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A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction

Hang Wang[1], Zhijian Zhang[1], George F Czapar[2], Mari KH Winkler[3] and JianGuo Zheng[4]

Abstract

Manure produced from confined animal farms can threaten public and environmental health if not managed properly. Herein, a full-scale commercial bioconversion operation in DeQing County, China for value-added swine manure reduction using house fly, *Musca domestica* L., larvae is reported. The greenhouse-assisted larvae bioreactor had a maximum daily treatment capacity of 35 m^3 fresh raw manure per day. The bioconversion process produced a fresh larvae yield of 95–120 kg m^3 fresh raw manure. This process provided an alternative animal foodstuff (having 56.9 and 23.8% protein and total fat as dry matter, respectively), as well as captured nutrients for agricultural re-utilization. Bioconversion reduced odour emission (characterized by 3-methylindole) and the *Escherichia coli* (*E. coli*) index by 94.5 and 92.0%, respectively, and reductions in total weight, moisture and total Kjeldahl nitrogen in solids were over 67.2, 80.0 and 76.0%, respectively. Yearly profit under this trial period ranged from US$33.4–46.1 per m^3. It is concluded that swine manure larvae bioconversion technology with subsequent production of value-added bio-products can be a promising avenue when considering a programme to reduce waste products in an intensive animal production system.

Keywords

Musca domestica, vermicomposting, waste reduction, animal production, economic return

Introduction

Confined swine operations throughout the world generate considerable amounts of manure, which can contaminate groundwater and pose a risk to public health (Aarnink and Verstegen, 2007). Although composting can transform organic wastes into bio-products which can be used as bio-fertilizers (Nakasaki and Marui, 2011), the economic benefits of conventional composting are often marginal due to the low value addition (Westerman and Bicudo, 2005). As the amount of land available for agriculture is scarce in densely populated areas such as the HangJiaHu district (one part of the Yangtze Delta) in China (Wu et al., 2009), few producers have adopted conventional composting as a means of manure management. From an environmental and economic perspective, alternative methods of animal manure reduction are needed to reduce waste and create financial benefits when incorporating improved manure management.

Vermicomposting is an example of advanced manure biotechnologies. Typically, this approach utilizes earthworms to stabilize organic materials and reduce sludge (Lenz et al., 2011; Monroy et al., 2009; Tamis et al., 2011; Westerman and Bicudo, 2005). Many fly species (*Diptera* spp.) are known to grow and develop naturally in animal wastes. For the breakdown of organic waste, larvae of the house fly, *Musca domestica* L., are often selected because of their short generation interval (Barnard et al., 1995, 1998). Under ambient temperatures of 25–30 °C, house flies will complete a life-cycle in 2 weeks or less (Figure 1(a)), while approximate 1 week is needed for the larval feeding period during the bioconversion process. Although the earthworm composting period during the feeding phase (Gupta and Garg, 2009; Lazcano et al., 2008; Monroy et al., 2009) is comparable to larvae bioconversion, input of additives (e.g. crop residue) may reduce the vermicomposting capacity and increase cost. House fly larvae have been shown to reduce manure mass, moisture content and manure dry matter content by 75, 89 and 35%, respectively, within a week-long developmental cycle (Barnard et al., 1998; Moon et al., 2001). In addition, the changes in microbial community structure in a larvae bioreactor may contribute anti-bacterial and anti-fungal compounds to

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compost, resulting in improved soil conditions (Jeon et al., 2011). Furthermore, house fly larvae produced during the bioconversion process can be harvested from waste residues and used as alternatives for protein-rich animal foodstuff (St-Hilaire et al., 2007), medicinal purposes (Marchaim et al., 2003), or even biodiesel and sugar production (Li et al., 2011). Larvae-driven bioconversion may be a promising biotechnology with remarkable advantages of waste reduction and value-added swine manure management.

Technical innovations for animal manure bioconversion using dipterous larvae have been used for almost 40 years (Miller and Shaw, 1969). For example, a 35% reduction (wet weight) in poultry waste, was accomplished with digestion using thermophilic anaerobic bacteria and house fly larvae (Marchaim et al., 2003). Researchers have considered the role of factors such as manure nutrient content and temperature on larval growth (Barnard et al., 1995; Moon et al., 2001), the optimization of larvae population density, and the bioreactor design for improving the efficiency of manure bioconversion (Barnard et al., 1998; Moon et al., 2001). Other researchers studied the effect of bioconversion on odour emission elimination (Bai et al., 2007; Elboushy, 1991) and control of bacterial pathogens (Erickson et al., 2004). These studies illustrate the mechanisms of animal manure bioconversion by house fly larvae, the main parameters for farm-scale bioreactor operation, and the potential benefit and commercial value for confinement animal producers. By far, few technical innovations were conducted to develop the full-scale larvae bioreactor with the temperature-regulated technology required for year-long operation.

With the technological innovation of a greenhouse-assisted bioreactor, a farm-scale operation for swine manure reduction using house fly larvae bioconversion was developed in 2008 in DeQing County, China. In this paper, insights into the technological feasibility, associated environmental risks, as well as the effectiveness and efficiency of bioconversion in terms of waste reduction (prior to and after larvae process) and economic benefit are provided.

