



Review

The use of fly larvae for organic waste treatment

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ABSTRACT

The idea of using fly larvae for processing of organic waste was proposed almost 100 years ago. Since then, numerous laboratory studies have shown that several fly species are well suited for biodegradation of organic waste, with the house fly (*Musca domestica* L.) and the black soldier fly (*Hermetia illucens* L.) being the most extensively studied insects for this purpose. House fly larvae develop well in manure of animals fed a mixed diet, while black soldier fly larvae accept a greater variety of decaying organic matter. Blow fly and flesh fly maggots are better suited for biodegradation of meat processing waste. The larvae of these insects have been successfully used to reduce mass of animal manure, fecal sludge, municipal waste, food scrapes, restaurant and market waste, as well as plant residues left after oil extraction. Higher yields of larvae are produced on nutrient-rich wastes (meat processing waste, food waste) than on manure or plant residues. Larvae may be used as animal feed or for production of secondary products (biodiesel, biologically active substances). Waste residue becomes valuable fertilizer. During biodegradation the temperature of the substrate rises, pH changes from neutral to alkaline, ammonia release increases, and moisture decreases. Microbial load of some pathogens can be substantially reduced. Both larvae and digested residue may require further treatment to eliminate pathogens. Facilities utilizing natural fly populations, as well as pilot and full-scale plants with laboratory-reared fly populations have been shown to be effective and economically feasible. The major obstacles associated with the production of fly larvae from organic waste on an industrial scale seem to be technological aspects of scaling-up the production capacity, insufficient knowledge of fly biology necessary to produce large amounts of eggs, and current legislation. Technological innovations could greatly improve performance of the biodegradation facilities and decrease production costs.

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1. Introduction

Increasing world population has necessitated the development of intensive confined animal feeding operations to satisfy the growing demand for animal protein. Large farms producing significant amounts of manure and other agricultural wastes are often concentrated in small areas without enough land available for proper waste disposal. This contributes to nutrient imbalances which sometimes result in deteriorating soil quality, water and air pollution (Westerman and Bicudo, 2005). Strict environmental regulations fostered considerable research into developing alternative waste management techniques. Sustainable agriculture depends on proper implementation of these new techniques.

Many insects naturally feed in organic wastes, incorporating the nutrients into their bodies and reducing the amount of waste material in the process. Coprophagous and carrion breeding flies play an important role in the recycling of organic matter in nature.

Lindner (1919) is probably the first who proposed the use of flies to recover nutrients, especially fat, from organic waste (human and animal excreta). Later, scientists showed that poultry manure may be artificially inoculated by house fly eggs, the newly-hatched larvae can be bred and harvested in a controllable manner and processed into meal fed to growing chicks (Calvert, 1979; Miller et al., 1974). Subsequent laboratory studies indicated that, despite relatively low yields of fly larvae (3.2% of fresh poultry manure on a wet basis), processing of manure by fly larvae is advantageous due to the high quality of fly protein, substantial reduction of manure mass, and conversion of manure residue into granular odorless material (Calvert, 1979; Morgan and Eby, 1975).

This paper summarizes currently available information about fly species and types of waste used for biodegradation, the technology of semi-natural and laboratory bioconversion systems, potential use and safety of the products and discusses the most recent advancement and perspectiveness of biodegradation of organic waste by fly larvae.

2. Fly species suitable for biodegradation of organic waste

Selection of suitable fly species is a very important factor determining success of biodegradation process. Size, behavioral characteristics, fecundity, duration of larval development, natural occurrence in the selected waste, pest status, adaptability to laboratory mass-rearing, and any species-specific requirements (e. g. adult diet) should be considered when selecting the optimum fly species for bioconversion.

2.1. The house fly, *Musca domestica* L.

House fly is a cosmopolitan species accompanying humans and livestock from tropical regions to the coldest areas in the world.

Adults are 6–9 mm long and feed on sebaceous fluids and also on most of the substrates where oviposition occurs (Hogsette and Farkas, 2000). House fly larvae can feed on a wide variety of decaying organic substrates, including animal manure and feed (Hogsette and Farkas, 2000).

The larvae develop through 3 larval instars. They grow fast; under optimum conditions pupation may occur after 3–5 days and adults emerge after another 4–5 days (Hogsette and Farkas, 2000). Pupation generally occurs in the dry upper layers of larval substrate. The development in warm regions during summer may be as fast as 7–10 days from eggs to adult and may extend to 40–49 days in cold environments (El Boushy, 1991).

The reproduction potential of house flies is great (Table 1). Under laboratory conditions, a maximum lifetime female reproductive output has been estimated to reach 729 eggs at 25 °C and 709 eggs at 30 °C (Fletcher et al., 1990). Due to the high population density, under mass-rearing conditions the fecundity is lower, reaching on average only 200–400 eggs per female in a 15-day egg collection period (Pastor et al., 2011). High reproduction rates, easy rearing in the laboratory and short development make the house fly an ideal insect for mass-rearing purposes.

The major disadvantage of the house fly is its pest status. It is a nuisance to both the man and animals and has been shown to transmit many pathogens, including parasites (Förster et al., 2007, 2009). The house flies can easily disperse several kilometers from the point of release (Hogsette and Farkas, 2000).

2.2. The black soldier fly, *Hermetia illucens* (L.)

Originally native to the Americas, the black soldier fly has been introduced to subtropical and tropical regions all over the world. Adults are large, conspicuous black flies up to 20 mm long. Larvae develop through 6 larval instars and generally grow to 18–20 mm (Rozkošný, 1997).

Adults are not strong fliers and spend most of the day resting on vegetation. Black soldier fly flourish at warmer temperatures, with almost all oviposition occurring at >26 °C (Tomberlin and Sheppard, 2002). Under laboratory conditions (greenhouse) mating usually occurs 2 days after eclosion and oviposition 4 days after eclosion (Tomberlin and Sheppard, 2002). Eggs are usually laid in crevices in dry locations near the larval substrate. Larvae can develop on a wide range of decaying plant and animal matter, including manure, food scrapes, municipal garbage, and rotting plant material (Diener et al., 2011a; Sheppard et al., 1994). Development of the

Table 1

Comparison of life histories (under optimum conditions) of some species of flies used for biodegradation. Note: Optimum conditions differ among the species.

Fly species	Common larval substrates	Duration of larval development (days)	Weight of pupae (mg/pupa)	Adult fecundity (eggs per female)	References
<i>Musca domestica</i>	Manure, garbage, food scrapes	3–5 ^a	5–21 ^{bc} (usually 12–16)	Up to 1000 (in several batches of 120–150 eggs) ^a , 200–400 in mass-rearing conditions ^c	^a Hogsette and Farkas (2000) ^b Barnard et al. (1998) ^c Pastor et al. (2011)
<i>Hermetia illucens</i>	Manure, rotting plant material, food scrapes	22–24 ^d	220 (natural) ^d 104–553 (laboratory) ^{de}	206–639 ^d , adults require special conditions for mating and oviposition	^d Tomberlin et al. (2002) ^e Nguyen et al. (2013)
<i>Lucilia sericata</i>	Carrion, meat, manure	3–4 ^f	13–63 ^g (usually 25–45)	172 (in the field) ^h	^f Grassberger and Reiter (2001) and Yehuda et al. (2011) ^g Tarone et al. (2011) ^h Hayes et al. (1999)
<i>Musca autumnalis</i>	Cattle manure	5 ⁱ	30 ^j	130 ^k	ⁱ Arends and Wright (1981) ^j Koo et al. (1980) ^k Wang (1964)

^{a–k} Superscript letters link data to references in the last table column.

black soldier flies from egg to prepupa lasts in laboratory conditions at 27 °C on average 22–24 days, from egg to adult on average 40–43 days (Tomberlin et al., 2002), but may take up to 4 months under less favorable conditions (Furman et al., 1959).

