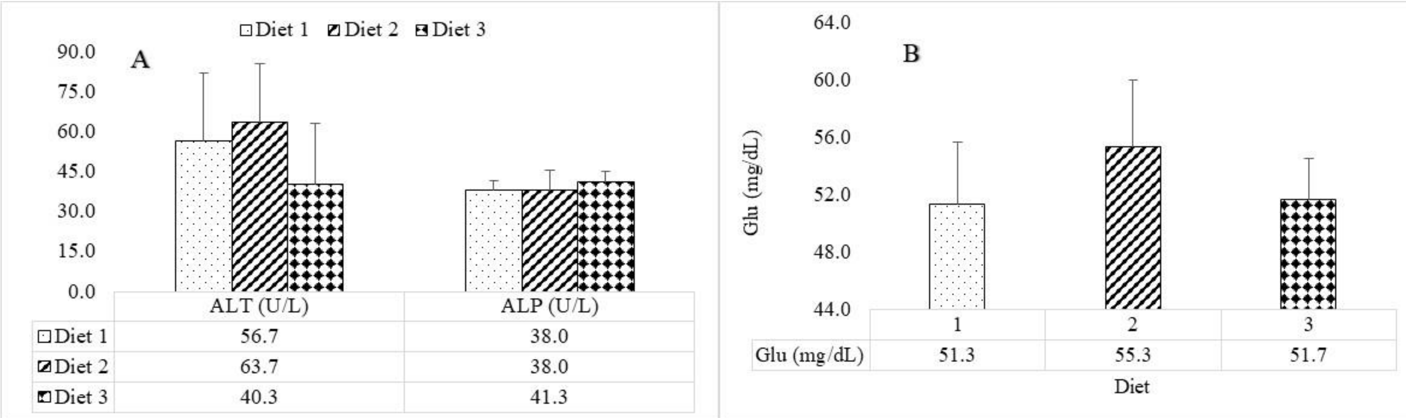

Figure 4. *ALT and ALP activities (**A**) and glucose content (**B**) in the blood of tilapia fed the experimental diets for 8 weeks.*

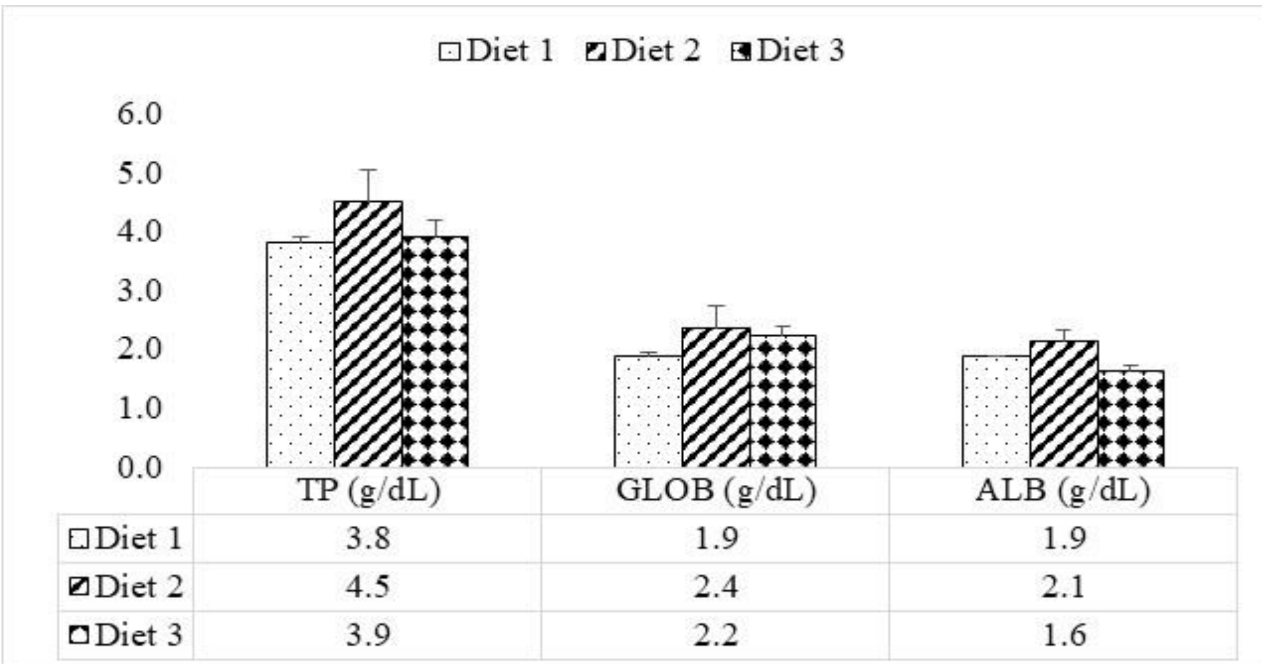

Figure 5. *Total protein (TP), globulin (GLOB) and albumin (ALB) contents in the blood of tilapia fed the experimental diets for 12 weeks.*