Materials and methods

Site description

A full-scale swine manure bioconversion project, using house fly larvae was installed by the GuoSheng Resource Insect & Biotechnology Company (GuoSheng company) in May 2007, located in DeQing County in the HangJiaHu district (southern part of the Yangtze Delta), ZheJiang Province, China (30°34′35.68″N, 120°13′26.30″E). BeiJingYuan is a finishing hog farm 200 m from GuoSheng, which has 35 swine barns and has raised approximately 32 500 hogs annually in the past 5 years. Through an agreement between the two neighbouring farms, all raw swine manure (with no solids–liquid separation) collected was directly transported from the BeiJingYuan farm to the bioconversion site of the GuoSheng company.

Description of bioconversion facility

Currently, the full-scale bioconversion treatment facility at the GuoSheng company processes approximately 25 m^3 of fresh swine manure per day. It possesses a 70 m × 30 m (L × W) nursery barn for house fly breeding and larvae developmental areas. The larvae bioreactors, which have a maximum daily treatment capacity of 35 m^3 day^{-1} of raw manure, are located in a 3800 m^2 greenhouse. The firm also consists of 10 pupation pools each measuring 3 m × 2 m × 30 cm (L × W × H) and two raw manure storage tanks with a total capacity of 70 m^3. In addition, there is a unit for secondary composting of larvae-treated residues and two driers (HC-15-09; HongCheng Biotech Company) capable of processing 4 m^3 of the harvested fresh larvae per day.

House fly production

The key procedures for house fly larvae bioconversion are detailed in Figure 1(a). The house fly colony (originally bought in 2007 from the JinJiang Biotech Company, China) from which eggs were collected for bioconversion purposes was reared in the insectary at 26–28 °C, 14 h : 10 h light : dark and 80% relative humidity. Adults were housed in cages measuring 4 m × 4 m × 3 m (L × W × H) and were fed a complete liquid formula (water content of 98%) consisting of sugar, milk powder, yeast extract, urea and rice soup (patent pending). The intended adult population density for each cage was approximately 4.8 million (1 fly per 10 cm^3). Oviposition media consisting of a semi-liquid mixture of sugar, milk powder, bran and an attractant (patent pending) was made available to adult house flies for 6 h. Eggs were collected from adult cages and transferred manually to vessels 60 cm × 40 cm × 12 cm (L × W × H) containing a wet mixture of bran and milk powder.

Larvae bioconversion

House fly larvae were collected approximately 20 h after eclosion, transported to specifically designed greenhouse-assisted larvae bioreactors (patent pending) (Figure 1(b)). Each bioreactor consists of three main components: greenhouse, larvae bioreactor and manure feeding system. Each greenhouse was 28 m × 5.5 m (L × W) and had a dome roof 3.5 m above the ground surface. The greenhouses were covered with 0.5 mm thickness plastic sheets, so that ambient temperatures during the winter and spring seasons were maintained at more than 20 °C. In order to avoid high temperature and excessive sunlight, both sides of the greenhouse were equipped with rolling shades. The larvae bioreactors were constructed as a series of ten cement-blocks with dimensions of 2 m × 5 m × 20 cm (L × W × H) (Figure 1(b)). These bioreactors provided the physical space required for raw manure bioconversion during the course of larvae growth and metabolism, which varied from 7, 6 and 5 days during the winter, autumn and spring, and summer, respectively. For the manure feeding, a 5-cm layer of raw swine manure was spread evenly on the surface of each

