ARTICLE IN PRESS

Waste Management xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions

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ARTICLE INFO

Article history:
Received 29 June 2017
Revised 22 November 2017
Accepted 14 January 2018
Available online xxxx

Keywords:
Hermetia illucens
Manure management
Sustainable agriculture
Indole

ABSTRACT

Volatile emissions from decomposing animal waste are known environmental pollutants. The black soldier fly, *Hermetia illucens* (L.), is being evaluated for industrialization as a means to recycle wastes and produce protein for use as food and feed. We examined the ability of black soldier fly larvae to reduce odorous compounds associated with animal wastes. Black soldier fly larvae were reared under laboratory conditions on poultry, swine, and dairy manure at feed rates of 18.0 and 27.0 g every other day until 40% reached the prepupal stage. Volatile emissions were collected and analyzed from freshly thawed as well as the digested waste when 90% of the black soldier fly larvae reached the prepupal stage. Volatiles were also collected simultaneously from manure not inoculated with black soldier fly larvae (non-digested) and held under similar conditions. Manure samples were analyzed for relative amounts of nine select odorous volatile organic compounds: phenol, 4-methylphenol, indole, 3-methylindole, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid and pentanoic acid. Black soldier fly larvae reduced emissions of all volatile organic compounds by 87% or greater. Complete reductions were observed for 2-methly propanoic acid in digested poultry manure, phenol, 4-methylphenol, indole and all five fatty acids in digested swine manure, and 4-methylphenol, indole, 3-methylindole and all five acids in digested dairy manure. This study is the first to identify volatile emissions from manure digested by black soldier fly larvae and compare to those found in non-digested manure. These data demonstrate additional benefits of using black soldier fly larvae as a cost-effective and environmentally friendly means of livestock manure management in comparison to current methods.

Capsule: Black soldier fly larvae are capable of altering the overall profile of volatile organic compounds and reducing levels of targeted odorous compounds in livestock manure.

1. Introduction

The decomposition of manure is responsible for environmental emissions, such as greenhouse gases, ammonia and other volatile organic compounds (VOCs), which are pollutants and pose potential health risks (FAO, 2009). Between 100 and 330 VOCs and volatile fatty acids are generated by concentrated animal feeding operations (CAFOs,) depending on management practices and the species of animal involved (Cai et al., 2015; Powers and Bastyr, 2004; Schiffman et al., 2001). The compounds most associated with or responsible for the odor of manure are phenols, indoles, alcohols, organic sulphides, and volatile fatty acids (Hales et al., 2012; Kuroda et al., 1996; El-Mashad et al., 2011). For example, Hales et al. (2012) found that 4-methylphenol was responsible for 67.3% of odor activity in dairy manure. The key odorous VOCs in poultry manure were butanoic acid, 3-methylbutanoic acid, dimethyl trisulphide, indole and skatole (Yasuhara, 1987). These VOCs, which are noxious odors, can also negatively affect humans by posing potential health risks to those living in communities surrounding animal farming facilities (PEW, 2008). VOCs responsible for strong odors contribute to higher levels of tension, depression, and anger experiences by those working or living in close proximity to areas with heavy animal farming (Barrett, 2006).

With the increasing amount of manure and the need for a sustainable method of management, fly (Diptera) larvae have become an alternative means to process this resource. Black solider fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae (BSFL) have been studied as a means of manure management because of

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https://doi.org/10.1016/j.wasman.2018.01.019
0956-053X/

Please cite this article in press as: Beskin, K.V., et al. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management (2018), https://doi.org/10.1016/j.wasman.2018.01.019

several beneficial abilities. First, BSFL reduce organic matter, such as livestock manure. Sheppard (1994) observed significant reductions in poultry manure (~50%) in one study, and in another study reported 56 and 42% reductions in dry weight, depending on whether manure had water added to it or not (Sheppard, 1983). Newton et al. (2005) observed a 39% reduction in the dry weight of swine manure processed by BSFL. Myers et al. (2008) documented a 58% and 33% reduction in dry matter of manure from BSFL fed 27 g and 70 g of dairy manure daily, respectively. In a study comparing poultry, swine, and dairy manure, the dry matter of all three manure types was reduced by ~37% (Oonincx et al., 2015).

In addition to the reduction of dry matter of manure, nutrients, which in excess can be detrimental to the environment, are decreased. BSFL reduce the nitrogen content of poultry manure by 62% (Sheppard, 1983). During the digestion of dairy manure, BSFL reduced concentrations of nitrogen and phosphorus by 30–50% and 61–70%, respectively (Myers et al., 2008). When BSFL were allowed to feed on poultry, swine, and dairy manure, nitrogen content was reduced by 80, 37, and 30%, respectively (Oonincx et al., 2015). Importantly, adult black soldier flies do not need to feed and their non-synanthropic nature has earned them the label of a non-pest species (Furman et al, 1959). However, to date, the impact of black soldier fly digestion of manure on VOC production has not been examined. The purpose of this study was to assess how the digestion of poultry, swine, and dairy manure by BSFL impacts select odorous VOCs.