Major advantage of the black soldier flies over house flies is their behavior. The black soldier flies tend to rest on vegetation and do not approach humans or animals. Moreover, their presence may contribute to biological control of the house fly by limiting house fly oviposition and reducing house fly larval numbers (Bradley and Sheppard, 1984; Furman et al., 1959; Sheppard et al., 1994). The females produce a single clutch of 206–639 eggs (Tomberlin et al., 2002). Adults do not require food for successful mating and oviposition, because they can utilize nutrients accumulated in their fat body during larval development (Sheppard et al., 2002). Although larval and pupal development lasts much longer than in the house flies, the resulting pupae are much larger (Table 1). Thus, larger amount of waste may be utilized by one larva compared to the house fly. Additionally, black soldier fly prepupae may be self-harvested by redirecting their natural search for pupation sites into collection bins without a need for complicated equipment (Diener et al., 2011a; Sheppard et al., 1994).

Despite these benefits, however, the utilization of black soldier flies in temperate and cold regions may be problematic. Laboratory rearing of black soldier flies is somewhat difficult. The adults are territorial and have complex mating behavior which includes courtship (Tomberlin and Sheppard, 2001). Moreover, they need sunlight for successful mating (Tomberlin and Sheppard, 2002). This problem has been partially solved by maintaining the adult colony in an environmentally-conditioned greenhouse with artificial plants (Sheppard et al., 2002); this however may be too expensive in colder climates where heating will substantially increase the costs. Some success with artificial light sources (quartz-iodine lamps) has been achieved but mating still reached only 61% and peak oviposition rates approached 62% of that in control (sunlight) variant (Zhang et al., 2010). Laboratory rearing on artificial diets is still far from perfect. Young larvae are very frail and easily succumb to molds when excess larval diet is present. Eclosion rate of laboratory-reared pupae is also generally lower than eclosion from 'wild' pupae (Tomberlin et al., 2002).

2.3. The green bottle fly, *Lucilia sericata* (Meigen)

L. sericata is a blowfly found in most parts of the world. The adults are slightly larger than the house fly, with green body and green–blue or golden metallic shine. Larvae are sarcophagous/

necrophagous and are most often associated with animal dung and dead animal remains. The larvae develop through 3 larval instars, followed by a pupa. Complete development from egg to adult may take as little as 12 days at 25 °C (Grassberger and Reiter, 2001).

Reproduction potential of this blow fly is great, however, given the short duration of adults in natural environments (less than 32% of females survive to the first oviposition), the mean lifetime fecundity has been estimated to be up to 172 eggs per female (Hayes et al., 1999; Table 1). Although lifetime fecundity was not explicitly measured under laboratory conditions, it is probably several times higher than that in the nature, given the high survival of adults under laboratory conditions (70–80% after 30 days at 25 °C; Zhang et al., 2009) compared to natural populations (mean: 46–53 day-degrees; Hayes et al., 1999).

The green bottle fly is a well studied blow fly species. It is an important ectoparasite of sheep, causing myiasis known as 'blow fly strike' (Wall et al., 2001). The capability of larvae to remove necrotic tissue and promote healing has been utilized in the treatment of chronic wounds (Sherman, 2009). To exploit its medicinal properties, methods for aseptic and sterile breeding of both adults and larvae have been developed (Tachibana and Numata, 2001; Zhang et al., 2009) which minimize the risk of disease transmission. Recent evidence has shown, however, that aside from its therapeutic applications, *L. sericata* larvae may be a useful tool in biodegradation of manure and waste from meat processing facilities (Marchaim et al., 2003; Nuov et al., 1995; Yehuda et al., 2011).

2.4. Face fly, *Musca autumnalis* L.

Face flies can be found in much of the Palearctic region (Europe and Asia) and were first detected in North America in 1952 (Krafsur and Moon, 1997). The adults are similar in size and appearance to the house fly, but feature bright gray parafrenal regions on the head (Hogsette and Farkas, 2000).

Face fly larvae can feed on a variety of organic substrates, but are found most often in cattle manure. The larvae develop through 3 larval instars before migrating to the surrounding soil and duff for pupation (Arends and Wright, 1981; Wang, 1964). Using face flies to recycle nutrients has primarily been researched in North America.

Face fly larvae pupation is strongly temperature dependent, with the larval stage ranging from 60 h at 40 °C to 21 days at 11 °C, and adults emerge after another 7.5 days at 25–30 °C (Wang, 1964). Within a temperature range of 13.8–34.7 °C,

developmental times from egg to adult ranged from 7.8 to 46.1 days (Moon, 1983). The larvae make maximum use of a feed source that is less than 5 cm deep, and, where possible, crawl from their feeding site and empty their gut prior to pupation, allowing the possibility for self harvest. Pupae are generally larger than house fly pupae with mean weight of 30 mg and are higher in ash, as they have a calcified puparium (Koo et al., 1980). Females produce 6–36 eggs at intervals of several days over a life of 1–3 months (maximum of about 130 eggs total); the first batch may be deposited as early as 4 days after eclosion (Krafsur and Moon, 1997; Wang, 1964). In cold environments, adults may enter a facultative prereproductive diapause (Krafsur and Moon, 1997).

A notable disadvantage of face flies is, as their name suggests, that they are pestiferous to cattle and horses, feed on their faces and may pose as vectors of several diseases, most notably pinkeye (infectious bovine keratoconjunctivitis), thelaziasis, and other diseases (Hogsette and Farkas, 2000). On the other hand, face fly pupae have a very favourable nutritional profile and may be excellent source of dietary calcium, phosphorus, magnesium and several other trace elements, and reported microbial counts of dried pupae (treated with hypochloric acid prior to drying) were well below tolerances for human food (Koo et al., 1980).

2.5. The common flesh fly, *Sarcophaga carnaria* (L.)

Adults are typically 8–18 mm long, grayish, with characteristic tessellation on the abdomen. Species identification of flesh flies, particularly *S. carnaria*, is problematic and requires microscopic examination of male terminalia (females and larvae of several flesh fly species appear morphologically identical). Maggots develop through three larval instars. In nature, the larvae seem to be parasitoids of earthworms (Kirchberg, 1961); under laboratory conditions maggots can be reared on decaying meat (Pape, 1987; Yehuda et al., 2011). Adults are often seen visiting flowers, carrion and feces (Pape, 1987). Due to difficulties with identification of flesh fly adults and larvae (partially discussed by Cherix et al., 2012), limited information is available about their life history and development.

3. Types of waste suitable for biodegradation by fly larvae

The ability of larvae to develop in certain organic wastes may differ. The house fly larvae develop well in manure of animals fed a mixed diet (swine, poultry, calf) but not in manures of

herbivores (cow, goat, horse) (Larraín and Salas, 2008; Zhemchuzhina and Zvereva, 1988). The black soldier fly larvae, on the other hand, seem to develop well in a larger variety of decaying matter and are also commonly found in rotting fruits and plant residues. Blowflies and flesh flies, as typical sarconecrophages, may be better suited for biodegradation of waste from meat processing facilities. Differences in larval development and yields were also observed in manure collected from the same kind of animals (pigs) reared in different animal houses within the same farm. This was probably the result of different composition of feed fed to the animals (fattening diet vs. standard diet), differences in manure handling and optional use of sawdust bedding for lactating sows (Čičková et al., 2012b).

