RESEARCH ARTICLE | AUGUST 18 2023

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AIP Conference Proceedings 2669, 030005 (2023)
https://doi.org/10.1063/5.0123980

Anaerobic Digestion of Sewage Sludge for Enhancing the Growth of Black Soldier Fly Larvae

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Abstract. The growth of black soldier fly larvae (BSFL) has been widely studied by feeding with myriad organic waste materials, transforming the wastes into a high-value added larval biomass via digestion and assimilation processes. One of the abundant organic wastes is a sewage sludge, comprising of complex organic substrates. Nevertheless, the presence of extracellular polymeric substances in the sewage sludge has been found retarding the digestion process of BSFL. As a result, the larvae are unable to assimilate the nutrients for their subsequent growth. In this regard, the anaerobic digestion of sewage sludge was adopted in this work to cleave the structures of sewage sludge in releasing more nutrients for BSFL feeding, whilst enhancing the larval growth. The different pH conditions amidst anaerobic treatment had plausibly changed the quantities of volatile fatty acids generated in which influencing the BSFL growth. The results showed that the larvae fed with the anaerobic treated sewage sludge had a better growth than those fed with fresh sewage sludge in terms of final total weight. The larval weights were measured at 2.05 ± 0.39 against 7.34 ± 0.97 mg/larva at dry weight basis while administering with fresh and anaerobically treated sewage sludge at pH 3, respectively, with an increment of almost 350%. Indeed, the highest larval weight was successfully achieved by using the sewage sludge having been pre-digested anaerobically at pH 3 for 8 days.

2nd International Conference on Engineering and Industrial Technology (ICEIT 2021)
in Conjunction with the Silpakorn International Conference on Total Art and Science (SICTAS) 2021
AIP Conf. Proc. 2669, 030005-1–030005-5; https://doi.org/10.1063/5.0123980
Published by AIP Publishing. 978-0-7354-4527-7/$30.00

INTRODUCTION

Sewage sludge has been generated as a by-product from municipal wastewater treatment plant every day and is projected to continuously increase due to the growing world's population [1, 2]. Currently, a massive volume of sewage sludge is managed by the various common techniques such as utilization for agricultural purpose, disposal into landfill site and incineration. However, the challenge of these traditional methods is the impacts on human health and environmental concern [1, 3]. The employment of insect larvae has become an interesting method for managing sewage sludge since the larvae have been found can valorize various organic wastes, including animal manure, food waste, and fruit and vegetable waste [4, 5, 6]. The generated larval biomass is also serving as valuable feedstock for protein and lipid that can be extracted from its biomass. The larval biomass is derived from the biotransformation process via its digestion system, i.e., consuming organic waste materials and converting into biomass [7]. Among the insects that have been studied for organic waste management, the black soldier fly larvae (BSFL) are the focal species in this work due to its strong capability to grow on a broad range of environmental conditions [8]. Moreover, the BSFL are not a pest and do not harm the human and other animals in its ecosystem [9]. Sewage sludge is considered as a carbon source since the dominant composition in sewage sludge is organic substrates, especially extracellular polymeric substance (EPS) [10, 11]. The EPS at the outer cell wall created is exuded by bacteria, comprising of various nutrition that can be used for larval growth, e.g., polysaccharides, proteins, lipids, and nucleic acids [12, 13]. Black soldier fly larvae (BSFL) had been proven that their biochemical compounds after consuming sewage sludge as a feed were free from heavy metals contamination [14]. However, BSFL fed with sewage sludge could be retard by the EPS as the slimy coating that is created will prevent the digestion by other organisms around the bacterial cells, resulting in the obstacle for larval digestion to go through EPS layer for interaction with the nutrition inside EPS cell [12, 15, 16]. The conversion of sewage sludge by BSFL in various works has been shown in Table 1. It can be noticed that BSFL could survive when feeding with sewage sludge. However, the larval growth performances were unsatisfactory and requiring improvement. Thus, the improvement of sewage sludge nutrition to enhance the larval growth was studied in this work. Anaerobic digestion of sewage sludge will be selected in this study since it is proven as a potential, economical and environmental-friendly method and widely used for organic waste treatment, including sewage sludge [1, 17]. As anaerobic treatment is operated under the absence of oxygen condition, sewage sludge will be biologically degraded [1]. In addition, the pre-treatment anaerobic of sewage sludge can be processed to accelerate the hydrolysis stage by many methods such as mechanical, thermal, chemical and biological process [1]. These pre-treatment techniques will break down the cell wall of EPS structure and release more organic matters which are expected to promote larval growth [18]. Volatile fatty acids (VFAs) which could promote larval growth would be produced from complex organic substrate through anaerobic process [19, 20]. In addition, various pH conditions would significantly affect the production of VFAs [19]. Thus, the suitable pH condition of anaerobic pre-treatment of sewage sludge prior administrating to BSFL would be investigated in this study, targeting to enhance the BSFL growth upon assimilation.