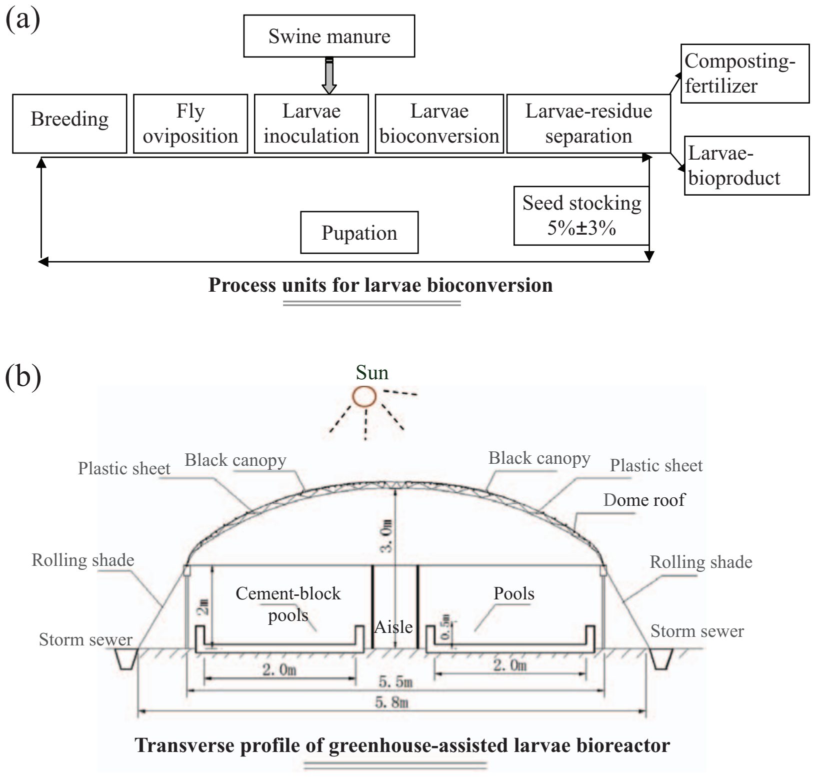

Figure 1. The process units for larvae bioconversion (a) and schematic drawing of the greenhouse-assisted larvae bioreactor (b) used in the full-scale swine manure bioconversion by house fly larvae.

bioreactor prior to adding the 20-hour-old larvae. The intended average larvae population density in each pool was 580 000 m^{-2}. The rearing density of larvae was determined following a previously reported protocol (Moon et al., 2001). During the 4 to 5 days following larvae incubation in each bioreactor, raw manure was fed at the rate of 25–30 kg m^{-2} day^{-1}. This resulted in an average depth of 8–10 cm of fresh manure applied prior to harvesting larvae from each bioreactor.

Larvae-residue separation and subsequent product polishing

Based on a negative phototaxis feature of late instar house fly larvae, residue in the mixture stacking was gradually and repeatedly removed by using bamboo-made besom manually during the daylight hours, before the phase of larvae pupation. The mature larvae left on the bottom of the bioreactor (after larvae–residue separation) were finally harvested, followed with secondary separation by using a sieve (sieve pore size 0.25 cm) for residuary solids removal. The fresh larvae collected by this process were dried for storage. The separated residues were further treated and stabilized by a conventional secondary aerobic composting technique (Nakasaki and Marui, 2011). Before secondary composting, the residues were stacked to an average depth of 1.5 m and fully covered by a double-layer plastic sheet for an additional 7 days of anaerobic digestion, ensuring that the remaining larvae were completely killed.

Sampling and laboratory analyses

Sampling and field investigations of full-scale swine manure bioconversion at the GuoSheng company were conducted during 2008 to 2010. Raw swine manure samples (~1.2 kg) were randomly collected from 3 m × 4 m × 1 m (W × L × H) fresh raw manure storage prior to use as feed in the bioreactor. Residue samples (~1.2 kg) were also collected, but from the pile shortly after larvae bioconversion. In addition, samples of fresh larvae (~0.5 kg) were collected and rinsed with tapwater. The samples were kept in portable freezer containers during transportation. All samples with three replicates were then frozen at −20 °C before chemical and biological examinations in the laboratory.

Samples of raw swine manure, manure residues and house fly larvae were transferred to the laboratory in HangZhou Center using the standard ‘Inspection and Testing for Quality and Safety for Agricultural and Genetically Modified Products’, Ministry of Agriculture, P.R. China (http://www.nybzjhz.com/index.asp), to measure the amounts of nutrients, proteins, amino acids, crude fat, fatty acids, heavy metals and ash. In accordance with the ‘Standardization Administration of the People’s Republic of China’ (http://www.sac.gov.cn/), the standard method coded as NY 525-2002 was used to test the levels of total Kjeldahl nitrogen (TKN), total phosphorus (TP), total potassium (TK) and organic matter (OM). Standard methods GB/T8573-1999 and LY/T1229-1999 were used to measure the levels of available P (AP) and available N (AN), respectively. The standard method

NY/T1110-2006 was conducted to test for heavy metal levels. Levels of crude protein, crude fat and ash in manure, manure residues and house fly larvae were tested by standard methods GB/T6432-1994, GB/T6433-2006 and GB/T6438-2007, respectively. Levels of amino acids [aspartic acid (ASP), threonine (THR), serine (SER), glutamic acid (GLU), proline (PRO), glycine (GLY), alanine (ALA), cystine (CYS), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), tyrosine (TYR), phenylalanine (PHE), histidine (HIS), lysine (LYS), arginine (ARG)] and fatty acids [myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3)] in larvae samples were measured by the standard methods coded as GB/T18246-2000 and GB/T17377-2008.

Odours consist of an extensive array of volatile organic compounds under different manure storage systems, among which 3-methylindole is a common and typical odorous molecular during organic biodegradation (Zhu, 2000). The intensities of offensive odour emitted from manure and residues were characterized by measuring the concentration of 3-methylindole (i.e. 3-MI or skatole) in the extracted liquid using gas chromatography (HP 5890A, USA). Fresh samples were primarily extracted by a solution mixed with 0.9% NaCl and 0.1% Tween-8 (Aldrich, USA). Under a standard atmospheric pressure of 45–55 kPa and 40 °C, the final concentrated liquid was obtained for 3-MI analysis. The *Escherichia coli* (*E. coli*) index was used to characterize the pathogen levels in manure and manure residues before and after larvae bioconversion, based on the reported method (Letourneau et al., 2010). Fresh samples were prepared by dilution in sterile sodium meta-phosphate buffer (2 g L^{-1}). *Escherichia coli* were enumerated by direct plating on mFC basal medium supplemented with 3-bromo-4-chloro-5-indolyl-β-D-glucuronide (100 mg L^{-1}). Colonies producing the characteristic blue colour indicative of β-glucuronidase activity were enumerated as *E. coli* in the unit of colony-forming units per gram (CFU g^{-1}).