Fly larvae have been traditionally employed to decompose poultry, dairy, beef, and pig manure (Eby and Dendy, 1978; Miller et al., 1974; Morgan and Eby, 1975; Newton et al., 1977). Manure and fecal sludge are generally poor in nutrients, and the amount of biomass obtained by fly biodegradation is relatively low (generally 20–80 g/kg on a wet matter basis; Tables 2 and 3).

However, it has been recently shown that fly larvae may be used even for biodegradation of other nutrient-rich wastes, such as food/restaurant waste, meat processing waste, abattoir waste, municipal garbage and market waste (Aniebo et al., 2008; Diener et al., 2011a,b; Yehuda et al., 2011; Zheng et al., 2012). These wastes have markedly higher nutrient content which can be assimilated by fly larvae. Here, the amount of fly biomass may easily reach 100–400 g/kg (Table 2). Plant residues, such as rice straw and plant meals left after oil extraction may also be digested by fly larvae alone or after inoculation with certain microbes and enzymes (Hem et al., 2008; Yang et al., 2012).

4. Systems design

4.1. Systems exploiting natural fly populations

The simplest and least expensive manure management systems utilize natural fly populations for the management of organic waste. Simple modifications of manure basins and animal houses are typically employed to support natural populations of flies in the vicinity of the farm, allow oviposition of flies directly in manure pits or lagoons and to redirect larval search for suitable pupation sites. While these systems are relatively cheap and very easy to implement, they can usually be considered only in warm regions

Table 2
Yield of fly biomass obtained by rearing the larvae in different kinds of organic waste. Values are expressed per 1 kg of fresh waste (wet matter basis).

Type of waste used	Fly species	Number of eggs inoculated	Yield of fly biomass ^a (g)	Weight of undigested residue (g)	References
Poultry manure	<i>H. illucens</i>	– (Natural)	24.4–45.3 (PP)	–	Sheppard et al. (1994)
	<i>M. domestica</i>	1000	22.4–26.8 (P)	703–847	Morgan and Eby (1975)
	<i>M. domestica</i>	N/A	30–40 (L)	N/A	Eby and Dendy (1978)
Pig manure	<i>M. domestica</i>	4400–11,000	43.9–74.3 (P)	180–650	Čičková et al. (2012b)
	<i>M. domestica</i>	5800 Young larvae ^e	105–120 (L)	350–450	Zhang et al. (2012)
Dairy (cow) manure	<i>M. domestica</i>	1000–1100	3.5–33.5 (P)	736–754	Morgan and Eby (1975)
Poultry waste I ^b	<i>L. sericata</i>	15,000	166.4 (L)	408.6	Yehuda et al. (2011)
	<i>L. sericata</i>	20,000	348.9 (L)	192.1	Yehuda et al. (2011)
Poultry waste II ^c	<i>L. sericata</i>	15,000	307.1 (L)	242.5	Yehuda et al. (2011)
Fish waste	<i>L. sericata</i>	20,000	118.3–412.8 (L)	150.2–440.3	Yehuda et al. (2011)
	<i>S. carnaria</i>	5000	395.7 (L)	206.5	Yehuda et al. (2011)
Concentrated pig slurry ^d	<i>M. domestica</i>	5500	–	360	Čičková et al. (2012b)

^a Larvae – L, prepupae – PP, pupae – P.

^b Skin, internal organs, and meat remains.

^c Ground bones, skin, internal organs, and meat remains.

^d Pig slurry concentrated by centrifugation and decantation to a moisture content of approximately 73%.

^e Based on the larval density of 580,000 larvae m⁻² and manure supply of 1 t m⁻².

Table 3
Yield of fly biomass and waste residue (dry matter basis) after biodegradation of manure by fly larvae.

Type of waste used	Fly species	Number of eggs for inoculation of 1 kg fresh (wet) waste	Yield of fly biomass (%)	Residue (%)	References
Poultry manure	<i>H. illucens</i>	–	7.8	–	Sheppard et al. (1994)
Pig manure	<i>M. domestica</i>	4400–11,000	4.8–8.1	–	Čičková et al. (2012b)
	<i>Chrysomya megacephala</i>	3250	9.58–9.64	74.45–83.71	Yang and Liu (2014)
Dairy manure	<i>H. illucens</i>	1000	5.67	46.97	Li et al. (2011b)
Municipal organic waste	<i>H. illucens</i>	– ^a	11.88	32	Diener et al. (2011a)
Defatted restaurant waste	<i>Boettcherisca peregrine</i>	3000	9.9	78.0	Yang et al. (2012)

^a Continual breeding system with larvae of various age.

with mild winters and the fly species to be used should pose minimum environmental and health risks (i.e. it must not be a pest). Close monitoring is advised to assure the presence of desired fly species in abundant numbers.

A system utilizing naturally occurring black soldier fly population is described by Sheppard et al. (1994). An experimental 460 hen caged layer house was modified to support breeding and self-harvesting of black soldier fly larvae and prepupae. A 30 cm deep, 1.1 m wide slightly sloped concrete basin was created under the cage batteries. A wall against the central walkway was vertical, while the wall toward the outside of the house was sloped at 40 ° and formed a ramp for prepupae leaving the manure basins. A plastic pipe with a 1.5 cm gap at the top was placed at the top of the slope. Migrating soldier fly prepupae entered the pipe through this slot and were directed to the holding containers. A stable black soldier fly population was initially established by releasing several liters of black soldier fly prepupae near the facility; these created a robust natural population in the vicinity of the layer house. Females returning to oviposit near manure accumulating in basins under the hen battery cages soon created a large larval population. Up to 150,000 prepupae were collected weekly during the highest peaks in June of 1991. Monthly yields of prepupae reached 47–78 kg of prepupae/460 hens in June–August and 7–22 kg of prepupae/460 hens in September–December, following a cleanout of the manure basins. Ten percent (by weight) of weekly collected prepupae were released at the facility for regeneration of the natural colony.

Notable advantages of this system include low cost, no special equipment, potentially high yields of prepupae, less labor, no external energy input, and elimination of house fly breeding. On the other hand, even in a warm climate environment, there were notable seasonal shifts in fly and larval numbers resulting in variable yields of prepupae and a sharp decrease in production following basin cleanouts.

In Thailand, Nuov et al. (1995) devised a system for mass-rearing of blowflies (*L. sericata*) in raw pig manure. The manure was placed in shallow concrete tanks (1.5 × 2.5 × 0.25 m) in 100 kg batches in the farm yard and inoculation was carried out by the wild ovipositing blowflies (*L. sericata*). The larvae were allowed to develop for 5 days. After this period they were separated on a wire screen (6 mm mesh size). Several smaller ponds with manure were left unharvested to ensure adequate numbers of ovipositing females. While cheap and easy to implement, this system presents a great risk due to the large number of uncontained blowflies which can easily spread in the vicinity and become nuisance to local inhabitants as well as a potential vector of various diseases.