2. Materials and methods

2.1. Acquisition of flies

The *H. illucens* larvae were from a colony established in 2014 from eggs from a colony maintained at the Coastal Plains Experiment Station, University of Georgia, (Tifton, GA) and are now maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University (College Station, TX). Adult flies were maintained in a 2.6 × 1.3 × 1.3 m wooden cage fitted with metal screening, in a greenhouse maintained at approximately 29 °C. Adults were allowed to oviposit in three 7.0 × 5.0 × 0.3 cm pieces of corrugated cardboard (Booth and Sheppard, 1984) held together with masking tape and placed on the lid of a 30.0 × 15.0 × 11.0 cm plastic shoe box containing one kilogram of Gainesville diet (Hogsette, 1992) saturated with water. A 13.0 × 5.0 cm portion of the lid was removed and replaced with metal screening on which the cardboard pieces were placed; this approach allowed volatiles to escape from the wet Gainesville diet and attract gravid flies, but prevented the flies from contacting and/or ovipositing directly into the media instead of the cardboard. The cardboard was removed from the cage after eight hours, and eggs were removed from cardboard using a sterile plastic spatula and weighed. One gram of eggs was then placed in a 0.5 L plastic container, covered with a paper towel secured with a rubber band, stored in a walk-in environmental chamber (29 ± 0.3 °C with 60 ± 5.1% relative humidity and 14:10 L:D cycle) and checked every 12 h until hatch. Two hundred grams of Gainesville diet at 70% moisture was added to the container once larvae eclosed. Newly-emerged larvae were allowed to feed for four days in the environmental chamber prior to use in the experiment.

2.2. Acquisition of manure

Three different livestock manure types were used in this study. Poultry manure was collected from layer hens housed at the Poultry Science Research, Teaching, and Extension Center at Texas A&M University in College Station, TX, USA. The hens were fed a mixture of corn and soybean meal that is considered typical layer diet, consisting of 18.5% crude protein, 2.5% crude fat and 2.4% crude fiber. Swine manure was collected from sows raised by Schroeder Genetics in Anderson, TX, USA. The sows were maintained on cubes containing 14.0% crude protein, 2.8% crude fat, and 6.5% crude fiber formulated for gilts, sows and adult boars. Dairy manure was collected from cows maintained at the Southwest Regional Dairy Center in Stephenville, TX, USA. The diet for these animals consisted of 16.1% crude protein, 5.0% crude fat and 28.1% crude fiber. The majority of this diet is composed of a mixture of corn silage (32.0%), ground corn (22.5%), and concentrate pre-mix (19.4%) composed of canola and soybean meal.

Each manure type was collected on site within 12 h of excretion, using a shovel and two 19 L buckets with lids, sterilized prior to use for manure collection. The manure was transported to the F.L.I.E.S. Facility where it was homogenized in the buckets and transferred to individual 3.78 L self-sealing plastic freezer bags and frozen at −20 °C until used. Manure was removed from the freezer and allowed to thaw for 24 h at room temperature before being used. Thawed manure was stored in a refrigerator at 4 °C.

2.3. Experiment design

One hundred 4-d-old larvae were placed in 88.7 ml plastic bathroom cups (Georgia-Pacific LLC, Atlanta, GA, USA) and assigned to one of the three manure types (poultry, swine, or dairy) and one of two feed rates (18.0 or 27.0 g every other day). Feed rates used were based off the methods of Myers et al. (2008) who used 300 larvae and feed rates of 27, 40, 54 and 70 g of manure per day. The feed rates used in this study are therefore modified from this due to only 100 larvae being used and feeding occurring every other day. Preliminary experiments were conducted to confirm the scalability of these feed rates (unpublished data).

Containers without larvae were used as controls and subsequently referred to as non-digested manure. These containers received manure assigned at a given feed rate in similar fashion to those with larvae. Three replicates for each feed rate and manure type with and without larvae were used. Containers were placed in a randomized block design among three levels of a shelving unit in the environmental chamber maintained at 29 ± 0.3 °C with 60 ± 5.1% relative humidity and 14:10 L:D cycle The experiment was replicated twice (Fig. 1).

Initially, larvae in each replicate were provided manure at the assigned amount and allowed to feed for four days. Larvae and contents of the cup were then transferred to a 1.89 L Reditainer™ EXTREME FREEZE™ deli container (Clear Lake Enterprises, Port Richey, FL, USA) and fed every other day. Manure was weighed directly into the containers using a Scout® Pro Balance (Ohaus, Parsippany, NJ, USA). Containers were then covered with a 25.4 × 25.4 cm piece of tulle to prevent contamination.

Containers were checked daily for post-feeding larvae (i.e., prepupae, Sheppard et al. 1994), which were removed and identified by the cuticular color shifting from opaque to black (May 1961). Additionally, to monitor the progress of larval feeding, manure in each container was shifted using forceps that had been sterilized with 70% ethanol. Separate forceps were used for each manure type to prevent cross contamination. Feeding of larvae terminated when 40% of the larvae reached the prepupal stage (Sheppard et al. 2002). VOCs were sampled from the digested group and a matching randomly selected sample from the non-digested group of the same manure type and feed rate, when approximately 90% of the larvae reached the prepupal stage. This level of pupation was selected as it would also represent industrialized production of BSFL.

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