TABLE 1. Comparison of sewage sludge and modified sewage sludge utilizations as the BSFL feeds.

<table>
<tr><th rowspan="2">Larval Feed</th><th colspan="2">Feeding amount</th><th colspan="3">Larval growth performance</th><th rowspan="2">Reference</th></tr>
<tr><th>Continuous [mg/larva/day]</th><th>Batch [mg/larva]</th><th>Larval weight increment [mg/larva]</th><th>Larval growth rate [mg/larva/day]</th><th>Larval final weight [mg/larva]</th></tr>
<tr><td rowspan="3">Fresh sewage sludge</td><td>40</td><td>-</td><td>-</td><td>-</td><td>70</td><td>[5]</td></tr>
<tr><td rowspan="4">-</td><td>125</td><td>-</td><td>-0.20</td><td>-</td><td>[12]</td></tr>
<tr><td rowspan="3">1250</td><td>-1.25</td><td rowspan="3">-</td><td rowspan="3">-</td><td rowspan="3">[14]</td></tr>
<tr><td>Sewage Sludge + Chicken Manure</td><td>1.25-2</td></tr>
<tr><td>Sewage Sludge + Wheat Bran</td><td>12.50</td></tr>
</table>

MATERIALS AND METHOD

Preparation of Anaerobically Digested Feeding Substrates

Sewage sludge was collected from municipal wastewater treatment plant in Malaysia and stored in freezer. Anaerobic pre-treatment of sewage sludge was prepared prior feeding to BSFL. Initially, the pH of fresh sewage sludge was measured at around 7 and this sludge was as well containing approximately 80% of moisture. Then hydrochloric acid (1 M) or sodium hydroxide (1 M) were used to adjust the pH of sewage sludge at various values; pH 3, 5, 7, 9, and 11. Each set up of sewage sludge was ensured to be conducted under the absence of oxygen condition by sealing with parafilm. Thereafter, pre-treated sewage sludge was left at room temperature for 8 days to undergo anaerobic digestion process.

BSFL Rearing

BSFL eggs were purchased from MLF Ingredient Sdn Bhd, Malaysia. The eggs were placed inside the container surrounding with waste coconut endosperm to serve as a larval food source after hatching from the eggs and closed the container with the lid equipped with the net to ensure the air ventilization within the container. After larvae emerged from the eggs, the 20 larvae with negligible weight were hand-picked and put in a plastic cup with diameter of 8 cm and height of 10 cm equipped with a cap to prevent larvae crawling outside the cup. Larvae would be then fed with 10 g (dry weight basis) at various pH of anaerobic digested sewage sludge. Each pH condition would be conducted in triplicate for more precise results. Larvae would be left in incubator to maintain the stability of temperature at 30°C and the moisture of larval feed was maintained at 80%. Once larvae were reared for 1 month, it would be separated from feed residue and deactivated in freezer at -20°C for 10 minutes before introducing in the oven at 65 °C until attaining a constant weight. Larval final weight will be then determined to study the impact on larval growth upon feeding with different pH pre-treated sewage sludge in term of larval final weight on dry weight basis. Whist, the leftover sewage sludge after separating from deactivated larvae would be introduced into the oven at 105°C until constant weight before recording as feed residue on dry weight basis as well to investigate the waste management by BSFL.

RESULTS AND DISCUSSION

As shown in Fig. 1, the larval growth once feeding with anaerobically pre-treated sewage sludge had resulted in a higher larval final weight than larvae fed with fresh sewage sludge (control sample). This could be ascribed by the presence of extracellular polymeric substance (EPS) which was a group of macromolecular compound formed by bacteria in sewage sludge [12, 15]. Even though the predominant constituents in EPS were polysaccharides, protein, or lipids which were nutritive for larval growth, the slime layer outside the EPS cells would act as a protective barrier to prevent the digestion by other microorganisms [12, 13, 15]. The efficiency of larval digestion process was therefore affected by this slime layer in order to penetrate through and assimilate the nutrients inside the EPS [21]. Accordingly, the larvae could not digest the nutrients effectively, leading to a small larval size which was measured merely at 2.05 ± 0.38 mg/larva of larval final weight on dry weight basis. Once the larvae were fed with anaerobically pre-treated sewage sludge, the larval growth varied differently with pH conditions employed during the anaerobic digestion. For all cases, the larval growths were still higher than the use of fresh sewage sludge. Larvae fed with anaerobically pre-treated sewage sludge at pH 3 had engendered the highest final larval weight, i.e., 7.34 ± 0.97 mg/larva on dry weight basis, followed by the larvae fed with pre-treated sewage sludge at pH 5, 11, 7 and 9. This could be ascribed to the effect of different volatile fatty acids (VFAs) formed from anaerobic digestion of sewage sludge prior to administrating to the BSFL. Pang et al. (2020) [19] had reported that the pH condition was playing a significant effect on the amounts of VFAs generated. The high VFAs content may bring bio-toxicity to larval feed and obstruct larval digestion process thereafter [19]. The results corresponding with Sun et al. (2012) [11] who revealed that VFAs content was obtained at the highest with anaerobic sewage sludge at pH 9, followed with pH 7, 5, 11 and 3. Thus, the lowest amount of VFAs from anaerobic digested sewage sludge which was observed from Sun et al. (2012) [11] could be describing the highest larval weight that were fed with anaerobic sewage sludge at pH3, while larvae fed with pH 9 could have the highest VFAs in larval feed, leading to the inhibition towards larval growth by the potential bio-toxicity.

