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Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production

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ABSTRACT

Modern dairies cause the accumulation of considerable quantity of dairy manure which is a potential hazard to the environment. Dairy manure can also act as a principal larval resource for many insects such as the black soldier fly, *Hermetia illucens*. The black soldier fly larvae (BSFL) are considered as a new biotechnology to convert dairy manure into biodiesel and sugar. BSFL are a common colonizer of large variety of decomposing organic material in temperate and tropical areas. Adults do not need to be fed, except to take water, and acquired enough nutrition during larval development for reproduction. Dairy manure treated by BSFL is an economical way in animal facilities. Grease could be extracted from BSFL by petroleum ether, and then be treated with a two-step method to produce biodiesel. The digested dairy manure was hydrolyzed into sugar. In this study, approximately 1248.6 g fresh dairy manure was converted into 273.4 g dry residue by 1200 BSFL in 21 days. Approximately 15.8 g of biodiesel was gained from 70.8 g dry BSFL, and 96.2 g sugar was obtained from the digested dairy manure. The residual dry BSFL after grease extraction can be used as protein feedstuff.

1. Introduction

Biofuel has been considered as an alternative option to reduce consumption of petroleum. Bioethanol and biodiesel are two kinds of biofuel. Biomass resources can be converted into biofuel. Biofuel production is still a relatively small scale in comparison to those refining fossil fuels. The difference is primarily caused by high production costs (Seungdo and Bruce, 2005).

Biofuel has been made from feedstock such as starch, vegetable oil, or animal fats (Marina et al., 2009). However, using the feedstock for fuel production instead of human food is unacceptable. The production cost is the major problem of biofuel, therefore it is important to develop cheap feedstock. An alternative way would be developed with cheap waste, such as dairy manure, to produce bioethanol (Liao et al., 2008; Predojević, 2008). Dairy manure was considered as a pollution source rather than a valuable resource which contains undigested organic matter to pollute the environment (Ann et al., 2002).

Currently no studies have been conducted on the hydrolysis of dairy manure after treatment with *Hermetia illucens* L. (Diptera: Stratiomyidae). *H. illucens*, which usually known as black soldier fly larvae (BSFL), are voracious consumers of dairy manure (Newton et al., 2005; Westerman and Bicudo, 2005). It can reduce manure dry matter by up to 56% (Sheppard, 1983). The black soldier fly is a non-pest insect distributed throughout the tropics and subtropics and is useful for managing large amount of animal manure and other biosolids. Its life cycle is divided into four stages (egg, larva, pupa and adult). Adults mate and then laid eggs in cracks near larval habitats (Sheppard et al., 1994). The eggs required about 102–105 h to hatch at 24 °C. Under appropriate conditions, the larvae can reach the prepupal stage in 2–3 weeks (Sheppard et al., 2002). BSFL which high in protein, are good feed for fish and chicken in both backyard and commercial purpose (Sheppard et al., 1994; Zuidhof et al., 2003; Erickson et al., 2004).

In this study, BSFL were inoculated into dairy manure for 21 days, the grease was extracted from dried BSFL, and it can be

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used as the material for biodiesel, and the digested dairy manure was hydrolyzed into sugars which can be further used.

2. Materials and methods

2.1. Biomass

H. illucens colony has been maintained for more than 10 generations at Huazhong Agricultural University, Wuhan, China. In this research, fresh dairy manure was obtained from the Dairy Center of Huazhong Agricultural University. About 1200 of 10 days old BSFL were inoculated into 1248.6 g fresh dairy manure (582 g dry wt) by triplicates. The cultivation conditions were temperature (27 °C), humidity (60–75%) in an open barrel with humid cotton gauze. After 21 days, most of the larvae prepupated and crawled out from the manure and the rest were picked out manually using sterile forceps. The larvae were inactivated at 105 °C for 5 min after being washed with distilled water, and then dried at 60 °C for 2 days. The full scale bioconversion procedure is shown in Fig. 1.

2.2. Production of biodiesel from BSFL grease

The grease was extracted from dried BSFL with petroleum ether using three methods. The first method was carried out by placing dried BSFL into a Soxhlet extractor with petroleum ether for 16 h. The second method was to immerse the dried BSFL into petroleum ether at room temperature for 48 h. The third method was to expose the dried BSFL in petroleum ether under ultrasound for 30 min. The extracted samples were dried at 60 °C for overnight, and extraction efficiency was calculated by weighing the samples before and after extraction. The extracted grease contained various kinds of impurities including water, pectin, phospholipids, and solid impurities. Therefore, it was necessary to be purified by adding 0.5% H_2SO_4.