Although the more natural systems require less investment than mechanized, intensive systems, they failed to develop into viable businesses primarily because there were no established markets for the larvae or larvae products. It is interesting to speculate that if commodity markets for larvae develop in the future,

that some lower cost, earlier demonstrated and proposed systems within animal housing may be revived.

4.2. Artificial rearing systems

Several authors have proposed controlled fly breeding to facilitate decomposition and bioconversion of organic waste at a stable rate. Such systems typically have a separate adult fly colony maintained under controlled conditions from which the eggs are collected and transferred to a biodegradation unit where the waste processing occurs. The initial costs of these systems are higher because of the need for infrastructure, special equipment and trained personnel. On the other hand, these systems offer several advantages over natural breeding systems: greater variety of fly species may be used (since the pests are contained there are negligible environmental and health risks), controlled egg seeding resulting in stable production of larvae/pupae, no seasonal shifts in fly numbers, and optimized yields of products.

Newton et al. (2005) described possible on-farm implementations of black soldier fly biodegradation. In one such system swine waste was collected on a special conveyor belt located below pigs which allowed for separation of manure solids; urine with excess water was moved for different processing. Manure solids were then conveyed to the larval basin containing larvae of mixed age. A 35° ramp along the opposing walls of the basin directed the wandering prepupae to gutters and collection containers. A small portion of the escaping prepupae was used to regenerate adult colony (reared in an insectary) and the rest could be used as feed. Newly-hatched larvae obtained from a laboratory *H. illucens* colony were used to maintain sufficient number of larvae in the manure basin. Moreover, if an efficient drainage system was employed for diverting urine and excess liquid, the black soldier fly larvae could be reared directly under the pig pens, reducing the need for a conveyor belt system (Newton et al., 2005).

Newton et al. (2005) also suggest that the black soldier flies may be used in high-rise layer hen houses by adapting an already described system of Sheppard et al. (1994). In addition to slight modifications of the manure basins, a small greenhouse attached to the southern wall of the layer house and separated from the manure pit by a curtain would easily accommodate adult *H. illucens* population without a need for laboratory rearing. Key factor for this system are favorable climatic conditions.

Diener et al. (2011b) researched the economic potential of a medium-scale biodegradation plant utilizing *H. illucens* to process market waste in conditions of Costa Rica with minimum initial investments. Adult black soldier flies were maintained in a small (2 × 3 × 2.5 m) greenhouse with nylon-netted side walls and plastic foil with a sun-shading net covering the top. The greenhouse was exposed to direct sunlight for about 8 h a day. Biodegradation was carried out in a former chicken pen (30 × 8 m) roofed by a corrugated metal sheet and enclosed by a wire net. Larvae were reared in trays (80 cm × 200 cm × 30 cm) made of zinc-coated steel

sheets. Two ramps at a 28° angle led from the base plate (100 × 80 cm) to the upper end of each shorter side panel to allow migration of prepupae at the end of their development. A plastic pipe (∅ 11 cm × 94 cm) with a longitudinal slit (5 × 80 cm) was fixed along the top of this edge and directed migrating prepupae to collection bins. Larval trays had to be placed on pieces of bamboo standing in water-filled plastic pots to prevent ant invasion. Full conversion of wastes by the larvae was rapid, and larval development took on average 18–27 days. Despite good bioconversion rates, however, the residue was very wet and gave off a foul-smelling odor. Overall yields of prepupae were lower than expected, probably as a result of zinc poisoning from the zinc-coated larval trays (Diener et al., 2011a). Anaerobic conditions developed at the bottom of larval trays due to stagnating liquid and hindered further larval development. A well-designed draining system and frequent feeding of small quantities of waste were suggested to further improve this biodegradation system (Diener et al., 2011a).

Eby and Dendy (1978) in their attempts to scale-up biodegradation of poultry and dairy manure by the house fly larvae demonstrated that a major obstacle for large-scale waste processing plants would be depth of the larval substrate. Larvae usually do not burrow deeper than about 7.5 cm into the medium unless supplied with air to promote aerobic conditions. When the depth of the worked-over medium increases to over 20 cm, the bottom zone can easily become anaerobic (Beard and Sands, 1973). Eby and Dendy (1978) tested a method of mass-rearing in an open cement mixer in approximately 46 kg batches with periodic tumbling without any serious negative effects on the larvae. Spent manure with the maggots could be dumped on screen-bottom trays in a 1.5 cm thin layer and placed under lamps to achieve about 90% separation of the larvae. However, further attempts to scale up and mechanize the system to process 1800 kg batches of manure did not yield satisfactory results. Incubation of fly larvae in a closed culture tank (3 m long, 1.2 m diameter, holding capacity 1800 kg) with air scrubbing and slow tumbling could not be controlled adequately to maintain suitable conditions for larval development.

A small-scale biodegradation facility capable of processing 500–700 kg of pig manure per week by the house fly larvae was described by Čičková et al. (2012b). The self-contained pilot plant contained an adult fly room (insectary), a larval (biodegradation) room and support area for maintenance tasks and staff. Adults emerging from ≈25,000 pupae were held in production cages and eggs were collected daily for a period of 15 days (starting on the 5th day since adult eclosion). Eggs were handled immediately and seeded on pig manure placed in shallow plastic trays with a holding capacity of 5 kg of manure. Larval trays with inoculated manure were held in an air-conditioned biodegradation room for 7–11 days. Although a method for separation of the larvae from manure residue was developed in the pilot plant (Čičková et al., 2012a), it was not used routinely since at the time of pilot plant operation there was no local market for house fly larvae or pupae. The larvae were typically left in manure to pupate and then killed by freezing for 4 days at –20 °C; the processed manure with killed pupae was air-dried, milled and packed for use as a fertilizer.

A patented, full-scale swine manure composting system utilizing house fly larvae to process the waste has recently been created in China. At its peak operation it is able to process 35 tons of raw swine manure per day (Zhang et al., 2012). The facility included a nursery barn for adult and larval rearing, as well as 3800 m² of greenhouses in which the biodegradation occurs. The facility also features support areas which include pupation pools to supply the adult colony with new insects, manure storage tanks with a total capacity of 70 m³ raw manure, and a unit where manure residue (after larval digestion) is subjected to secondary aerobic

composting. Two driers are installed to process and dry 4 m³ of fresh larvae per day.

Adults were maintained in an insectary in large production cages (4 m × 4 m × 3 m; 4.8 million adults per cage) and fed a liquid formula of water, sugar, milk, and other ingredients (Wang et al., 2013). The flies were allowed to oviposit for 6 h and eggs were incubated on a medium of milk and bran until the larvae hatched. The newly-hatched larvae were transferred to manure basins at a density of about 580,000 larvae m⁻², and 25–30 kg m⁻² of raw swine manure was added daily. The larvae were allowed to develop for 5–7 days according to ambient temperature. Manure residue was removed manually with a besom and larvae further cleaned by sieving. Collected manure residue was stabilized by aerobic composting and larvae were sold as feed (Wang et al., 2013).

