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Biofuel Produced from Solid-State Anaerobic Digestion of Dairy Cattle Manure in Coordination with Black Soldier Fly Larvae Decomposition

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Abstract

This study was conducted to evaluate the feasibility of applying a two-step biological treatment process, solid-state anaerobic digestion (SSAD) and black soldier fly larvae (BSFL) composting, for treating dairy cattle manure. Biogas from SSAD of dairy cattle manure, and the digestate of SSAD was fed to BSFL. In turn, BSFL can be fed to animals as a protein supplement. Adjustment of pH and 30% inoculation ratio (IR_{30}) during SSAD produced the highest theoretical methane yield, 626.1±28.7 L CH_4/kg $VS_{des,}$ with an ultimate methane yield of 96.81±2.0 L CH_4/kg VS_{load}. For BSFL composting, the groups with a feeding rate of 75 and 100 mg/day/larvae had the highest body weight change, which was 969.6±28.4 and 984.1±177.6%, respectively. The combination process of SSAD and BSFL composting increases the incentive for dairy cattle manure treatment enabled higher waste removal efficiency, and produced more valuable products.

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1. Introduction

With a growing population to approximately 9.7 billion in the year 2050 and income increase, worldwide production of milk is estimated to grow from 580 million tons in the year 1999 to 1043 million tons in 2050, and global meat production is estimated to increase from 229 to 456 million tons [1]. In 2015, there were 146,030 heads of dairy cattle in Taiwan [2]. Dairy cattle manure is commonly treated using both wastewater treatment and composting technology in Taiwan. A three-step wastewater treatment system that includes: solid-liquid separation, anaerobic digestion, and aeration; is used to treat dairy and piggery wastewater [3]. Composting is used to treat mainly the solid fraction of dairy cattle or pig manure. In fact, the solid fraction of dairy cattle manure could also be used as a feedstock for anaerobic digestion.

Biogas from anaerobic digestion (AD) of pig and cattle manure mainly consists of CH_4 (60−76%), CO_2 (18−30%), and trace amounts of H_2S [4−7]. The methane found in biogas burns with a blue flame and has a calorific value of 4500−5000 kcal/m^3 [8]. Anaerobic digestion can be divided into different types by total solid content (TS), temperature, and ways of feeding the digester [9]. Based on the TS content, AD can be classified into liquid anaerobic digestion, which contains less than 15% TS and is applied for treating wastewater [10], and SSAD, which contains more than 15% TS [11].

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Liquid AD is a technology that had been used for a long time, while SSAD to treat municipal solid waste was initially installed in Europe and is gradually increasing since the 1990s [12]. Compared to SSAD, liquid AD generates a large amount of wastewater as well as sludge production [13, 14]. In contrast, SSAD generates a lower amount of wastewater and requires less energy for mixing as well as heating [9, 15]. Due to the high content of TS, mixing and handling of feedstock for SSAD is more difficult when compared to liquid AD [16]. Also, inhibitors such as volatile fatty acids (VFAs) and ammonia have a higher tendency to inhibit the anaerobic digestion process [11]. Digestate produced from SSAD may contain high organic nutrient contents that enable it to be used as a feedstuff for black soldier fly larvae.

The black soldier fly (BSF) (*Hermetia illucens*) can colonize a wide range of habitats like manure, dead animal remains, and decomposing vegetables [17−19]. Thus, they are proposed to be a key on manure recycling in farm waste management and to reduce manure bulkiness while producing valuable feed for chicken, pigs, and even fish [20, 21]. Myers et al. (2008) stated that BSFL were capable of reducing manure total solids by 33−58% when daily fed with 27−70 g dairy cattle manure [22]. Available phosphorus and nitrogen are reduced by 61−70% and 30−50%, respectively, with some differences across treatment. BSFL fed with municipal organic waste achieved 65.5−78.9% of waste removal efficiency according to the daily waste added into the system [23]. BSFL was

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able to reduce hen manure accumulation by 50% of a 100,000-hen poultry house [24]. Several studies had shown that activity of BSFL could inactivate *E. coli* and *S. enterica* subspecies in different substrates [25−27]. The goal of this study was to conduct a pilot study of SSAD and BSFL composting of dairy cattle manure.

2. Materials and methods

2.1. Inoculum for SSAD

Dairy cattle manure from solid/liquid separation of wastewater from the National Taiwan University (NTU) dairy farm was used to be the sole substrate for SSAD reactors. The sludge from the anaerobic digesters of NTU dairy farm was used as the initial inoculum for enrichment of SSAD reactors. After three batches of enrichment process (about 90 days), the methanogenically activated (MeA) mixture from the previous batches of SSAD were utilized as the inocula for the further SSAD experiments.