Waste reduction calculation and gross profit analysis

Data collected from raw manure and residue samples were expressed as the mean plus standard errors. Percentage reductions for those parameters were calculated by dividing the difference between the mass prior to and after bioconversion during a batch operation by the mass prior to bioconversion, then multiplied by 100. The current data reported in this paper were collected during 2008 to 2010, and these field investigations were based on yearly average capacities of manure bioconversion and dry larvae production. The main sources of income were due to larvae products and residue bio-fertilizer whereas total production costs included labour, electricity, water, substrate for fly nutrition and other supplementary costs (e.g. greenhouse repairs, packing material). The profit was calculated by deducting total inventory cost from the sum of incomes.

Results and discussion

Larvae production and nutrimental value

Temperature inside the greenhouses (Figure 1(b)) was considerably higher (25–45 °C) than the prevailing outside air temperatures (2–39 °C). A fresh larvae production rate of 95–120 kg m^{-3} of fresh raw manure and an accompanying fresh manure residue rate of 350–450 kg m^{-3} were attained during each year (Figure 2). By comparison, the rate of fresh larvae production at this farm was approximately 10% greater than that obtained in a laboratory-scale test using rearing medium (Marchaim et al., 2003).

The moisture level of fresh larvae averaged 74.8% whereas dry larvae averaged 7.5% (Table 1). Crude protein in biomass of fresh and dry larvae, ranged from 10.4 to 18.5% (with an average of 14.6%) and 55.3 to 61.2% (with an average of 56.9%), respectively. These values are basically in line with other house fly larvae studies (61.4% of crude protein in dry biomass) (Elboushy, 1991), but significantly higher than those

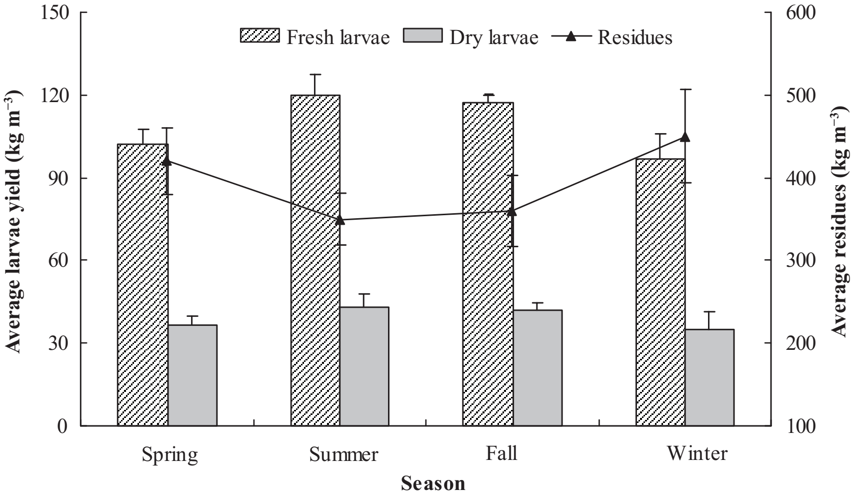

Figure 2. The average productivity of larvae and residues per m^3 of fresh raw swine manure in the full-scale swine manure bioconversion using house fly larvae.

Table 1. Comparison of physical–chemical parameters of larvae harvested from the full-scale swine manure bioconversion using house fly larvae.

Parameters		Fresh larvae			Dry larvae		
		Range	Average	*n*[a]	Range	Average	Criterion[b]
Moisture	%	68.3–77.8	74.8 ± 6.4	6	4.22–11.42	7.50 ± 1.38	–
Ash	%	1.04–2.23	1.93 ± 0.47	8	5.67–11.03	8.61 ± 1.22	–
Crude protein	%	10.4–18.5	14.6 ± 2.4	5	55.3–61.2	56.9 ± 2.4	–
Crude fat	%	4.04–4.96	4.73 ± 1.26	4	12.5–29.4	23.8 ± 3.8	–
Crude fibre	%	1.02–2.23	1.21 ± 0.26	3	7.04–7.69	7.32 ± 1.21	–
TKN[c]	%	–	–	3	7.16–5.74	6.73 ± 0.36	–
TP	%	0.087–0.178	0.125 ± 0.008	3	1.02–1.59	1.25 ± 0.08	–
Copper (Cu)	mg kg^{-1}	4.34–6.23	5.34 ± 0.23	4	23.2–87.5	56.3 ± 11.2	≤ 125
Cadmium (Cd)	mg kg^{-1}	0.009–0.018	0.014 ± 0.003	4	0.096–0.310	0.127 ± 0.032	≤ 0.50
Lead (Pb)	mg kg^{-1}	0.006–0.010	0.008 ± 0.002	4	0.089–0.110	0.092 ± 0.005	≤ 5.0
Mercury (Hg)	mg kg^{-1}	0.000–0.003	0.002 ± 0.001	3	0.012–0.014	0.013 ± 0.003	≤ 0.10
Chromium (Cr)	mg kg^{-1}	0.036–0.053	0.046 ± 0.011	4	0.341–0.782	0.438 ± 0.068	≤ 10.0
Zinc (Zn)	mg kg^{-1}	20.8–27.5	24.6 ± 4.9	4	198–312	251 ± 43	–
Arsenic (As)	mg kg^{-1}	0.020–0.031	0.025 ± 0.007	4	0.253–0.452	0.336 ± 0.045	≤ 2.0