4.3. Mechanization of fly larvae production

Much, if not all of the interest in fly culture for treatment of manure and waste can be traced to observations of massive populations of wild flies associated with animal production or waste handling and disposal. This is especially true for poultry, where the beginning developments of modern housing separated the animals from their manure, allowing flies to propagate under largely undisturbed conditions. Some of the first reports on black soldier fly were in reference to their displacing massive populations of house fly larvae in manure accumulations beneath caged hens (Furman et al., 1959; Tingle et al., 1975). Such observations led to the design of manure basins that allowed the collection of larvae from beneath hens, and later, from under pigs (with the addition of urine drains) (Sheppard et al., 1994; Newton et al., 2005). Such systems were primarily seasonal, as they depended on wild oviposition, were self-harvest and provided limited opportunity for management intervention. As a result, the culture of flies for waste treatment/resource recovery from animal manures has moved toward intensively managed systems away from the animals. These fly culture systems and proposed systems offer continuous production and are most often built around some level of mechanization. Significant advancement was achieved in automation of insect-based waste processing. Unfortunately, the technological solutions were rarely published.

One way of surveying how mechanization and potential mechanization of fly, larvae, or pupae production has developed is to look at patents on the subject. Calvert et al. (1973) described a static device utilizing light for harvesting housefly larvae from chicken manure. Eby and Morgan (1977) described a system consisting of a larvae culture drum reactor linked to a perforated screen belt for harvesting the larvae as the reactor was emptied. Sorokoletov (1985) developed a convenient system for collection of house fly eggs from production cages. Olivier (1998, 1999) described a conveyor belt with a device for adding and distributing suitable waste to the belt; followed by a means of depositing fly eggs onto the waste; a means of removing the larvae from the waste and off the belt; and finally a means of removing the treated waste from the belt. Kappelt and Levenhagen (1998) described stackable trays for incubation and growth of insect larvae in artificial environments. Endencia and Endencia (2000) described a static device using a box and screens to culture and harvest larvae. Tedders and Blythe (2001) described a device for rapidly loading and evenly distributing insect eggs into individual culture containers. Olivier (2002, 2003) described a system consisting of a disposal track (with bins) where waste was processed by larvae at the surface and treated material was removed from the bottom using sub-surface scrapes, such that treated material is removed without disturbing the larvae. This material is further treated using earthworms and composting. Olivier (2004) described systems utilizing culture containers without moving parts, with ramps for collecting

migrating black soldier fly larvae. The containers can be used for individual households with the larvae fed manually, or many culture containers fed by mechanical means.

In addition, there are several more recent patent applications, including Newton and Sheppard (2013) which describes a system consisting of automatically fed, stacked culture basins which are harvested using a vacuum system (demonstrated by operating a four basin system for 1 month); Courtright (2014) which describes a system for production and collection of fly eggs, particularly related to black soldier flies; and Milian (2010) that describes a multiple belt system for producing house fly larvae. Another patented housefly system (Mijanovic, 2007), with three belts in series, was tested in Denmark (Johansen and Hinge, 2010). Many more examples of fly rearing technology from around the world can be located by searching internet patent sites, such as Patentfish (2014).

5. Advantages of biodegradation by fly larvae

Biodegradation of organic waste is fast; depending on the fly species the process may take 4–27 days, which compared very favourably to traditional composting methods and aerobic and anaerobic digestion systems. Moreover, bioconversion of waste with dipteran larvae may result in significant production of fly biomass and digested manure, which can be sold and thus create revenue (Wang et al., 2013).

5.1. Production of fly biomass

Many studies have shown that fly larvae and pupae grown on organic waste may become valuable feedstuff for fish, chicken and pigs, either in the dried form (maggot meal) or as live larvae (Bondari and Sheppard, 1981; Dordević et al., 2008; El Boushy, 1991; Koo et al., 1980; Newton et al., 1977; Nuov et al., 1995; Ogunji et al., 2008). Protein isolated from the fly larvae and pupae compares favorably with soybean or meat meal traditionally used in the feed formulations. Insects may also serve as a good dietary source of mineral salts (Khusro et al., 2012; Koo et al., 1980). St-Hilaire et al. (2007) have shown that the fatty acid profile of black soldier fly prepupae may be improved by adding fish offal to the larval diet. This may provide an opportunity to improve nutrient composition of fly biomass by manipulation of the larval diet.

Proportion of major nutrients in fly larvae may differ between species (Table 4). Dry matter content of black soldier fly prepupae

has been shown to be 33–44% (Diener et al., 2009; Sheppard et al., 1994 – although Bondari and Sheppard, 1981 observed 17% dry matter content of black soldier fly larvae), house fly larvae and pupae 26–32% (Čičková et al., 2012b; El Boushy, 1991; Wang et al., 2013), blowfly *L. sericata* larvae 28–30% and pupae 23% (Nuov et al., 1995; Yehuda et al., 2011), and flesh fly *S. carnaria* larvae 25% and pupae 35% (Yehuda et al., 2011). The black soldier fly larvae/prepupae contain a larger proportion of fat compared to house flies, blow flies and flesh flies, which in turn contain more protein, and face fly pupae are rich in mineral salts (Table 4). Insect diet can greatly affect body composition; the percentage of fat may differ in the same species if larvae are fed different diets (Yang et al., 2012) and also in different developmental stages (Aniebo and Owen, 2010). In the black soldier flies, the weight of the chitinous prepupal skin accounts for about 10% of total prepupal weight (Tomberlin et al., 2002).

Although experience with maggot meals was generally good, several authors point out that it is necessary to consider amino acid and trace element composition of the insect feed, as nutrient content or balance may not meet guideline recommendations (Khusro et al., 2012; Newton et al., 1977). Some advocate extraction of fat from the larvae (Diener et al., 2011b; Fasakin et al., 2003) to create a high-protein meal and suggest alternative use of the larval fat, e.g. for production of biodiesel (Li et al., 2011a).

It should be kept in mind that although fly larvae can accelerate the reduction of pathogenic organisms (*Escherichia coli* and *Salmonella* sp.) in manure and feces (Erickson et al., 2004; Lalander et al., 2013; Wang et al., 2013), several studies have shown that potentially harmful microorganisms may survive in both the larvae/pupae and the processed waste (Lalander et al., 2013; Yehuda et al., 2011) and live insects served as feed may thus act as vectors of bacterial and viral diseases (Khusro et al., 2012). Processing of larvae into separate protein meal may address these issues by killing the bacteria during the drying and extraction steps. Experiments have also shown that larvae feeding on contaminated sources may accumulate heavy metals, such as cadmium (Diener et al., 2011b).

The large amount of fat present in fly larvae and pupae (particularly in the black soldier fly prepupae) has been shown to be valuable feedstock for production of biodiesel. During the procedure, the fat is extracted from larvae by petroleum ether and modified by acid-catalyzed esterification of free fatty acids (to decrease the acidity of crude fat) and alkaline-catalyzed transesterification (Li et al., 2011a). Produced biodiesel compares favorably with plant-based biodiesels and meets selected criteria of EN14214

Table 4
Nutritional value of dried fly larvae and pupae obtained by rearing the flies in different types of organic waste.