Due to the acid value of BSFL grease (AV = 8.7), a two-step method was chosen for biodiesel production in which H_2SO_4-catalyzed pretreatment was introduced to reduce the acid value, and NaOH-catalyzed transesterification was carried out subsequently (Veljković et al., 2006). The reactor was filled with BSFL grease and heated to the desired temperature (73 °C) in a water bath. Methanol/oil at a ratio of 8:1 and 1% w/w H_2SO_4 as the catalyst were added. After 2 h, the mixture was poured into a funnel for separation of the biodiesel and neutral oil from the water phase. The upper layer was transferred to the reactor, and methanol/oil at a ratio of 8:1 and 0.8% w/w NaOH as a catalyst were added for transesterification. The mixture was stirred, and reacted for 30 min in a water bath at 65 °C. Then the mixture was poured into another funnel for separation. The biodiesel was washed with 80 °C distilled water until the washing liquor was neutral.

2.3. Manure pretreatment and enzymatic hydrolysis

2.3.1. Single factor test for high sugar production parameters of acid pretreatment

To compare fresh dairy manure with digested dairy manure by BSFL, the sugar production was determined after being treated by acid pretreatment and enzymolysis. Diluted hydrochloric acid (HCl) is suitable for pretreatment of dairy manure (Liao et al., 2007, 2008). The main factors were temperature, reaction time, acid concentration and substrate concentration. For each pretreatment, the manure was mixed with HCl (0.5–8%), then pretreated under a range of conditions: time (1–5 h), substrate concentration (10–100 mg/L) and temperature (50–90 °C). The supernatant was determined for sugar analysis after centrifugation, and the manure was washed with water to a neutral pH. In order to optimize the pretreatment conditions, the $L^9(4^3)$ orthogonal design was chosen for this work (Zhu et al., 2006).

2.3.2. Enzymatic hydrolysis

Pretreated dairy manure was transferred into Erlenmeyer flasks with sodium acetate buffer (pH = 4.8). Cellulase (Ningxia Heshibi Biology Co. Ltd., China) was added at an enzyme loading of 20 mg/g substrate (0.53 ± 0.03 FPU/mg) (Zhu et al., 2006). Tetracycline was added at a concentration of 40 μg/mL. The hydrolysis was carried out in the incubator shaking at 50 °C and 150 rpm for 48 h, and the supernatant was sampled for sugar analysis (Yu et al., 2009).

2.4. Phase-contrast microscope observation

Samples including the fresh and digested dairy manure were prepared for phase-contrast microscope observation to compare the structure differences of the fresh and the digested dairy manure.

2.5. Analysis methods

The composition of the biodiesel was determined by GC/MS (Thermo-Finnigan, USA) equipped with a polyethylene glycol phase capillary column (Agilent, USA) (Zullaikah et al., 2005). The fresh and digested dairy manure were dried to constant weight at 65 °C for 2 days. The samples were treated with phosphate buffer, chloroform–methanol, DMSO, 4 M KOH, acetic acid–nitric acid and H_2SO_4 (Peng et al., 2000). And the sugar in the supernatant was analyzed by colorimetric assays. The total sugar is consisted from the pretreatment and from the hydrolysis. Conversion rate of sugar was calculated by the following formula:

$$\text{Conversion rate } (\%) = [S \times 0.9/(m \times c)] \times 100\%$$

S, is the content of sugar (g); m, is dry weight of the dairy manure (g); c, is the carbohydrates content of dairy manure (%).