[a]Refers to the number of samplings during investigation.
[b]Refers to 'Hygienical Standard for Feeds'-in poultry section.
[c]TKN and TP refer to total Kjeldahl nitrogen and total phosphorus.

house fly larvae grown on poultry droppings (36% in dry larvae) (Ogunji, 2008). In addition, the crude protein content from the present tests was relatively higher than those observed in black soldier fly larvae reactors (40–45% of protein contents) (Diener et al., 2009; St-Hilaire et al., 2007). With the exception of Chilean anchoveta fishmeal (Glencross et al., 2007), the protein contents of dry larvae in the present study were similar to or higher than other protein sources used for animal feedstuffs (e.g. soybean meal, earthworm) (Loh et al., 2009; Sun et al., 1997). Essential amino acids (THR, VAL, LET, LEU, PHE and LYS) accounted for approximately 48.5% of the total amino acids and high percentages of GLU, ASP and HIS were also found (Figure 3). Despite the greater total protein levels found in aquatic worms (*Lumbriculus variegates*, 63%) (Elissen et al., 2010), the house fly larvae harvested in the present study appeared to be of higher nutritional value due to the balanced amino acid composition.

The average crude fat was determined to be about 4.73 and 23.8% in fresh larvae and dry larvae, respectively (Table 1). These figures were comparable with (Marchaim et al., 2003) or higher (Elboushy, 1991) than other larvae bioreactors that utilized poultry and fish processing wastes, but were somewhat lower than that of black solider larvae (33% of crude fat in dry larvae) (St-Hilaire et al., 2007). Meanwhile, the unsaturated fatty acids among the crude fat were composed of C16:1, C18:1, C18:2, C18:3, amounting to 16.2, 26.5, 18.3 and 1.79% of the total, respectively (Figure 3). Moreover, the total percentages of those unsaturated acids (62.8%) exceeded the average contents in black solider fly larvae (Li et al., 2011) and commercial fishmeal (St-Hilaire et al., 2007; Glencross et al., 2007). As these unsaturated fatty acids are regarded as important supplemental ingredients for animal metabolism (McKenzie, 2001), it is obvious that the tested larvae containing higher level of unsaturated fatty acids in the present study possessed a remarkably competitive edge in comparison with the regular source of animal foodstuff. In addition, dry larvae ash levels averaged about 9% (Table 1), indicating a substantial quantity of mineral elements were also available.

Efficiency of waste reduction for manure bioconversion

The raw swine manure (Table 2) had a yellow-brown colour, with visible agglomeration (i.e. poor homogenization) and an offensive odour with faint ammonia emissions. In contrast, the bioconverted residues appeared dark-brown in colour, with a non-compacted texture, low moisture, good homogenization, with a less-offensive odour (and earthy smell).

During steady-state operation, the physical and chemical parameters of biomass before and after bioconversion changed considerably (Table 2). The moisture content in the residues reduced to 47.6% in comparison with that of raw manure (75.5–85.6%). Further analysis (Table 3) indicated average waste reductions of 67.2% in total weight and 80.0% in moisture. These values are comparable with and/or greater than that reported in laboratory-scale larvae bioconversion by a number of investigators (Barnard et al., 1998; Lenz et al., 2011; Li et al., 2011; Marchaim et al., 2003; Moon et al., 2001; Myers et al., 2008) and greater than earthworm vermireactors (Gupta and Garg, 2009; Lazcano et al., 2008). In addition, reduction via mass of metabolisms is calculated by mass of total reduction minus mass of harvested worm (Bai et al., 2007). A range of 57 to 70% of the reductions was achieved by the metabolism in larvae. Obviously, the larvae intervention along with the associated bio-activity