Trial 1 substrate had a lower percentage of extractable oil at 3.6% rather than 8.88% for Trial 2. Reduced extractable oil in Trial 1 may be due to a more prolonged process of methanogenesis in the stage 2 digestion process or increased structural breakdown within the substrate due to higher anaerobic activity. A higher level of extractable oil in Trial 2 may suggest greater potential for bioconversion of n-3 and/or n-6 fatty acids by the BSFL than Trial 1, though further analyses is needed in assessing amino acid profile of biodigestate reared larvae. Furthermore, a harvest period with introduction of biodigestate could potentially degrade chitin structure development, similar to anaerobic bacteria observed in salt marsh environments (Boyer, 1987). However, concerns of chitin- often considered as a nutritional additive- may not be significant especially when using mechanical methods for feed development. Proximate nutrition content from this rearing trial provides a potential connection to form a closed sustainable system between waste management, energy production, and food production, where aquaculture sludge may be managed through for meso/thermophilic digestion (Madariaga and Marín, 2017).

In conclusion, by supporting the Nile Tilapia diet, the BSFL shows significant promise to close a gap of sustainability in an integrated process with anaerobic digestion, while its use of alternative feed and waste management may positively impact emission of greenhouse gases (GHG) especially in supporting methane sequestration. Any potential GHG emission in the substrate from active biodigestate is curtailed by larval reduction of the substrate, a vermiculture & digestate management process more environmentally friendly and economically supportive of food production and waste management. BSFL reared on the byproducts of distilling and renewable biogas seem to show high-potential to be implemented in a more closed-loop sustainable system between aquaculture and green-energy production via waste management.

Conclusion on Viability of the System

Additional industrial-scale study is required to implement the closed-loop system as suggested by this study. However, based upon an analysis of the vermiculture pilot study and results of aquaculture feeding trials, this study suggests great promise for a sustainable closed-loop system between anaerobic biodigestion, vermiculture production, aquaculture production, crop production, and distilling & subsequent stillage wastes. Given favorable economic conditions surrounding the use of stillage waste and most processes of "upcycling" value from wastes, private or public efforts are certainly warranted to develop such a system in a replicable manner and at an industrial scale. This may reduce methane emissions and wastes from each of the component practices and external inputs, in addition to proving more sustainable means of higher crop yields, and a renewable energy source provided from biogas. Further implementation and design of carbon-capture technologies which may yield residual products useful in the agricultural sphere or in other component practices of the sustainable closed-loop is also warranted to further total carbon reduction. This too may be critical for large-scale development where net carbon-impact may be more fully assessed for this system to be fully considered in its capability as a potential combatant against greenhouse gas emissions. With the critical link qualitatively and quantitatively established in this study via aquaculture and vermiculture, the potential impact of this closed-loop system should be a subject of great excitement by the sustainability and related scientific communities. The results of this study urge large-scale implementation for further study.

REFERENCES

Adeniyi OV, Folorunsho CY (2015). Performance of Clarias gariepinus (Burchell, 1822) fed dietary levels of black soldier fly, Hermetia illucens (Linnaeus, 1758) prepupae meal as a protein supplement.Int J Res Fish Aquat 5:89–93.

A.M. Ziganshin, T. Schmidt, F. Scholwin, O. Il'inskaya, H. Harms, S. Kleinsteuber (2011). Bacteria and archaea involved in anaerobic digestion of distillers grains with solubles. Appl. Microbiol. Biotechnol., 89 , pp. 2039-2052

Barton, BA (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. Integrative and Comparative Biology. 42(3): 517-525.

Bondari, K. and Sheppard, D. C. (1987). Soldier fly, Hermetia illucens L., larvae as feed for channel catfish, Ictalurus punctatus (Rafinesque), and blue tilapia, Oreochromis aureus (Steindachner). Aquaculture Research, 18: 209-220. doi:10.1111/j.1365-2109.1987.tb00141.x

Boyer, JN (1987). End products of anaerobic chitin degradation by salt marsh bacteria as substrates for dissimilatory sulfate reduction and methanogenesis. Applied Environmental Microbiology. Dec; 52(6): 1415-8.