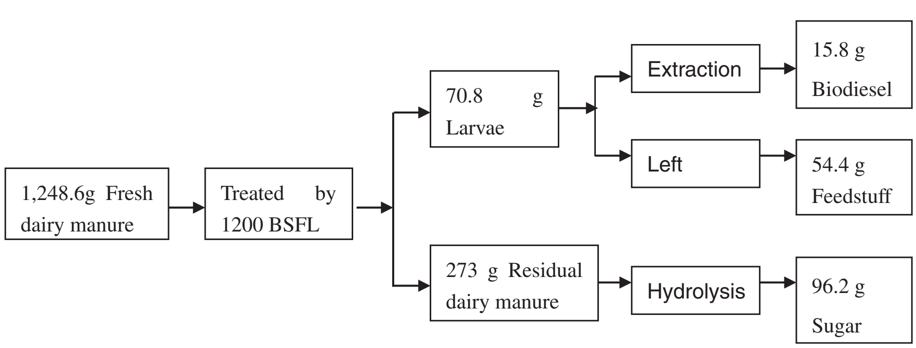

Fig. 1. Process of bioconversion of dairy manure by BSFL.

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Table 1
Physical and chemical properties of grease of BSFL.

Iodine value (gI/100 g)	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Melt point (°C)	Peroxide value (meq/kg)
96 ± 2.4	8.7 ± 0.4	157.5 ± 6.2	5 ± 0.3	0.03 ± 0.01

3. Results and discussions

3.1. Grease extraction and quality from BSFL

About 1200 BSFL reduced 1248.6 g of fresh manure to 273.4 g dry digested dairy manure within 21 days, and BSFL converted the nutritive material into protein and fat from the fresh dairy manure. About 70.8 g dried BSFL was obtained in the end. The results showed that the extraction rate of immersing method was 22.9%, the Soxhlet extraction method was 21.9%, and the ultrasonic method was 15.7%. Totally, 16.4 g of BSFL grease was gotten from the 70.8 g dry BSFL.

BSFL grease is yellow with a peculiar smell. The iodine number indicated the amount of unsaturation in the BSFL grease. Saponification value represents by the milligrams of potassium hydroxide, and it is used for the measure of the average molecular weight of the BSFL grease. Melt point is referred to the freezing point of the grease. Peroxide value indicated the level of rancidity during storage. Acid value reflects the amount of free fatty acid in grease. The results demonstrated that BSFL grease was suitable for the production of biodiesel (Table 1).

There were 10 kinds of fatty acids had been detected, including palmitic acid, oleic acid and linolenic acid, etc. The carbon number ranged from 10 to 22, as shown in Table 2 (Li et al., 2010). The grease consisted of 58.2% saturated fatty acid and 39.8% unsaturated fatty acid.

3.2. Biodiesel produced from BSFL grease

Biodiesel has lower toxicity and more biodegradable compared to petroleum diesel. Biodiesel is a renewable fuel produced from a wide range of vegetable oils and animal fats, which can be used to fuel diesel vehicles, providing energy security and emissions and safety benefits. According to the standard EN 14214, the quality of biodiesel demonstrates that BSFL grease is a suitable material for biodiesel production (Gerhard, 2005). The density, flash point, kinematic viscosity of the biodiesel is within the standard of EN 14214, as Table 3 (Li et al., 2010) showed. Biodiesel from the grease of BSFL has a higher acid value, and other factors are similar to those previously reported (Prafulla et al., 2010). This biodiesel was produced from organic waste, and it needs no farmland as compared to rape and soybean oil.

Table 2
The fatty acid composition of BSFL grease.

Fatty acid	Retention time (min)	The numbers denote the number of carbons and double bonds	Relative content (%)
Capric acid	5.8	C10:0	3.1 ± 0.03
Lauric acid	7.3	C12:0	35.6 ± 0.1
Myristic acid	8.8	C14:1	7.6 ± 0.1
Pentadecanoic acid	9.7	C15:0	1.0 ± 0.06
Palmitoleic acid	11.2	C16:1	3.8 ± 0.2
Palmitic acid	11.6	C16:0	14.8 ± 0.4
Oleinic acid	15.5	C18:1	23.6 ± 0.3
Linolenic acid	15.8	C18:2	2.1 ± 0.3
Stearic acid	16.1	C18:0	3.6 ± 0.1
Noadecanic acid	17.9	C19:1	1.4 ± 0.02
Decosanic acid	19.9	C22:1	1.4 ± 0.03

Table 3
Analysis data of the quality parameters of biodiesel from the BSFL grease.