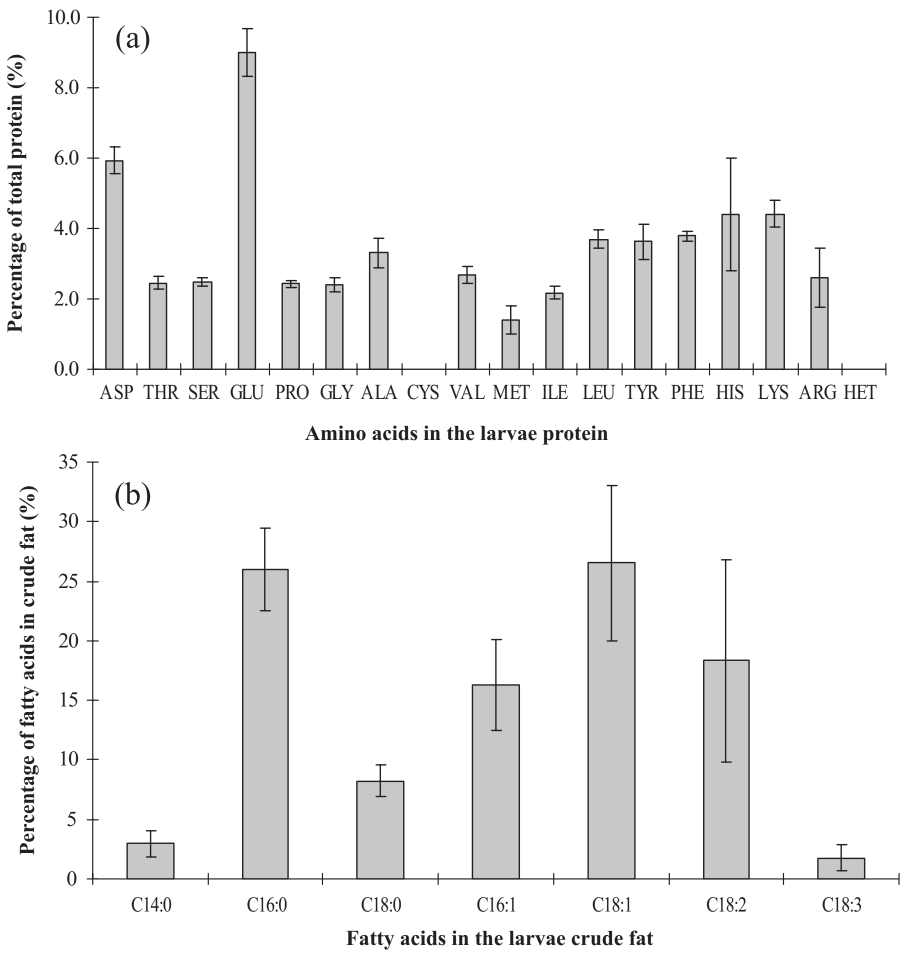

Figure 3. The percentages of different amino acids (a) and fatty acids (b) in the protein and crude fat of dry house fly larvae harvested from the full-scale swine manure bioconversion by house fly larvae.

Table 2. Comparison of physical–chemical parameters of the biomass before and after the bioconversion of house fly larvae. Units are expressed as original matter.

		Raw swine manure			After bioconversion		
		Range	Average	*n*[a]	Range	Average	*n*
Moisture	%	75.7–85.6	78.3 ± 5.4	8	40.7–53.5	47.6 ± 1.6	8
Organic matter	%	28.7–49.6	32.5 ± 12.4	8	45.6–58.4	53.3 ± 4.7	8
Crude fibre	%	14.3–21.2	17.2 ± 2.8	4	19.8–21.8	20.5 ± 2.7	4
Crude fat	%	4.05–5.21	4.61 ± 0.54	4	0.94–1.72	1.35 ± 0.43	4
TKN	%	1.35–3.66	2.99 ± 0.65	8	1.87–3.02	2.20 ± 0.31	8
AN	%	0.487–0.645	0.575 ± 0.079	8	0.318–0.702	0.441 ± 0.125	8
TP	%	0.92–3.02	1.82 ± 0.54	8	1.85–3.21	2.86 ± 0.36	8
AP	%	0.632–1.173	0.832 ± 0.434	8	0.843–1.451	1.154 ± 0.073	8
TK	%	1.34–2.13	1.79 ± 0.12	8	1.21–1.87	1.48 ± 0.06	8
AK	%	0.762–1.872	1.384 ± 0.431	8	0.862–1.652	1.324 ± 0.026	8
Copper (Cu)	mg kg^{-1}	98–850	313 ± 230	3	332–943	415 ± 212	3
Cadmium(Cd)	mg kg^{-1}	0.065–3.69	0.274 ± 0.076	3	0.039–0.987	0.342 ± 0.106	3
Lead (Pb)	mg kg^{-1}	0.034–1.742	0.952 ± 0.430	3	0.761–9.023	6.03 ± 3.23	3
Mercury (Hg)	mg kg^{-1}	0.091	0.091	1	0.039	0.039	1
Chromium (Cr)	mg kg^{-1}	21.3–31.0	27.8 ± 1.7	3	23.8–40.2	34.8 ± 2.1	3
Zinc (Zn)	mg kg^{-1}	650–1406	980 ± 320	3	790–935	856 ± 12	3
Arsenic (As)	mg kg^{-1}	4.56–9.53	5.72 ± 0.34	3	4.32–6.51	5.52 ± 0.30	3
Odour (3-MI)	mg kg^{-1}	35.1–45.7	40.4 ± 7.5	2	0.84–3.67	2.24 ± 1.41	3
E. coli index	CFU × 10^6 g^{-1}	2.16–4.56	3.77 ± 0.62	2	0.25–0.36	0.30 ± 0.08	2

[a] *n* refers to the number of samplings during the investigation.