2.2. SSAD reactor design

Acrylic anaerobic digester (19 cm i.d. × 115 cm Height) in triplicates with a working volume of 37 L was used in this study (Fig. 1). Each digester was equipped with an acrylic inner sieve vessel (17 cm i.d. × 87cm Height). The inner sieve vessel was utilized to load solid dairy cattle manure and the leachate was allowed to drain to the bottom of the digester. A thermostatic recirculation water batch (BH-230D-W, Yih-Der Co., Taipei, Taiwan) was equipped outside the digester to maintain the digester at a 36±1°C. Each

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digester had an independent leachate recirculation magnet pump (speed: 2800/3100 rpm, Model: MD-10K-NL, Iwaki Co., Tokyo, Japan), which was used to recirculate the leachate from the side port at the bottom of the digester to the side port at the top of the digester through rubber tubes (18 mm o.d × 8 mm i.d.). A digital programming timer (Model: OTM304, Max Star Electric Co., Ltd., Taichung, Taiwan) controlled the leachate recirculation and the recirculation frequency was every 20 min for 20 sec recirculation process (approximately 3.8L leachate/recirculation).

2.3. Preliminary study of SSAD by fresh cattle manure

A preliminary study was performed in an acrylic anaerobic digester (19 cm i.d. × 115 cm Height) with fresh cattle manure (5 kg) as the sole feedstock. The operation conditions were the same as the time course experiments.

2.4. Time course experiment of SSAD

A two-step biological treatment process was carried out for this study (Fig. 2). Two groups of initial pH (7.8 and 5.2−5.5) and three groups of inoculation ratios (IR) (50, 30, and 10%) were designed to evaluate efficiency of SSAD experiments. The group with 50, 30, and 10% inoculation ratio are referred to IR_{50}, IR_{30}, and IR_{10}, respectively. Every time course experiment of SSAD was conducted for a 14-d period, daily biogas yield was collected and measured by applying water displacement method with a 6-L glass gas collector. The MeA mixture from the initial SSAD reactor as inocula were added

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manually into a laundry mesh bag (60cm × 60cm) by the inoculation ratios of 50, 30, and 10% (w/w) respectively, the total weight of the mixture was 5 kg. The laundry mesh bag with the mixture of dairy cattle manure and the MeA mixture was inverted several times to mix completely before placing into the inner sieve vessel of the SSAD reactor.

The initial volume of the recirculation tap water for the experimental sets of 50, 30, and 10% was 2.5 L. For the sets with pH adjustment, the pH value of tap water was adjusted to 7.8 by using the mixture of 0.1 M $NaHCO_3$ and 0.1 M K_2CO_3 solution. The pH-adjusted tap water was then added into the SSAD reactors for performing time course experiments. The SSAD reactors were then sealed with screws to ensure an airtight environment and operated for a 14-d-period time course experiment. The liquid samples, leachate, from the SSAD reactors were taken and analyzed periodically.

2.5. Black soldier fly larvae

The 10-day-old black soldier fly larvae were received from a commercial pig farm, 5000 pigs on farm, with a self-reproduction housing of black soldier fly larvae, which located in Chang-Hua County, Taiwan. The black soldier fly larvae are fed on distillers' grains or soymilk residue.

2.6. Black soldier fly experiment

After SSAD experiments, solid digestate was removed and dried in an oven for a week at 65°C. After drying, the solid digestate was grinded and screened through a 20-

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mesh screen. The solid digestate was then stored in a fridge at 4°C and ready to be used for performing BSFL composting. The TS content of the dried digestate was adjusted to obtain a 15−30% total solid content before distributing it into plastic containers (29.5 cm L ×22 cm W × 11 cm H) based on different feeding rates (25, 50, 75, and 100 mg/day/larva).

Four hundred, 10-day-old, BSFL were weighted and spread out onto the solid digestate of plastic containers placed in an incubator at 30°C during the experimental periods. After a 14-d period, the BSFL were collected from the solid residue of digestate. The collected BSFL and solid residue were weighted and analyzed for total solid (TS), volatile solid (VS), and nitrogen content determination.

2.7. Analysis.

Biochemical oxygen demand (BOD), chemical oxygen demand (COD), TS, and VS of samples were determined according to the Standard Method for the Examination of Water and Wastewater.[28] The values of pH in samples were determined by a pH meter (PH200, CLEAN instruments Co., Ltd, New Taipei City, Taiwan). Electrical conductivity (E.C.) of samples was determined by a conductivity meter (WalkLAB, Trans Instrument Ltd., Singapore).