Nutrient (dry matter basis)	Black soldier fly		House fly			Blowfly <i>L. sericata</i>		Face fly	Flesh fly <i>S. carnaria</i>	
	Larvae	Prepupae	Larvae ^a	Larvae ^b	Larvae	Larvae ^c	Pupae ^c	Pupae	Larvae ^c	Pupae ^c
Crude protein (%)	42.1	43.2	43.45	59.48	63.99	51.8	57.5	51.7	61.8	65.2
Crude fat (%)	34.8	28	14.3	6.66	24.31	32.5	23.6	11.3	22.1	17.8
Crude fiber (%)	7.0	–	–	11.53	–	–	–	–	–	–
Moisture (%)	7.9	–	8.25	–	5.28	–	–	–	–	–
NFE (carbohydrate) (%)	1.4	–	19.66	8.08	1.25	10.7	11.2	8.1	11.6	13.3
Ash (%)	14.6	16.6	–	14.24	5.16	4.6	7.7	28.9	4.4	3.7
Calcium (%)	5.0	5.36	0.36	5.96	2.01	–	–	2.48	–	–
Phosphorus (%)	1.5	0.88	–	1.05	1.32	–	–	2.74	–	–
Waste used for rearing	Cattle feces, urine slurry	Pig manure	Chicken manure	Chicken manure	Chicken manure, powdered milk, sugar	Poultry waste	Poultry waste	Cattle manure	Fish waste	Fish waste
Reference	Newton et al. (1977)	Newton et al. (2005)	Fasakin et al. (2003)	Dordević et al. (2008)	Hwangbo et al. (2009)	Yehuda et al. (2011)	Yehuda et al. (2011)	Koo et al. (1980)	Yehuda et al. (2011)	Yehuda et al. (2011)

^a Full-fat maggot meal, oven dried.

^b Oven dried house fly larvae.

^c Based on the moisture levels presented in Yehuda et al. (2011).

Table 5

Yield of biodiesel obtained from fat extracted from the larvae/prepupae of several fly species reared on different wastes. Values are expressed per 1 kg of waste (dry matter basis).

Fly species	Waste	Fat content of dried larvae (%)	Conversion of fat into biodiesel (%)	Yield of biodiesel (g/kg of waste)	References
<i>Ch. megacephala</i>	Restaurant waste	24.4–26.29	87.71	–	Li et al. (2012)
	Swine manure	20.0–21.1	–	1.6 ^a	Yang and Liu (2014)
<i>H. illucens</i>	Defatted restaurant waste + rice straw + Rid-X	35.7–39.6 ^b	–	43.8	Zheng et al. (2012)
	Dairy manure	23.2 ^c	–	2.6	Li et al. (2011b)
<i>B. peregrine</i>	Defatted restaurant waste	31.1	92.3	32.4	Yang et al. (2012)

^a Based on 73% moisture content of fresh manure.^b Depending on the ratio of restaurant waste, rice straw and Rid-X dosage; optimum conditions: 70% restaurant waste, 30% rice straw, and 0.35% (w/w) Rid-X inoculum.^c 16.4 g of fat extracted from 70.8 g dry larvae; the fat was converted to 15.8 g biodiesel.

(Li et al., 2011b, 2012; Zheng et al., 2012). The amount of insect fat differs among the species, leading to variable yields of biodiesel (Tables 4 and 5).

In addition to the use of larvae for feed and biodiesel production, several authors have shown that the larvae of numerous flies, including house flies, blow flies and the black soldier flies, produce biologically active (antibacterial, antitumor, growth-stimulating) substances which can be easily extracted from larval bodies and may be interesting for the pharmaceutical industry (Choi et al., 2012; Hou et al., 2007; Kawabata et al., 2010).

5.2. Waste management

Fly larvae developing in animal manure extract nutrients and by tunnelling the substrate they improve its structure. The changes in manure during biodegradation, however, cannot be solely attributable to the maggot action but rather to the interaction of fly larvae with microflora (bacteria, yeast and fungi) already present in manure. The main contribution of fly larvae to biodegradation seems to be mechanical aeration which results in increased loss of water, ammonia and favouring the growth of aerobic microorganisms (Beard and Sands, 1973). Some bacteria present in larval substrate

were shown to enhance larval development while others proved to be detrimental (Beard and Sands, 1973; Zurek et al., 2000). Experimental inoculation of the feed with either conspecific larval gut bacteria (Yu et al., 2011) or a commercial bacterial product (Zheng et al., 2012) improved both bioconversion efficiency and growth rate of larvae and manipulation of waste bacterial flora may thus be a powerful tool to influence the outcome of biodegradation.

During biodegradation by fly larvae the temperature of the substrate rises, pH changes from neutral to alkaline, ammonia release increases, activity of some enzymes within medium shifts markedly, and moisture decreases (Beard and Sands, 1973; Zhu et al., 2012; Zvereva, 1984; Table 6). Humidity and odor emissions in biodegraded manure are markedly decreased (El Boushy, 1991; Wang et al., 2013).

Manure processed by fly larvae has a loose granular structure with earthy odor and is suitable for use as an organic fertilizer (Sorokoletov, 2006; Kováčik et al., 2010). Further treatment of manure residue by aerobic composting considerably reduces volume. Manure residue may be also utilized as a soil amendment to reduce the numbers of cysts, eggs and juveniles of several species of potato cyst nematodes (Renčo et al., 2011).

Table 6

Changes in selected parameters of organic waste following biodegradation by fly larvae.

Waste	Fly species	Experimental scale	Total mass (%)	Moisture (%)	Dry matter (%)	Other changes	References
Poultry manure	<i>M. domestica</i>	Lab (100 g samples)	–59.1 to –80.3 ^a	–48.8 to –70.6 ^a	–31.9 to –39.6 ^a	–	Barnard et al. (1998)
		Simulated field (4760 g) ^b	–72.4 to –75.8 ^a	–85.3 to –89.8 ^a	–34.5 to –39.5 ^a	–	Barnard et al. (1998)
		Lab (4 kg sample)	–45	–58	–52	Organic matter –80%, no changes in ash content	Miller et al. (1974)
Pig manure	<i>M. domestica</i>	Pilot scale (5 kg/tray, 700 kg/week)	–72.8 ^c	–88.15 ^c	–18.45 ^c	–	Čičková et al. (2013)
	<i>H. illucens</i>	Small scale (manure from 12 pigs)	–	–	–56	Reduction of N (–55.1%), P (–44.1%), K (–52.8%), C (–62.4%) and other elements	Newton et al. (2005)
Dairy manure	<i>H. illucens</i>	Lab (27–70 g manure per day)	–	–	–33.2 to –58.2	Reduction in P (–62% to –71%) and N (–30% to –50%)	Myers et al. (2008)
Human feces	<i>H. illucens</i>	Lab (370 g samples)	–68.1	–	–73	Reduced count of <i>Salmonella</i> spp.	Lalander et al. (2013)
Fecal sludge	<i>H. illucens</i>	Medium-scale	–	–	–54.7	–	Diener et al., 2011a
Market waste	<i>H. illucens</i>	Medium-scale	–	–	–59.4	–	Diener et al., 2011a
Municipal organic waste	<i>H. illucens</i>	Medium-scale ^d	–	–	–68.0	–	Diener et al., 2011a,b

^a Depending on the larval density.^b 340 g Daily for a period of 2 weeks.^c Manure without sawdust, control treatment, based on 78.35% and 34.00% manure moisture at the beginning and end of the experiment, respectively.^d 1.1 kg of fresh waste daily for 21 days, then 1.5–4.6 kg of fresh waste daily for 34 days.

Zhu et al. (2012) showed that further treatment of fly-digested manure residue by aerobic composting may lead to faster loss of phytotoxicity and, during thermophilic phase of the composting, help to eliminate pathogens. An additional treatment step for waste decomposed by the black soldier fly larvae is also recommended by Lalander et al. (2013) to inactivate pathogenic organisms (bacteria, viruses, nematodes) before reuse in agriculture.