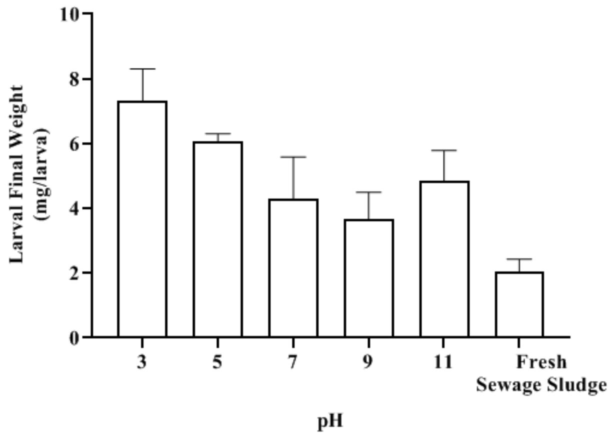

FIGURE 1. Comparison of larval weights upon feeding with different pH of anaerobically digested sewage sludge.

In term of organic waste management by BSFL, the results had shown that no significant effect of BSFL fed with pre-treated sewage sludge at different pH as well as fresh sewage sludge were observed, i.e., utilization of BSFL to dispose sewage sludge had no affect by any anaerobic pre-treatment. The feed residue left after harvesting larvae was around 9.5 g on dry weight basis. This circumstance, i.e., different larval final weight but the same amount of sewage sludge was left after BSFL consumption could be described mass by a balance system between larval biomass, metabolism of larvae and feed residue as presented in Fig. 2 [4]. As larval feed was considered as an inlet stream in this mass balance model while outlet stream could be divided into three parts; mass loss for metabolism process, feed residue, and larval biomass, the relationship between mass loss through metabolism was indirect proportion with larval biomass when fed BSFL with the same amount of total feed (10 g dry weight basis) and feed residue was left at the amount (9.5 g on dry weight basis) as well [4]. Hence, an increasing of larval final weight was influenced by the lower mass loss during metabolism.

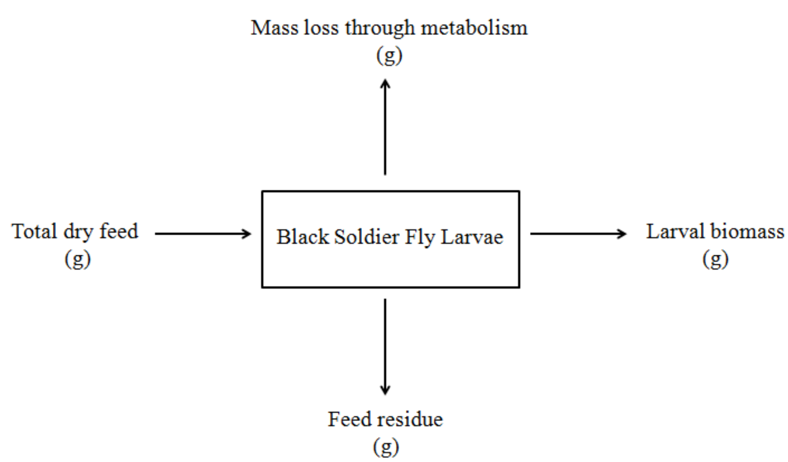

FIGURE 2. Mass balance model of BSFL fed with sewage sludge and conversion into biomass [4].

CONCLUSION

Black soldier fly larvae could grow by assimilating sewage sludge as larval feed. However, larvae fed with fresh sewage sludge had resulted in unsatisfied growth due to the presence of extracellular polymeric substance which could inhibit the larval assimilation of nutrients. Hence, anaerobic pre-treatment of sewage sludge was introduced in this study at various pH to improve the larval growth. The results showed that larval growth in terms of larval final weight could be fortified once sewage sludge was treated with anaerobic digestion prior to feeding to larvae. Larval fed with anaerobically digested sewage sludge at pH 3 had achieved the highest larval final weight at 7.34 ± 0.97

mg/larva on the dry weight basis in comparison with larval weight fed with fresh sewage sludge which could merely attain 2.05 ± 0.38 mg/larva on dry weight basis.

ACKNOWLEDGEMENTS

The financial supports being received from Universitas Muhammadiyah Surakarta, Indonesia via External Grant with the cost centre of 015ME0-246 and Yayasan Universiti Teknologi PETRONAS (YUTP) with the cost centre of 015LC0-341 are gratefully acknowledged.

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