Biogas samples were analyzed for its composition by gas chromatography (Master GC, DANI Instruments, Marlborough, MA, USA), which was equipped with a thermal

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conductivity detector (TCD) and Carboxen 1010 PLOT capillary column (30 m × 0.53 mm × 0.25 µm film thickness; Supelco Analytical of Sigma-Aldrich Co., PA, USA). Helium was used as carrier gas with a flow rate of 10 mL min^{-1}. The oven temperature was increased from 40 to 180°C with a 20°C min^{-1} increasing rate. The injector and detector temperature were set at 200°C. Sample injection volume was 250 µL and was injected using Pressure-Lok® analytical syringes (VICI, Valco Instruments Co., Ltd., Houston, TX, USA). Calibration curves of methane, carbon dioxide, and nitrogen gas were obtained by external standard method, and the calibration curves correlation coefficient were >0.9974.

For volatile fatty acids analysis, 2 mL of liquid sample was mixed with 500 µL of 20% H_3PO_4. The mixture was next centrifuged (Z 36HK, Hermle Labortechnik GmbH, Germany; radius of rotor = 10 cm) for 20 minutes at 4°C with 15000 rcf to remove solids in the solution. The supernatant was filtered through 0.2 µm filter and transferred to a 2-mL gas chromatography vial. Before injection, 200 µL supernatant was moved into a 300-µL gas chromatography vial and 50 µL of a 2000 mg/L crotonic acid solution was added as an internal standard. The injection volume of the sample was 1µL. The identification of VFAs of the effluent was conducted using gas chromatography (Agilent GC 7820A, Agilent Technologies) equipped with a flame ionization detector (FID) and a Nukol capillary column (30 m × 0.25 mm × 0.25 µm film thickness, Supelco). The injector and

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detector were set at 180°C, and the oven temperature was increased from 80 to 180°C with a 10°C/min increasing rate. Helium was used as carrier gas with a flow rate of 0.7 mL/min. Hydrogen and airflow rate were set at 30 and 400 mL/min, respectively.

Liquid samples from anaerobic digester were diluted and filtered through 0.2 µm filter. 10 mL of the filtered sample were analyzed using ion chromatography (Metrohm, 883 Basic IC Plus, Switzerland) [29].

2.8. Determination of methane productivity.

Methane productivity can be measured in terms of volatile solids (VS) destroyed, VS loaded, or volume (Møller et al., 2004) [33]. Thus, theoretical methane yield (B_u) and ultimate methane yield (B_o) were defined in terms of either VS destroyed (L CH_4/kg VS_{des}) or VS loaded (L CH_4/kg VS_{load}) based on either the actually bio-degraded or total load VS contents of the substrate mixture, respectively, by the SSAD process.

2.9. Statistical analysis

The solid-state anaerobic digestion and black soldier fly larvae composting experiments were conducted in triplicates. One-way ANOVA analysis was performed using Origin 9.1 software to compare the result using Tukey's test with a significance level of 0.05.

3. Results and discussion

3.1. Preliminary study of SSAD by fresh cattle manure

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The SSAD time course experiment was carried out for 37 days. The preliminary results showed that two peaks of biogas yield occurred during Days 1 to 7 and Days 30 to 34 (Fig. 3A). Nitrogen content in the biogas decreased from 63.8 to 19.2%. However, Methane content increased from 8.5 to 52.7% for the 37-d period. The results implied that denitrification was dominant in the anaerobic digester during Days 1 to 7 and methanogenesis became dominant after Day 16 (Fig. 3B). In order to accelerate the SSAD process, various addition ratios of inocula (digestate fiber from previous experiments) were tested as well as the operation conditions. Theoretically, the more digestate fiber you inoculate, the faster SSAD process can be achieved. Thus, the following study aimed to accelerate the SSAD process under optimal IR.

3.2. Characterization of sludge, sludge/dairy cattle manure mixture, and digestate

The initial and final weight of dairy cattle manure and MeA mixture was 5 and 4.1−4.8 kg and the volume of initial and final liquid was 2.5 and 2.7−4.0 L, respectively for SSAD experiment (Table 1). Table 1 showed that the weight of the solid substrate mixture decreased, but the volume of the leachate mixture increased in the 14-d experimental period. Tables 2 and 3 present characteristics of TS and VS contents of the MeA mixture, dairy cattle manure and MeA (CMeA) mixture, and digestate, respectively. After SSAD process, the TS and VS contents of the solid digestate and liquid digestate ranged from 0.24 to 18.1% and 32.4 to 96.2%, respectively (Tables 2 and 3). There was

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not significantly different between solid and liquid parts of either TS or VS before/after SSAD process among all IR sets.