Table 3. The mass balance of the full-scale swine manure bioconversion by house fly larvae, from 2008 to 2010.

	Total mass			Larvae	Mass of metabolism	
	Fresh manure (*a*)	Residue (*b*)	Reduction (*c*: %)^a	(*d*)	Amount (*e*)^a	Percentage (*f*: %)^a
	2008					
Total weight (m^3 year^{-1})	4332	1420	67.2	407	2505	57.8
Moisture (m^3 year^{-1})	3384	676	80.0	305	2403	71.0
Crude fat (m^3 year^{-1})	199	19.2	90.4	26.2	154	77.3
Crude fibre (m^3 year^{-1})	745	291	60.9	8.11	446	59.9
TKN (m^3 year^{-1})	130	31.2	76.0	2.42	95.9	74.1
TP (m^3 year^{-1})	42.8	32.9	23.1	1.43	0.77	1.78
TK (m^3 year^{-1})	22.7	18.6	18.1	–	–	–
	2009					
Total weight (m^3 year^{-1})	4880	1462	70.0	579	2840	58.2
Moisture (m^3 year^{-1})	3813	696	81.7	434	2683	70.4
Crude fat (m^3 year^{-1})	225	19.7	91.2	37.2	168	74.7
Crude fibre (m^3 year^{-1})	839	300	64.2	11.4	528	62.9
TKN (m^3 year^{-1})	146	32.2	77.9	3.32	110	75.7
TP (m^3 year^{-1})	48.2	33.9	29.7	2.01	4.40	9.10
TK (m^3 year^{-1})	25.7	21.6	16.0	–	–	–
	2010					
Total weight (m^3 year^{-1})	8030	2510	68.7	942	4578	57.0
Moisture (m^3 year^{-1})	6274	1195	81.0	706	4373	69.7
Crude fat (m^3 year^{-1})	370	33.9	90.8	60.6	276	74.5
Crude fibre (m^3 year^{-1})	1381	515	62.7	18.6	848	61.4
TKN (m^3 year^{-1})	240	55.2	77.0	5.42	179	74.7
TP (m^3 year^{-1})	79.3	58.2	26.6	3.23	4.29	5.42
TK (m^3 year^{-1})	45.7	39.6	13.3	–	–	–

$^a c = (a - b)/a \times 100\%$; $e = a - (b + d)$; $f = e/a \times 100\%$.

catalysed the main portion of waste reduction in this study. The significant waste reduction can contribute to an easier handling of residues. Due to water losses via physical evaporation and biological transpiration under relatively high temperature in greenhouse, organic matters and crude fibre levels increased in the residues. However, waste reductions of over 90 and 60% were found in crude fat and crude fibre (Table 3).

Although fluctuations in nutrient composition (TKN, AN, AP, TK and AK) during the course of bioconversion were nominal (Table 2), reductions of TP and TKN by larvae bioconversion ranged from 23.1 to 29.7% and 13.3 to 18.1%, respectively (Table 3). These values were much lower than those reductions in total weight and moisture, whereas TKN and TP in residues (Table 2) were found to have average values of 2.20 and 2.86%, respectively. These residues, having optimal nutrient levels, are favorable qualities for use as bio-fertilizers.

Economic feasibility and valued-add contribution

Over 85% of total larvae products were sold to local fish and poultry feedstuff manufacturers and nearly 10% of the totals were sold to a Bio-Tech company in ShangHai, China for R&D on chitosan and antibacterial peptide products. The remaining dry larvae product was applied to fishponds affiliated with the GuoSheng company for aquatic farmers. The residues were further composted under a technique for secondary composting coupled with semi-continuous turning (Nakasaki and Marui, 2011), then sold to local fertilizer dealers.

Both the treatment capacity of raw manure (8030 m^3 year^{-1}) and the production of dry larvae (942 m^3 year^{-1}) in 2010 were nearly double those in 2008 (4332 and 407 m^3 year^{-1}, respectively) (Table 3). The harvested bio-products, such as house fly larvae and manure residues increased remarkably, yielding both economic profits and environmental benefits. The value of larvae increased from 137 000 US$ year^{-1} in 2008 to 347 000 US$ year^{-1}. Similarly, the compost income also reached 16 400 US$ year^{-1} in 2010. Total input costs were estimated at 77 000 US$ year^{-1} in 2008 and increased to 161 000 US$ year^{-1} in 2010. Labour was the primary input cost, accounting for 94% of all costs in 2008 and 92% of total costs in 2010. It is necessary to further innovate technologies for improving larvae productivity as well as extending the potential of larvae utilization (chitosan and antibacterial peptides), so that the relative economic gain per labour input would be more attractive to other confineed livestock producers. The net profit from the operation was 67 900 US$ year^{-1} in 2008 and 210 000 US$ year^{-1} in 2010. As shown in Table 4, the economic returns for larvae accounted for about 95.9% of the total income, with the overall income per m^3 of raw manure ranging from 33.4 to 46.1 US$ m^{-3}. This is nearly

Table 4. Average yearly economic return of the full-scale swine manure bioconversion by house fly larvae.