	EN 14214	Biodiesel from BSFL grease
Density (kg/m^3)	860–900	872 ± 0.3
Ester content (%)	96.5	97.2 ± 1.4
Flash point (closed cup) (°C)	120	121 ± 2.6
Water and sediment (mg/kg)	500	300 ± 3.7
Kinematic viscosity at 40 °C (mm^2/s)	2.5–6.0	4.5 ± 0.1
Acid number (mg KOH/g)	0.50	0.8 ± 0.2
Methanol or ethanol (m/m)	0.2%	Not detected
Distillation	Not mentioned?	91 ± 1.87% at 360 °C

Table 4
Variation of selected factors in the fresh and digested dairy manure.

	Fresh dairy manure	Digested dairy manure
Dry matter (%)	46.5 ± 0.2	54.4 ± 0.3
Cellulose (%)	38.6 ± 0.3	21.3 ± 0.4
Hemicellulose (%)	18.3 ± 0.4	12.9 ± 0.3
Lignin (%)	23.2 ± 0.09	31.1 ± 0.1
Total nitrogen (%)	1.7 ± 0.2	0.96 ± 0.2
pH (units)	8.2 ± 0.1	7.3 ± 0.1

3.3. Componential changes of dairy manure after conversion by BSFL

BSFL converted most of nutrient from the fresh dairy manure into fat and protein. No odor was smelled from digested dairy manure. The components of the fresh and digested dairy manure are presented in Table 4. About 50% of cellulose and 29.4% of hemicellulose were reduced within 21 days. The relative content of lignin increased because of the degrading of cellulosic materials. Hemicellulose and cellulose in the digested dairy manure represent a potential source of carbohydrates which are capable to produce fermentable sugar.

The nitrogen in the fresh dairy manure was mainly from proteins bonded with the cell wall of the forage and proteins associated with lignin; both of proteins were difficult to be removed by simple washing (Liao et al., 2004). BSFL reduced 43.6% nitrogen of the fresh manure, and pH of manure decreased from 8.2 to 7.3.

3.4. The orthogonal test for optimal parameters of pretreatment

The digested manure was passed through a 40 mesh to eliminate the influence of size on the following treatment. The orthogonal test $L^9(4^3)$ table was designed to determine the optimal pretreatment conditions for sugar yield. Four factors: acid concentration (A), temperature (B), reaction time (C) and substrate concentration (D), were optimized by the orthogonal experiment. And every parameter has 3 levels to be optimized.

Different pretreatment conditions have different effects on production yield of sugar. Fig. 2A shows the results of single-factor experiment on of acid concentration. The production of sugar increased with the concentration of the acid. But higher acid concentration could lead to much pollution. Hence, 0.5%, 1% and 2% were selected for further optimization. As far as the pretreatment temperature is concerned, the higher the temperature, the higher the sugar production, but higher temperature implied more energy requirement. As shown in Fig. 2B, the controlled temperatures in water bath were 70 °C, 80 °C and 90 °C. When the time continued to lengthen, the sugar yield increased little. Because longer time could lengthen production cycle, 2–4 h of pretreatment time was adopted in the present work as Fig. 2C showed. The production of sugar increased with substrate concentration, and the chosen results were 20 mg/L, 40 mg/L, 60 mg/L as Fig. 2D showed.