3.3. Effect of pH adjustment and inoculation ratio (IR) on TS and VS removal

The initial pH values were 5.6, 6.1, and 5.9 for the groups of IR_{10}, IR_{30}, and IR_{50}, respectively. After SSAD process, the final pH values were 5.3±0.1, 5.6±0.1, and 5.4±0.1 for the groups of IR_{10}, IR_{30}, and IR_{50} without pH adjustment, respectively.

The groups with pH adjustment had a higher TS removal efficiency (14.3±1.1−18.4±2.8%) compared to the groups without pH adjustment (6.1±0.5−9.8±2.1%). There was not significant difference in TS removal among the three groups with pH adjustment ($p > 0.05$) (Fig. 4A). However, there was significant difference in TS removal of $IR_{50}$with pH adjustment and the three groups without pH adjustment ($p < 0.05$) (Fig. 4A). Similarly, the groups with pH adjustment had a higher VS removal efficiency (14.9±0.6−20.2±2.4%) compared to the groups without pH adjustment (5.9±0.9−11.3±1.9%). The VS removal efficiency of the IR_{50} with pH adjustment (20.2±2.4%) was significantly higher than the IR_{30} and IR_{10} groups ($p<0.05$) (Fig. 4B). In addition, VS removal of IR_{50} with pH adjustment was significantly different from the three groups without pH adjustment ($p < 0.05$) (Fig. 4B). The VS removal efficiency during anaerobic digestion is usually due to conversion of VS content in the substrate into VFAs through hydrolysis, and the VFAs are converted into methane gas through

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methanogenesis [30]. Experimental results showed that the cumulative biogas yield of groups with pH adjustment were higher than the groups without pH adjustment, because of the groups with pH adjustment reduced more VS content to generate biogas (Figs. 4B and 5B).

3.4. Effect of pH adjustment and IR on daily and cumulative biogas yield

All groups with pH adjustment reached daily biogas yield peak on Day 2 to 4, except for group IR_{10} without pH adjustment which reached its peak on Day 7 (Fig. 5A). Experimental results showed that daily biogas yield trend significantly differs between the groups with and without pH adjustment. The delay of daily biogas yield peak in the group IR_{10} without pH adjustment was probably due to unfavorable pH value and insufficient inoculation ratio leading to slower start-up of methanogenesis.

Group IR_{50} with pH adjustment had the largest biogas peak value, 21.7±0.2 L/kg VS_{load}/day, after the peak of biogas yield on Day 3, the daily biogas yield decreased gradually to 5.81±0.2 L/kg VS_{load}/day on Day 14 (Fig. 5A). While the peak value of daily biogas yield was 17.3±0.3 and 11.7±0.3 L/kg VS_{load}/day for group IR_{30} and IR_{10} with pH adjustment, respectively. The peak value of daily biogas yield was 6.4±0.3, 6.9±0.2, and 4.5±0.4 L/kg VS_{load}/day for group IR_{50}, IR_{30}, and IR_{10} without pH adjustment, respectively.

Experimental results implied that pH was a key parameter that influenced the peak

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sizes; indeed, the groups with pH adjustment had a larger peak size from 21.7 to 11.7 L/kg VS_{load}/day, where groups without pH adjustment had a small peak size from 6.9 to 4.5 L/kg VS_{load}/day. The cumulative biogas yield of groups with pH adjustment (124.6±5.8−164.6±1.9 L/kg VS_{load}) were all significantly higher than groups without pH adjustment (37.7±1.9−45.5±2.6 L/kg VS_{load}) ($p<0.05$) (Fig. 5B).

Within groups with pH adjustment, daily biogas yield peak size of the group IR_{50} was significantly larger than the groups IR_{30} and IR_{10} ($p<0.05$). The results implied that the higher the inoculation ratio was, the higher daily biogas yield peak achieved in 6d. Although group IR_{50} with pH adjustment had the highest daily biogas yield peak, group IR_{30} with pH adjustment had the same amount of cumulative biogas yield with group IR_{50} with pH adjustment ($p > 0.05$) (Fig. 5). Group IR_{30} with pH adjustment had 3.5 kg of fresh substrate, while group IR_{50} with pH adjustment had only 2.5 kg of fresh substrate. Therefore, group IR_{30} with pH adjustment had a larger quantity of fresh substrate (1 kg) than group IR_{50} with pH adjustment, which enabled group IR_{30} with pH adjustment to maintain daily biogas yield at a higher level compared to group IR_{50}. Thus, this might result in the faster peak of daily biogas yield of the group IR_{50} with pH adjustment and the slower peak of daily biogas yield of the group IR_{30} with pH adjustment.