			2008	2009	2010
Income (*a*)	Larvae (dry)[a]	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	137	206	354
	Compost fertilizer	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	7.82	8.77	16.4
Cost (*b*)	Labour	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	72.4	96.1	148
	Electricity	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	1.44	1.71	2.51
	Tap water	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	0.34	0.47	0.79
	Substrate	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	0.98	1.88	3.29
	other	$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	1.76	4.29	6.20
Profit (*a* – *b*)		$(\mathrm{US\$} \times 10^3)\ \mathrm{year}^{-1}$	67.9	110	210

[a] The average purchasing price of dry larvae ranged from 1.25 US\$ kg^{-1} in 2008 to 1.43 US\$ kg^{-1} in 2010.

triple the return from conventional manure composting in the USA (Westerman and Bicudo, 2005). As a result, the income from larvae was 17 to 23 times greater than the income from residue compost sales (Table 4). The feasibility of raw manure bioconversion using larvae in the present study demonstrated a successful use of technological initiatives for swine manure management. House fly larvae fed by protein-rich poultry waste and fish-processing waste converted 22 to 35% of the total waste into fresh larvae biomass (Marchaim et al., 2003). As the saprophagous larvae will utilize many types of rotting organic matter, this greenhouse-assisted bioreactor could potentially be applied to other waste sources, resulting in a broader scope of regional waste-derived recycling.

Implication on environmental risk of manure bioconversion

Heavy metals added to the feedstuff and their subsequent excretion in manure from the swine digestion system pose a debatable threat to the world environment (Aarnink and Verstegen, 2007). High levels of heavy metals, including Cu, Cr and Zn, along with trace amounts of Cd, Hg and Pb were found in both raw manure and residue samples (Table 2). Although the total amount of heavy metals in the residues may be higher than background levels in agricultural soils, most of the bio-available form of heavy metals are transformed into inactive forms via soil fixation (Kan and Meijer, 2007; Mohamed et al., 2010) thereby minimizing biological uptake by crops. The risks of heavy metals to both food-web and environment could also be reduced by fertilizing residues during the fallow season.

Bioaccumulation of heavy metals in both earthworms (Huang et al., 2009) and aquatic worms (Elissen et al., 2010) has been reported for vermicomposting processes which were fed heavy metal-containing mediums, such as sludge. Heavy metals such as Cu and Zn might be assimilated and stored as metal-complexes or chelated with chitosa in intact cells (Gyliene et al., 2002; Huang et al., 2009). Fortunately, the levels of heavy metals in the sampled larvae biomass (Table 1) were uniformly lower than the ‘Hygienical Standard for Feeds’ (GB 13078-2001) and therefore acceptable as a poultry feedstuff. In addition, by comparing the data in Tables 1 and 2, the heavy metal concentrations in larvae remained lower than those in the manure, demonstrating the lower metal bioaccumulation capacity of larvae. These values were also lower when compared with the aquatic worm bioconversion process (Elissen et al., 2010).

Similar to the roles of intestinal microflora in the manure digestion process in black soldier fly larvae (*Hermetia illucens*) and earthworm (*Eisenia fetida*) (Aarnink and Verstegen, 2007; Jeon et al., 2011), the levels of odor and *E. coli* index in residues (Table 2) were reduced by 94.5 and 92.0%, respectively. For example, black soldier fly larvae effectively reduced pathogenic bacterial counts (*E. coli*) in animal wastes by three to four orders of magnitude (Liu et al., 2008). Another factor may include the bio-oxidation by the joint action of worms/larvae and micro-organisms during vermicomposting and/or bioconversion (Lazcano et al., 2008; Westerman and Bicudo, 2005), leading to a remarkable reduction of the pathogenic bacterial (particularly for anaerobic micro-organisms) population and the stimulation of the biodegradation of odorous organic compounds (such as 3-MI and volatile fatty acids). As a whole, the activities and metabolisms of house fly larvae in the present study showed a multiple-functional bioprocess for swine manure reduction.

Conclusions

In summary, this greenhouse-assisted larvae bioreactor demonstrated the successful operation of a full-scale value-added swine manure bioconversion and waste reduction at the GuoShen farm. This one-week-duration bioreactor generated a fresh larvae production rate of 95–120 kg m^{-3} of fresh raw manure, providing nutritious larvae as alternative animal feed which contented crude protein and crude fat in dry biomass at averages of 56.9 and 23.8%. The reduction of the total weight and moisture reduction were 67.2 and 80.0%, and the 3-methylindole (representing odour) and *E. coli* index (representing pathogen) were reduced by 94.5 and 92.0%, respectively. Due to the relative abundances of total Kiehldahl nitrogen (average of 2.20%) and total phosphorus (average of 2.86%), the residue after larvae bioconversion (at rate of 350–450 kg m^{-3}) could be re-utilized as soil amendment for agriculture. The income per fresh raw manure ranged from 33.4 to 46.1 US\$ m^{-3}, to which the larvae products contributed roughly 95% of total income. The results of the present study show that the house fly larvae bioreactor is a robust option for value-added bioconversion of swine manure and as a waste reduction programme.

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