6. Economic analysis and commercial development

Although several studies have addressed the economics of utilizing fly larvae for biodegradation, in most instances, these analyses consist of either extrapolating lab or pilot-size experiments to a commercial setting or developing an economic case study with a considerable number simplifying assumptions.

To date, the primary uses for fly larvae have been in the treatment of waste. Newton et al. (2005) suggest that, under conditions of North America, *H. illucens* larvae used for direct processing of manure in poultry houses could return \$25,000 per house per year with minimal initial investments and maintenance costs.

Zering et al. (2006) concluded that the adoption of black soldier fly (BSF) technology held significant economic promise as a method to dispose of swine manure in North Carolina. The physical results of that trial indicated that BSF larvae favorably reduced pig waste solids. However, the economic results of this trial were tempered considerably by the lack of a steady-state operating system, the lack of an established national market for vermicompost, and uncertainty regarding the approval of dried black soldier fly larvae as a feedstuff.

For the treatment of municipal organic waste, Diener et al. (2009) established that a ~1100 m² plant with 5 permanent workers would be needed to process 3 tons of organic waste (wet weight) per day. Initial costs for building the plant and infrastructure in conditions of Costa Rica was estimated to be \$85,000 and yearly running costs, including salaries, to be \$35,700. On the other hand, such a plant would be, based on the preliminary results, able to produce ~150 kg of BSF prepupae (dry weight) per day which, if sold for \$1000 per ton (current price of fishmeal), would yield annual revenue of \$55,000 (Diener et al., 2009). This option may be attractive particularly for small businesses in low- or middle-income countries.

The findings from Diener et al. (2009) were adapted to determine the commercial viability of black soldier flies in producing biodiesel and poultry feed in Tanzania (Agrawal et al., 2011). This study was unique in that the focus of the paper was to evaluate the economics of utilizing BSF larvae to biodegrade human waste in Dar es Salaam, Tanzania. The authors concluded that utilizing the larvae to biodegrade human waste was financially feasible. They recommended selling the resulting larvae from the degradation process as high-grade animal feed and crude oil feedstocks in the first and second years of the project and then reinvesting the accruing profits in the necessary equipment to produce biodiesel. Estimated annual profits from selling the animal feed and biodiesel were 63,362 US\$ produced from the waste of three latrines per day. Even though the anticipated profits were considerable, the study's authors noted that there were several challenges or risks that must be overcome in order for this project to be successful. Among others they noted that, "one of the biggest threats to the sale of BSF larvae as chicken feed could be the negative perception in the use of insects grown on (human) waste". They also noted other obstacles such as: permits to import BSF in Tanzania (it is not native to that country), certification regarding the nutritional analysis of BSF feed, and research on the safety in use of BSF as a feed.

In China, a biodegradation plant with an average processing of 25 m³ of raw pig manure daily has been built. Economic analyses

showed that while annual costs were 72,400–148,000 US\$ of which about 95% was labor, the products may be sold for 144,800–370,400 US\$. Majority of the revenue (95–96%) were created by selling the larvae. Thus, a net profit of 67,900–210,000 US\$ could be created annually (Wang et al., 2013).

In addition to the research projects, several companies with fly larvae-based waste processing technology entered the commercial sector. Among the best known, the South African company Agri-Protein (www.agriprotein.com) operates a plant for recycling of food waste by BSF larvae. Among the products available for sale are feed products – dehydrated larvae (Whole Dried Larvae™), a high-protein larvae meal (MagMeal™), oil extracted from the larvae (MagOil™), as well as soil conditioner (MagSoil™).

A Canadian company EcoSpace Engineering Ltd. perfected and patented a system for processing of organic waste (manure) by fly larvae, called Milinator (<http://www.ecospace-eng.com>). The technology is based on the space project originally developed in Russia during the Soviet era. Main products of the company are the nutrient-rich larvae and organic fertilizer (Cyclorganic).

While several studies have documented the potential for this technology and several companies have entered the market, the primary impediments to its commercial adoption include regulatory barriers to larvae and larvae products as a feedstuff, as well as a lack of established (publicly reported prices and reasonable estimates of quantities) markets for compost and vermicompost. The uncertainties of these output prices make financial forecasting difficult and as a result, potential investors are reticent to venture into this technology.

It is worth noting that two American livestock producers interviewed for this paper (who wish to remain anonymous) view feeding BSF larvae as a "gray area" in terms of regulation. That is, even though BSF larvae have not been formally cleared as a livestock feed for swine, poultry, or fish in the US, it is not expressly prohibited. Moreover, larvae are a natural food source for these species. These producers reason that even though feeding BSF larvae is a perfectly legitimate and defensible practice in their eyes, the lack of formal government clearance makes any acknowledgment that they utilize this technology to feed food animals financially risky.

We posit that this mindset is not uncommon in the US among pasture-based alternative and sustainable livestock producers. In fact, given the growing local, natural, and pasture-raised livestock sector (Martinez et al., 2010), we assert that there is actually a small but growing "gray market" for fly larvae in these types of systems.

7. Current problems and future perspectives

Presently, the major obstacles associated with the production of fly larvae from organic waste seem to be technological aspects of scaling-up the production capacity and production of necessarily large amounts of fly eggs, as well as lacking information about fly biology which is necessary for successful laboratory rearing (Diener et al., 2011a; Eby and Dendy, 1978).

7.1. Technological problems associated with scaling up of waste processing capacity

The major factor limiting the volume of organic waste which can be processed by fly larvae is the depth of substrate. Typically, the fly larvae do not burrow deeper than 7.5–10 cm into the substrate. This is caused by low levels of oxygen and resulting anaerobic conditions in deep layers of the media. Industrial-scale waste processing by fly larvae would thus require a large number of shallow trays or basins, which would increase handling and loading costs or space requirements of the facility. Eby and Dendy (1978)

tried several different approaches to increase the depth of larval medium. Forced delivery of air into larval substrate through a system of copper pipes with air outlets set 7.5 cm apart allowed the larvae to process manure in the full depth of the incubation box (1.22 m). However, several problems were encountered with this system. The larvae, when ready to pupate, entered the air holes and migrated through the pipes all the way to the filter of air pump. Manure in the vicinity of air holes became dry and was not utilized by the maggots. Ultimately, loading and unloading of the system was problematic due to the large number of air pipes and there were no practical means to collect the larvae. A better approach proved to be periodic tumbling of larvae with substrate in standard cement mixers. Once the larvae matured, the spent manure residue with larvae could be easily unloaded on screen-bottom trays to allow light-induced separation of maggots. A specially designed closed culture tank capable of handling 1800-kg batches of medium however could not provide adequate conditions for larval development (Eby and Dendy, 1978). Still another option to increase waste batch volume is to use tall containers with perforated walls through which the larvae are inoculated (Ivanov et al., 1980). The mesh covering sides of the container can supply the maggots with sufficient amount of oxygen during their development and may serve as a convenient separator through which larvae migrate prior to their pupation.

Another important issue which would have to be addressed by large-scale facilities is management of volatile byproducts and noxious gases released during larval processing of organic waste. Several authors pointed out that, even under small- to medium-scale operations, the release of various volatiles, particularly ammonia, can be intensive and may pose health hazards for the staff (Iñiguez-Covarrubias et al., 1994; Čičková et al., 2012b). Fast air exchange provided by powerful fans may not be sufficient and may inadvertently contribute to air pollution in the close vicinity of the facility. However, to date, there have been no reports on any technological solutions employed to remove odor and/or noxious volatile metabolites from the air in insect-based biodegradation facilities. If the technology is to be employed on an industrial scale, the development of a reliable and efficient air scrubbing system is mandatory to eliminate health risks for the staff and make this method of waste management environmentally sound.