3.5. Biogas yield for the groups without pH adjustment

The groups without pH adjustment had very low pH value either before (pH =

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5.6−6.1) or after SSAD (pH = 5.4−5.6,). Accumulation of VFAs tends to lower the pH value and might inhibit anaerobic digestion as well as biogas production (Figs. 5 and 6A). It was far lower than optimum pH value (6.8−7.2). When the concentrations of propionic acid exceeded 900 mg/L, anaerobic digestion process cab ne inhibited [31]. After SSAD process, propionic acid concentrations (885±11−2276±25 mg/L) of the groups without pH adjustment were near or higher than the inhibition concentrations stated by the study of Wang et al (2009) (Fig. 6B) [31].

3.6. Effect of IR on VFAs concentration for the groups with pH adjustment

The results showed the groups with lower inoculation ratios accumulated more VFAs than the groups with higher inoculation ratios (Fig. 7A). The highest total VFAs peaks were on Days 2 and 3 for the groups of IR_{10} (1234±23 mg/L), IR_{30} (913±38 mg/L) and IR_{50} (412±38 mg/L), respectively (Fig. 7A). These results indicate that the groups with higher inoculation ratios consumed VFAs at a higher rate. According to study of Ward et al. (2008), rapid VFAs accumulation was an indicator for an overload of organic loading rate [32]. Group IR_{10} showed the highest total VFAs accumulation and lowest cumulative biogas yield compared to groups IR_{30} and IR_{50}. It indicated there was an overloading organic loading rate, which is inhibiting the methanogenesis process.

All acetic acid concentration peaks on Day 2 (172±26−614±42 mg/L) for all IR groups and it dramatically decreased right after the peaks until the end of anaerobic

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digestion (Fig. 7B). Propionic acid concentration peaks for the groups IR_{10}, IR_{30}, and IR_{50} were 747±148, 530±49, and 186±20 mg/L on Day 7, 4, and 2, respectively (Fig. 7C). According to the study of Ward et al. (2008), pH value tends to drop during the early stage of anaerobic digestion due to high hydrolysis activity and low methanogenesis activity [32]. The intermediates of anaerobic digestion such as VFAs might lower the pH, if sufficient alkalinity were unavailable. However, the pH could be recovered when the VFAs were consumed by methanogens. In this study, the pH values of all groups dropped from pH = 7.8 to 6.3 in 2 d while the VFAs accumulated in the anaerobic digester. And the pH value increased gradually from Day 3 to 14, while the VFAs were slowly consumed for biogas yield (Fig. 7D).

The group IR_{10} had the lowest pH value in the early stage due to higher VFAs accumulation and organic loading rates comparing to the other groups with pH adjustment. Except for the group IR_{10}, the groups IR_{30} and IR_{50} had better performance in biogas yield because these groups were able to maintain the pH in the ideal range (pH = 6.8−7.2) for methanogenesis [8].

3.7. Effect of pH adjustment and IR on methane concentration

The groups with pH adjustment reached 60% methane concentrations were on Days 4, 5, and 9 for the group IR_{50}, IR_{30}, and IR_{10}, respectively (Fig. 8A). This result indicated the group $IR_{50}$with pH adjustment had a faster methanogenesis start-up and resulted in

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faster increase of methane concentration. However, in this study, among the groups with pH adjustment, average methane concentrations of the groups IR_{30} and IR_{50}, 57.6±0.6 and 55.3±0.7%, respectively, were not significantly different ($p>0.05$) while the group IR_{10} had the lowest average methane concentration (51.0±0.3%) ($p<0.05$). These results implied that IR_{10} may be not sufficient for batch SSAD to obtain a rapid start up.

Although the groups IR_{30} and IR_{50} with pH adjustment had the highest average methane concentrations, the group IR_{30} with pH adjustment achieved higher theoretical methane yield (626.1±28.7 L CH_4/kg VS_{des}) and ultimate methane yield (96.81±2.0 L CH_4/kg VS_{load}) than the group IR_{50} with pH adjustment ($p<0.05$) (Table 4). These results implied that groups IR_{30} and IR_{50} with pH adjustment had enough amounts of inocula and optimal pH conditions to obtain higher average methane concentration over the 14-day SSAD experimental period. However, group IR_{50} with pH adjustment had a sharp decrease of biogas yield after daily biogas yield peak occurred, due to insufficient fresh substrate to be utilized for biogas yield. This resulted in lower theoretical methane yield (432.9±54.9 L CH_4/kg VS_{des}) and ultimate methane yield (86.8±1.0 L CH_4/kg VS_{load}) than that of group IR_{30} with pH adjustment ($p<0.05$) (Table 4).