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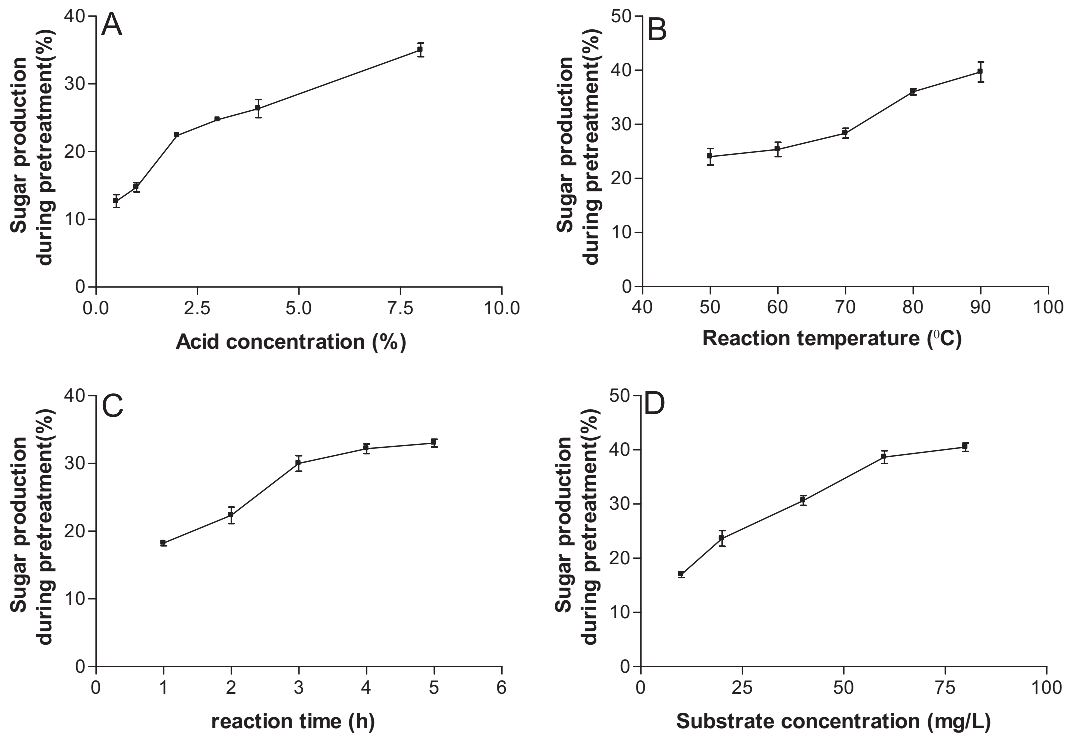

Fig. 2. Single-factor effect of acid concentration, temperature, time and substrate concentration on pretreatment hydrolysis. (A) Effect of acid concentration, condition: temperature (60 °C), reaction time (2 h) and substrate concentration (20 mg/L). (B) Effect of temperature, condition: acid concentration (2%), react time (2 h) and substrate concentration (20 mg/L). (C) Effect of time, condition: acid concentration (2%), temperature (60 °C) and substrate concentration (20 mg/L). (D) Effect of substrate concentration, condition: acid concentration (2%), temperature (60 °C) and reaction time (2 h).

Table 5
The analysis of orthogonal experiment design results.

	A	B	C	D	Pretreatment hydrolysis	Enzymatic hydrolysis	Total sugar
1	1(0.5)	1(70)	1(2)	1(20)	25.6 ± 0.1	9.4 ± 0.2	35.9 ± 0.3
2	1(0.5)	2(80)	2(4)	2(40)	31.1 ± 0.1	11.9 ± 0.2	43.1 ± 0.3
3	1(0.5)	3(90)	3(6)	3(60)	45.8 ± 0.04	12.6 ± 0.3	58.4 ± 0.4
4	2(1)	1(70)	2(4)	3(60)	27.5 ± 0.2	8.5 ± 0.1	36.0 ± 0.3
5	2(1)	2(80)	3(6)	1(20)	37.8 ± 0.2	9.3 ± 0.2	46.2 ± 0.3
6	2(1)	3(90)	1(2)	2(40)	42.1 ± 0.3	12.1 ± 0.1	54.1 ± 0.5
7	3(2)	1(70)	3(6)	2(40)	30.9 ± 0.2	16.2 ± 0.3	47.1 ± 0.5
8	3(2)	2(80)	1(2)	3(60)	33.0 ± 0.2	15.6 ± 0.2	48.6 ± 0.4
9	3(2)	3(90)	2(4)	1(20)	42.8 ± 0.2	13.4 ± 0.1	56.2 ± 0.3
K1	137.4	119.0	138.7	138.3			
K2	136.3	137.8	135.3	144.3			
K3	151.8	168.6	151.6	142.9			
k1	45.8	39.7	46.2	46.1			
k2	45.4	45.9	45.1	48.1			
k3	50.6	56.2	50.5	47.7			
R	5.2	16.5	5.5	1.9			
S	B > C > A > D						

Ki represents the sum of sugar yields of sugar at level i (%); ki represents the average sugar yields of sugar at level i (%); R represents the sum of deviation squares.

The analyses of orthogonal experiment design were listed in Table 5. From the results, the influential order was temperature > substrate concentration > acid concentration > time. The optimal combination parameters of the processing technology were A3, B3, C3 and D2, namely, temperature (90 °C), substrate concentration (60 mg/L), acid concentration (2%), and time (3 h).