7.2. Design and operation of fly biodegradation facilities

Another important issue which must be considered is the large amount of heat and volatile by-products that are released during the most intensive phases of biodegradation. The difference between the temperature of substrate with larvae and ambient environment may be as large as 12.5 °C (Zvereva, 1984), which places substantial demands on the air conditioning system.

To provide additional security for the biodegradation facility, egg-production and biodegradation areas could be separated into smaller subunits, for example each with a processing capacity of 1 t of raw manure. Such structure would allow easier management of some potentially serious problems, e. g. infectious fly diseases or invasion by parasitoids which could seriously threaten fly colonies. Independent management of these subunits would allow convenient quarantine of affected areas with little impact on the remaining operation.

7.3. Quality control procedures

Development of insects is a dynamic process influenced by a complex of biotic and abiotic factors. Since biodegradation of waste by fly larvae depends on controlled mass-rearing of these insects, suitable quality control procedures should be established in the

biodegradation facilities to avoid deterioration of fly strain which could result in a collapse of waste processing.

Routine procedures should involve continuous monitoring of the health and egg productivity of adult fly colony, as well as basic parameters of larval development and physicochemical parameters of end products. Additionally, laboratory populations should be periodically out-crossed to reduce problems (decreased fitness levels) associated with continuous inbreeding (Reed and Bryant, 2001; Day et al., 2003).

The flies are susceptible to a number of pathogens, particularly entomopathogenic fungi, which can threaten an adult colony (Čičková et al., 2012b). Decreased fecundity of adults is often indicative of problems encountered during larval development, e. g. poisoning (Diener et al., 2011a), inadequate environmental conditions and/or food supply of adults (Pastor et al., 2011).

Larval development should be assessed on a regular basis. Duration of larval development, larval mortality, size (weight) of fly larvae/pupae obtained at the end of biodegradation, and eclosion rates of adults are crucial parameters which can be used to monitor biodegradation process and indicate potential problems, e. g. low hatching rates of eggs, qualitative changes of waste fed to larvae (changes of its nutritional value, moisture content, possible contamination, etc.), infestation of pupae by parasitoids, or unfavorable environmental conditions (temperature, moisture, overcrowding). There should be conscious effort to improve these outcomes to develop more efficient rearing procedures for the larvae (Čičková et al., 2013).

Ensuring standard conditions for bioconversion as well as monitoring quality of final products (fly biomass and waste residue) should be mandatory for every biodegradation facility to ensure stable processing capacity and high quality and safety of the products (larvae and waste residue).

7.4. Legislative affecting insect-based waste processing

With the exception of honey bees, silk worms and some endangered or threatened species, laws and regulation concerning insects primarily address control and elimination. With minor exception, food and feed laws and regulations address insects under the topic of sanitation and hygiene, and treat insects as filth or adulterant. The Food and Agriculture Organization of the United Nations maintains a database, FAOLEX (2014), which constitutes what may be the world's largest electronic collection of national laws and regulations on food, agriculture and renewable natural resources. Searches of the database for "fly", "larvae" and "insect" did not find laws or regulations specific to the use of insects as a feed ingredient.

A Codex Alimentarius (2014) standard on the use of insects as food and feed ingredients could serve as a reference for national legislation on insect production and use as food and feed, from both safety and quality viewpoints.

A search of the database of the Animal Legal and Historical Center (2014) using several insect related terms failed to identify any law or court case directly related to the use of insects as animal feed, other than use as bait. The first step in developing a feeding rule for insect products in North America would be obtaining an Ingredient Definition from the American Association of Feed Control Officials (AAFCO, 2014). Information on sponsoring a new definition, the rationale, and other tasks necessary to have a new ingredient definition approved and published is included on the AAFCO website. In a Brief summary; the AAFCO investigator works with the sponsor to submit information to the U.S. Food and Drug Administration for review. A favorable review results in the issuance of a regulatory discretion letter, which allows use of the ingredient; as long as it is used within the limits of the definition and no problems develop.

Under European Union regulations, insects would be allowed in feed only as processed animal protein (PAP). Past feed crises caused by the presence of dioxins in animal feeds and particularly outbreaks of bovine spongiform encephalopathy (BSE) resulted in prohibition of PAP derived from mammals in animal feeds with the exception of hydrolyzed proteins by Regulation (EC) 999/2001. Regulation (EU) 56/2013 further prohibits feeding of PAP to all non-ruminant farm animals, other than fur animals, while allowing the use of PAP derived from non-ruminants, other than fishmeal, to feed aquaculture animals.

Insects mass-reared for feeding purposes would be classified as “farmed animals” (Smith and Pryor, 2013). As such, waste materials of animal origin allowed for feeding to farmed animals are limited by Regulation 1069/2009 to Category 3 material. This includes (but is not limited to) carcasses, blood and parts of slaughtered animals fit for human consumption which did not show any signs of disease communicable to humans or animals; products of animal origin no longer intended for human consumption for commercial reasons or due to defects during manufacturing or packaging; hatchery by-products; aquatic animals and animal by-products from aquatic animals (except sea mammals) from establishments manufacturing products for human consumption, and adipose tissue from animals slaughtered in a slaughterhouse. The feeding of catering waste or products derived from catering waste, which are also classified as Category 3 material, is prohibited to farmed animals other than fur animals. Feeding of terrestrial animals and farmed fish of a given species other than fur animals with processed animal protein derived from the bodies or parts of bodies of animals of the same species is also prohibited.

Under Regulation (EC) 1069/2009, the only permitted use of Category 2 material (including, but not limited to manure, products of animal origin declared unfit for human consumption due to the presence of foreign bodies, animals and parts of animals that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes) related to insect-based waste processing is for manufacturing of organic fertilizers or soil improvers.

Both Category 2 and 3 material may be used as feed for maggots and worms for fishing bait.

Further regulations may apply if the insects grown on waste would be used for isolation of substances of medicinal or veterinary importance.

To allow commercial development of insect-based waste processing in Europe, existing legislation would have to be relaxed. Additional regulations would have to be considered to minimize environmental risks of the technology as well as address some of the newly-emerging issues, such as animal welfare during insect mass-rearing (Smith and Pryor, 2013).

8. Conclusions

Although significant success has been achieved in the laboratory, practical application of fly biodegradation technology has been impeded by the space needed for a full-scale operation as well as technological aspects of the bioconversion (Diener et al., 2011a; Eby and Dendy, 1978; Morgan and Eby, 1975). Deeper understanding of saprophagous fly biology and improved rearing methods for larvae and adults are needed to make the method as feasible as other alternatives of waste management.

While the house fly and black soldier fly seem to be the best candidates for biodegradation, further study of various fly species and their life histories might contribute to a greater variety of wastes suitable for biodegradation. Development of flightless fly strains with acceptable egg production characteristics might

further reduce the risk of potential pests invading the environment (Beard and Sands, 1973).

Technological innovations could greatly improve performance of the biodegradation facilities and decrease production costs. Additional information should be provided to assess safety of the products, especially toxicological and microbiological safety of maggots.

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