The study of Møller et al. (2004) showed that the theoretical methane yield and ultimate methane yield of dairy cattle manure were 468±61 L CH_4/kg VS_{des} and 148±41 L CH_4/kg VS_{load}, respectively [33]. The theoretical methane yield of the group IR_{30} with

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pH adjustment (626.1±28.7 L CH_4/kg VS_{des}) was comparable to Møller's study (468±61 L CH_4/kg VS_{des}) [33]. While the ultimate methane yield of the group IR_{30} with pH adjustment (96.81±2.0 L CH_4/kg VS_{load}) was slightly lower than Møller's study (148±41 L CH_4/kg VS_{load}) [33]. The experimental results implied that the methane productivity of SSAD in this study was comparable to other SSAD studies.

3.8. Effect of pH adjustment and IR on cumulative and daily methane yield

The group IR_{30} with pH adjustment had the highest cumulative methane yield (96.8±2.0 L CH_4/kg VS_{load}) on Day 14 than the groups IR_{10} (69.2±3.7 L CH_4/kg VS_{load}) and IR_{50} (86.8±1.0 L CH_4/kg VS_{load}) with pH adjustment ($p<0.05$). However, the groups without pH adjustment had lower cumulative methane yield (12.1±2.5−16.0±1.7 L CH_4 /kg VS_{load}) on Day 14 compared to groups with pH adjustment regardless inoculation ratios ($p<0.05$) (Fig. 8B).

The daily methane yield was calculated by dividing cumulative methane yield with the days of cumulative average methane yield. The daily methane yield of the groups IR_{30}, and IR_{50} reached the maximum (7.7±0.3 and 8.1±0.1 L/kg VS_{load}/day) on Day 5 and 8 then decreased to 6.9±0.1 and 6.2±0.1 L/kg VS_{load}/day on Day 14, respectively (Fig. 8A). However, the daily methane yield of the group IR_{10} reached the maximum (4.9±0.3 L/kg VS_{load}/day) on Day14 (Fig. 9A). Overall the maximum daily methane yield of the groups IR_{30} and IR_{50} with pH adjustment were insignificantly different ($p>0.05$), but it was

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significantly different from the group IR_{10} either with or without pH adjustment ($p<0.05$) (Fig. 9B). The daily methane yield of the groups without pH adjustment was about 0.02±0.01−1.2±0.1 L/kg VS_{load}/day (Fig. 9A).

Although the maximum daily methane yield did not differ from the groups IR_{30} and IR_{50} with pH adjustment, the group IR_{50} with pH adjustment achieved maximum daily methane yield about 3 days earlier than that of the group IR_{30} with pH adjustment. The results implied that the groups with higher inoculation ratio might have a shorter start-up time than the groups with lower inoculation ratios. The group IR_{10} with and without pH adjustment both reached maximum daily methane yield on Day 14 and the daily methane yield could be higher if the experiments were conducted more than 14 days, indicating there were insufficient inoculation ratio to reach maximum daily methane yield in 14-days experiment (Fig. 9).

3.9. Effect of different feeding rate (FR) on black soldier fly larvae weight change

The group 100 mg/day/larva and the group 75 mg/day/larva showed the highest weight change of 984.1±177.6 and 969.6±28.4%, respectively ($p>0.05$) than the other groups. Results indicated the substrate was able to support larvae growth to 969.6±28.4% in 14 days when the feeding rate reached 75 mg/day/larva (Fig. 10). While group 25 mg/day/larva showed the lowest weight change of 189.8±19.4%, indicating 25 mg/day/larva feeding rate was not sufficient to support BSFL growth. However, BSFL

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weight change may up to 7681% when BSFL was fed digestate of corncob and pig manure according to the study of Li et al. (2015) [13]. The weight change of BSFL in this study was much lower than the study of Li et al. (2015) [13]. This may due to limited nutrients that can be utilized by BSFL from SSAD digestate of dairy cattle manure, which mainly consisted of fibrous content.