C. Eskicioglu, K.J. Kennedy, J. Marin, B. Strehler (2011). Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions. Bioresour. Technol., 102, pp. 1079-1086.

David Zaks, Niven Winchester, et. al (2011). Contribution of Anaerobic Digestors to Emissions Mitigation and Electricity Generation Under U.S. Climate Policy. Environmental Science Technology, 45 (16): 6735-6742. Web: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155279/

Diener, S., Studt Solano, N.M., Roa Gutiérrez, F., et al. (2011). Biological Treatment of Municipal Organic Waste using Black Soldier Fly Larvae. Waste Biomass Valorization 2: 357.

Dortmans (2015). Valorization of Organic Waste - Effect of the Feeding Regime on Process Parameters in a Continuous Black Soldier Fly Larvae Composting System. Masters thesis. Industrial ecology royal institute of technology, Stockholm, Sweden.

FAO (2016). The State of World Fisheries and Aquaculture (2016). Contributing to food security and nutrition for all. Rome. 200 pp.

Gustavsson, Jenny & Cederberg, Christel & Sonesson, Ulf (2011). Global Food Losses and Food Waste. Save Food at Interpack Düsseldorf, Germany.

He, Qingyao, Shi, Mingfei , Liang, Feihong, Xi, Long, Cheng, Xin, and Yan, Shuiping (2019). A Sustainable Process Concerning Negative CO2 Emission and Profit Increase of Anaerobic Digestion. Sustainable Chem. Eng., 7, 2, 2276–2284.

Janet Kelly, Barry Kornstein, Ryan Marshall (2017). The Economic and Fiscal Impacts of the Distilling Industry in Kentucky. University of Louisville, Urban Studies Institute prepared for the Kentucky Distillers Association. Web: https://kybourbon.com/wp-content/uploads/2017/03/Kentucky-Bourbon-Economic-Impact-Study-2017-Final.pdf

Johan Lund (2014). Aquaculture Effluents as Fertilizer in Hydroponic Cultivation: A Case Study Comparing Nutritional and Microbiological Properties. Beatrix Alsanius, SLU, Institutionen för Biosystem och teknologi, Accessed at: https://core.ac.uk/download/pdf/19883686.pdf

Kentucky Department for Environmental Protection (2017). Distilling By-products: Simplifying Compliance. Division of Compliance Assistance, accessed at: https://eec.ky.gov/Environmental-Protection/Compliance-Assistance/DCA%20Resource%20Document%20Library/DistillersGrainsontheFarm.pdf

Lievens, S., Poma, G., De Smet, J., Van Campenhout, L., Covaci, A., & Van Der Borght, M. (2021). Chemical safety of black soldier fly larvae (Hermetia illucens), knowledge gaps and recommendations for future research: a critical review. Journal of Insects as Food and Feed, 1-14.

Madariaga, S. T. and Marín, S. L. (2017). Sanitary and environmental conditions of aquaculture sludge. Aquac Res, 48: 1744-1750. doi:10.1111/are.13011

Mirzoyan, N., Parnes, S., Singer, A., Tal, Y., Sowers, K., & Gross, A. (2008). Quality of brackish aquaculture sludge and its suitability for anaerobic digestion and methane production in an upflow anaerobic sludge blanket (UASB) reactor. Aquaculture, 279(1-4), 35-41.

Médale, F., R. Le Boucher, M. Dupont-Nivet, E. Quillet, J. Aubin, and S. Panserat (2013). Des aliments à base de végétaux pour les poissons d'élevage. INRA Prod. Anim. 26:303–316.

Mikucka, W., Zielińska, M. Distillery Stillage: Characteristics, Treatment, and Valorization. Appl Biochem Biotechnol **192,** 770–793 (2020). https://doi.org/10.1007/s12010-020-03343-5

Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., & El Naggar, M. H. (2012). Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresource technology, 111, 122-126.

Newton, G. L., D. C. Sheppard, D. W. Watson, G. J. Burtle, C. R. Dove, J. K. Tomberlin, and E. E. Thelen (2005). The Black Soldier Fly, Hermetia illucens, as a manure management/resource recovery tool. Symposium on the state of the science of Animal Manure and Waste Management: 5-7.

Olsen, R.L., and M.R. Hasan (2012). A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends Food Sci. Technol. 27:120–128.

Panuccio, M. R., Papalia, T., Attinà, E., Giuffrè, A., & Muscolo, A (2019). Use of digestate as an alternative to mineral fertilizer: effects on growth and crop quality. Archives of Agronomy and Soil Science, 65(5), 700-711.