3.5. Enzymatic hydrolysis of pretreated manure

There is a bottleneck in the enzymatic hydrolysis of dairy manure. The pretreatment is a very important factor in ethanol production because the crystalline cellulose is highly structured and resistant to enzymatic hydrolysis. Current pretreatment methods are used to remove most of the lignin and hemicellulose (Yu et al., 2009). Dairy manure is rich in carbohydrate, such as cellulose and hemicellulose which can be converted into biofuel (Wen et al., 2004).

Table 6
Total production of sugar from dairy manure.

	Fresh dairy manure	Digested dairy manure
Acid hydrolysis	48.1 ± 0.2	22.3 ± 0.2
Enzymatic hydrolysis	10.8 ± 0.1	12.9 ± 0.3
Total sugar	48.9 ± 0.1	35.1 ± 0.1

The fresh and digested dairy manure were pretreated under the optimal hydrolysis condition. The total production of sugar is shown in Table 6. Through the comparing experiments, it was found that BSFL had consumed part of the lignocelluloses in the fresh manure. Consequently during acid pretreatment, the production of sugar from digested dairy manure (22.3%) was less than that from fresh dairy manure (48.1%). Whereas, the production of sugar from digested dairy manure (12.9%) was more than that from fresh dairy manure (10.8%) during enzymatic hydrolysis. Altogether 96.2 g sugar was obtained from the digested dairy manure.

Physical changes had been taken place during BSFL conversion, such as surface area, porosity, and pore size. It is an ideal pretreatment for manure in the mild conditions; the digested manure is more susceptible to enzymes due to structure destruction of cellulose. The crystalline cellulose had been decreased from 8.6 ± 0.21% to 3.3 ± 0.17%, which indicated that the degree of crystallinity had been reduced after digestion. Phase-contrast microscope was used to observe the structural change of digested dairy manure. The cell wall of straw in the digested dairy manure had been broken, which expose the cellulose and enhance accessibility of enzymes. The

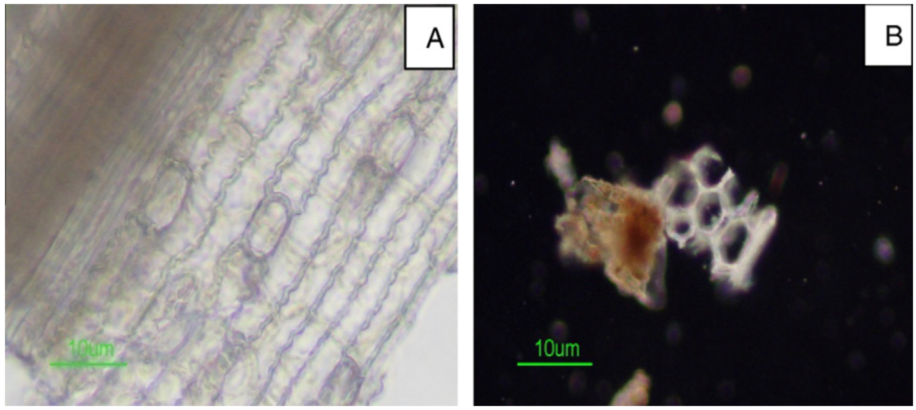

Fig. 3. Comparison of manure surface between fresh dairy manure (A) and digested dairy manure (B) under phase-contrast microscope.

structural difference, as shown in Fig. 3, led to the production of sugar from digested dairy manure was more than that of fresh dairy manure during enzymatic hydrolysis. Sugar can be gotten from digested manure, which can be used as a sugar source for other industrial products, particularly for ethanol fermentation.

4. Conclusions

This study suggests that *H. illucens* holds a high promise for converting waste manures into a valuable commodity, which is in accordance with the concept of circular economy. The bioconversion of dairy manure by BSFL to produce biodiesel and sugar was investigated in this study. In short, 15.8 g of biodiesel can be processed from 1248.6 g fresh dairy manure by 1200 BSFL in 21 days, the residual larvae (54.4 g) can be used as animal feedstuff. Furthermore, 96.2 g sugar was obtained from the digested dairy manure.

Compared with energy plant, *H. illucens* has high reproductive capacity and short lifecycle, while the energy plants need long lifecycle and plenty of land which can avoid conflicts between human food use and industrial use of crops. From comprehensive analysis of society, economy and environment, it can be concluded that BSFL can recycle waste into clean energy, and reduce environmental pollution of the manure.

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