3.10. Effect of different FR on removal efficiency of SSAD digestate

The removal efficiency of TS, VS, and nitrogen content among the groups with different feeding rate was not significantly different in this study ($p>0.05$) (Table 5). For all groups with different feeding rates, average removal efficiency of TS (9.1±2.1−13.1±2.5%), VS (9.0±1.9−13.4±2.6%), and nitrogen content (8.4±1.4−11.7±3.1%) was 10.4±2.3, 10.3±2.3, and 9.5±3.2%, respectively (Table 5). However, the weight change of those 400 BSFL for the groups of 25, 50, 75, and 100 mg day/larva was significantly different (189.8±19.4, 349.1±66.4, 969.6±28.4, and 984.1±177.6%, respectively) ($p<0.05$) (Table 5). The removal efficiency of total solids, volatile solids, and nitrogen content did not reduce while the feeding rate increased from 25 to 100 mg/day/larva. These results implied that there would be limited nutrients, crude protein, which might be utilized by BSFL for supporting growth on SSAD digestate originated from dairy cattle manure.

4. Conclusions

Experimental results showed great potential and feasibility for biogas yield, BSFL biomass production, and waste reduction of dairy cattle manure by the two-step bioconversion. For SSAD process, pH adjustment and sufficient amount of inocula as well as retention time was essential for producing biogas efficiently. For BSFL composting, feeding rate for BSFL higher than 75 mg/day/larvae was necessary for larvae development when the BSFL grew on dairy cattle manure digestate. In the future, SSAD and BSFL composting could be one of the options to treat dairy cattle manure in an economic feasible way, thus generating more valuable renewable resources by this bioconversion system.

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References

1. Steinfeld, H.; Gerber, P.; Wassenaar, T.; Castel, V.; Rosales, M.; Haan, C.D. Livestock's long shadow: environmental issues and options. Food and Agriculture Organization of the United Nations (FAO), Rome. **2006**. (http://www.fao.org/3/a-a0701e.pdf)
2. COA. Cattle production report, Council of Agricultures (COA), Taiwan, ROC , **2016** (From, http://agrstat.coa.gov.tw/sdweb/public/inquiry/InquireAdvance.aspx). (in Chinese)
3. Su, J.J., Liu, Y. L., Shu, F. J., Wu, J. F. Treatment of piggery wastewater treatment by contact aeration treatment in coordination with the anaerobic fermentation of three-step piggery wastewater treatment (TPWT) process in Taiwan. *J. Environ. Sci. Heal.* **1997**, 32A(1), 55−73.
4. Su, J.J.; Liu, B.Y.; Chang, Y.C. Emission of Greenhouse Gas from Livestock Waste and Wastewater Treatment in Taiwan. *Agr. Ecosyst. Environ*. **2003**, 95, 253−263.
5. Su, J.J.; Chang, Y.C.; Chen, Y.J.; Chang, C.K.; Lee, S.Y. Hydrogen sulfide removal from livestock biogas by a farm-scale bio-filter desulfurization system. *Water Sci. Technol.* **2013**, 67 (6), 1288−1293.
6. Su, J.J.; Chen, Y.J.; Chang, Y.C. A study of a pilot-scale biogas bio-filter system for

utilization on pig farms. *J. Agr. Sci.* **2014**, 152(2), 217−224.

7. Su, J.J.; Chen, Y.J. Monitoring of sulfur dioxide emission resulting from biogas utilization on commercial pig farms in Taiwan. *Environ. Monit. Assess.* **2015**, 187(1), 4109 (total 8 pages).

8. Hilkiah Igoni, A.; Ayotamuno, M.J.; Eze, C.L.; Ogaji, S.O.T.; Probert, S.D. Designs of anaerobic digesters for producing biogas from municipal solid-waste. *Appl. Energ.* **2008**, 85, 430−438.

9. Kothari, R.; Pandey, A.K.; Kumar, S.; Tyagi, V.V.; Tyagi, S.K. Different aspects of dry anaerobic digestion for bio-energy: An overview. Renew. *Sust. Energ. Rev.* **2014**, 39, 174−195.

10. de Laclos, H. F.; Desbois, S.; Saint-Joly, C. Anaerobic digestion of municipal solid organic waste: Valorga full-scale plant in Tilburg, The Netherlands. *Water Sci. Technol.* **1997**, 36, 457−462.

11. Ge, X.M.; Xu, F.Q.; Li, Y.B. Solid-state anaerobic digestion of lignocellulosic biomass: Recent progress and perspectives. *Bioresour. Technol.* **2016**, 205: 239−249.