P.R. Shukla, J. Skea, R. Slade, R. van Diemen, E. Haughey, J. Malley, M. Pathak, J. Portugal Pereira (eds.) Technical Summary (2019). In: Climate Change and Land: An IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. In press.

Rust, M.B. (2002). Nutritional physiology. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. The Academic Press, New York, pp. 368–446.

Sánchez-Muros, M.J., De Haro, C., Sanz, A., Trenzado, C.E., Villareces, S., Barroso, F.G. (2015). Nutritional evaluation of Tenebrio molitor meal as fishmealsubstitute for tilapia (Oreochromis niloticus) diet. Aquacult. Nutr., http://dx.doi.org/10.1111/anu.12313 (in press)

S.D. Meester, D. Jens, V. Filip, A. Peene, H. V. Langenhove, J. Dewulf (2012). The environmental sustainability of anaerobic digestion as a biomass valorization technology. Bioresource Technology, 121, October 2012, 396-403.

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., ... & Borgemeister, C (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Scientific reports, 9(1), 1-13.

Slorach, P. C., Jeswani, H. K., Cuéllar-Franca, R., & Azapagic, A (2019). Environmental sustainability of anaerobic digestion of household food waste. Journal of environmental management, 236, 798-814.

Snow, A. M., Ghaly, A. E. & Snow, A (2008). A Comparative Assessment of Hydroponically Grown Cereal Crops for the Purification of Aquaculture Wastewater and the Production of Fish Feed. American Journal of Agricultural and Biological Sciences, 3(1), 364-378. https://doi.org/10.3844/ajabssp.2008.364.378

F. A. Sebastião, D. Nomura, R. Sakabe, F. Pilarski (2011). Hematology and Productive Performance of Nile Tilapia (Oreochromis niloticus) Naturally Infected With Flavobacterium columnare. Brazillian Journal of Microbiology. Jan-Mar; 42(1): 282-289.

Tacon, A.G.J., and M. Metian (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285:146–158.

Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumnser SM (2002). Rearing methods for the Black Soldier Fly (Diptera: Strastiomyidae). Medical Entomology 39(4):695-698.

Sheppard DC, Newton GL, Thompson SA (1994). A value added manure management system using the black soldier fly. Bioresource Technology 50:275–279.

Stamer, A (2015). Insect proteins--a new source for animal feed: The use of insect larvae to recycle food waste in high-quality protein for livestock and aquaculture feeds is held back largely owing to regulatory hurdles. EMBO reports 16: 676–680

Stamer A, Stefan Wesselss , Ralph Neidigk , G. Hoerstgen-Schwark (2014). BSF larvae-meal as an example for a new feed ingredients' class in aquaculture diets. Proceedings of the 4th ISOFAR Scientific Conference. 'Building Organic Bridges', at the Organic World Congress 2014, 13-15 Oct., Istanbul, Turkey (eprint ID 24223).

K C, Surendra & Olivier, Robert & Tomberlin, Jeffery & Jha, Rajesh & Khanal, Samir (2016). Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renewable Energy. 98. 10.1016/j.renene.2016.03.022.

US Environmental Protection Agency (2021). Inventory of U.S. Greenhous gas emissions and Sinks. Accessed at: https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2019

Vutai, V., Ma, X. C., & Lu, M. (2016). The Role of Anaerobic Digestion in Wastewater Management. US Environmental Protection Agency EM (Pittsburgh, Pa.), (September 2016), 12.

Van Tung, T., Tran, Q. B., Phuong Thao, N. T., Vi, L. Q., Hieu, T. T., Le, S., Tuan, N. Q., Sonne, C., Lam, S. S., Hai, L. T., & Le, Q. V. (2021). Recycling of aquaculture wastewater and sediment for sustainable corn and water spinach production. Chemosphere, 268, 129329. https://doi.org/10.1016/j.chemosphere.2020.129329

Webster, C. , Rawles, S. , Koch, J. , Thompson, K. , Kobayashi, Y. , Gannam, A. , Twibell, R. and Hyde, N. (2016). Bio-Ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, Hermetia illucens, along with poultry by-product meal and soybean meal, as total replacement of fish meal in diets for Nile tilapia, Oreochromis niloticus. Aquacult Nutr, 22: 976-988. doi:10.1111/anu.12316

Xiao, Xiaopeng & Jin, Peng & Zheng, Longyu & Cai, Minmin & Ziniu, yu & Yu, Jeffrey & Zhang, Jibin. (2018). Effects of black soldier fly (Hermetia illucens) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (Pelteobagrus fulvidraco). Aquaculture Research. 10.1111/are.13611.