12. Baere, L.D.; Mattheeuws, B. Anaerobic digestion of MSW in Europe. *BioCycle* **2010**, 51, 24−26.

13. Li, W.; Li, Q.; Zheng, L.; Wang, Y.; Zhang, J.; Yu, Z.; Zhang, Y. Potential biodiesel

 doi:10.20944/preprints201812.0354.v1

and biogas production from corncob by anaerobic fermentation and black soldier fly. *Bioresour. Technol*. **2015**, 194, 276−282.

14. Pezzolla, D.; Maria, F.D.; Zadra, C.; Massaccesi, L.; Sordi, A.; Gigliotti, G. Optimization of solid-state anaerobic digestion through the percolate recirculation. *Biomass Bioenerg*. **2017**, 96, 112−118.
15. Xu, F.Q.; Wang, Z.W.; Tang, L.; Li, Y.B. A mass diffusion-based interpretation of the effect of total solids content on solid-state anaerobic digestion of cellulosic biomass. *Bioresour. Technol.* **2014**, 167, 178−185.
16. Degueurce, A.; Tomas, N.; Le Roux, S.; Martinez, J.; Peu, P. Biotic and abiotic roles of leachate recirculation in batch mode solid-state anaerobic digestion of cattle manure. *Bioresour. Technol.* **2016**, 200, 388−395.
17. Nakamura, S.; Ichiki, R.T.; Shimoda, M.; Morioka, S. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. *Appl. Entomol. Zool.* **2016**, 51, 161−166.
18. Sheppard, D.C.; Tomberlin, J.K.; Joyce, J.A.; Kiser, B.C.; Sumner, S.M. Rearing methods for the black soldier fly (*Diptera: Stratiomyidae*). *J. Med. Entomol.* **2002**, 39, 695−698.
19. Martínez-Sánchez, A.; Magaña, C.; Saloña, M.; Rojo, S. First record of *Hermetia illucens* (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. *Forensic*

Sci. Int. **2011**, 206, e76−e78.

20. Bondari, K.; Sheppard, D.C. Soldier fly, *Hermetia illucens L.*, larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquac. Res.* **1987**, 18, 209−220.

21. Newton, G.L.; Booram, C.V.; Barker, R.W.; Hale, O.M. Dried *Hermetia Illucens* larvae meal as a supplement for swine. *J. Anim. Sci.* **1977**, 44, 395−400.

22. Myers, H.M.; Tomberlin, J.K.; Lambert, B.D.; Kattes, D. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environ. Entomol.* **2008**, 37, 11−15.

23. Diener, S.; Studt Solano, N.M.; Roa Gutiérrez, F.; Zurbrügg, C.; Tockner, K. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valori.* **2011**, 2, 357−363.

24. Sheppard, D.C.; Newton, G.L.; Thompson, S.A.; Savage, S.A. value added manure management system using the black soldier fly. *Bioresour. Technol.* **1994**, 50, 275−279.

25. Erickson, M.C.; Islam, M.; Sheppard, C.; Liao, J.; Doyle, M.P. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Enteritidis in Chicken manure by larvae of the black soldier fly. *J. Food Protect.* **2004**, 67, 685−690.

26. Lalander, C.; Diener, S.; Magri, M.E.; Zurbrügg, C.; Lindström, A.; Vinnerås, B.

Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) − From a hygiene aspect. *Sci. Total Environ*. **2013**, 458–460: 312−318.

27. Liu, Q.; Tomberlin, J.K.; Brady, J.A.; Sanford, M.R.; Yu, Z. Black soldier fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. *Environ. Entomol.* **2008**, 37, 1525-1530.
28. APHA. *American Public Health Association (APHA)* **1995**, Washington, DC, USA.
29. Su, J.J.; Huang, J.F.; Wang, Y.L.; Hong, Y.Y. Treatment of duck house wastewater by a pilot-scale sequencing batch reactor system for sustainable duck production. *Poultry Sci*. **2018**, 97, 3870–3877.
30. Song, Y.C.; Kwon, S.J.; Woo, J.H. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. *Water Res.* **2004**, 38, 1653−1662.
31. Wang, Y.; Zhang, Y.; Wang, J.; Meng, L. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass Bioenerg.* **2009**, 33, 848−853.
32. Ward, A.J.; Hobbs, P.J.; Holliman, P.J.; Jones, D.L. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.* **2008**, 99: 7928−7940.
33. Møller, H.B.; Sommer, S.G.; Ahring, B.K. Methane productivity of manure, straw

 doi:10.20944/preprints201812.0354.v1

and solid fractions of manure. *Biomass Bioenerg.* **2004**, 26, 485−495.