Utilization of *Hermetia illucens* Larvae as A Bioconversion Agent to Reduce Organic Waste

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Abstract. The larvae of *Hermetia illucens*, known as Black Soldier Fly (BSF), are utilized to reduce various types of organic waste by means of bioconversion. The purpose of this study was to evaluate the efficiency of the larvae in reducing and converting mixtures of fresh food waste (FW) with other organic waste types, i.e., immature windrow composting material (IW), residue windrow composting material (RW), and fresh cow manure (CM) at three different composition ratios. The growth rate and protein content of the larvae were also determined in this study. A specific amount of organic waste mixtures was fed to the BSF larvae. The results suggest that the type of organic waste given to larvae affected the overall waste reduction and larval growth at a certain extent. The composition ratio of FW with the other wastes used was 90:10, 70:30, and 60:40 (w/w). The highest reduction percentage was at the ratio of 90:10 achieved for FW mixed with IW, FW mixed with RW, and FW mixed with CM, i.e. 82.2%, 79%, and 74.5% respectively. The protein content of larvae in those waste mixtures was 54.6%, 63.1%, and 48.75% respectively. The final residue from the decomposition of the waste by BSF larvae was also observed in this study.

Keywords: *Hermetia illucens*, organic waste, waste reduction

1. Introduction

Organic waste is the dominant component of municipal solid waste typically generated in many cities in Indonesia, including Surabaya, reaching up to 71.96% in this current decade [1]. This waste characteristic offers the opportunity of BSF larvae application in reducing the organic waste constituents as high as 50% or even higher in a shorter time compared to the conventional composting methods. Previous studies have examined that the bioconversion process by BSF larvae was able to reduce organic waste by 66.4-78.9% in food waste mixture [2], as an example, and up to 56% in manure waste [3]. The reduction in the mixture of food waste and human faeces was 68% (85% on wet basis) [4]. The waste loads entering the landfill can be eventually decreased when the method is sustainably implemented [5]. Furthermore, the residues from the decomposition of waste by BSF larvae can be used as fertilizer and soil amendment, while the BSF larvae can also be further processed for animal feed [6]. BSF larvae convert organic waste into nutrient content as biomass, with protein content of the larvae around 30-48%, depending on the type of waste fed [7]-[8].

2. Material and Methods

The study was conducted in a laboratory scale with duplicate experiments, except the control experiment (Table 1). The BSF larvae used were six days old. The process of decomposition of
organic waste by BSF larvae were carried out for 12 days, which, according to the larval phase, would be before the pre-pupae formation. 300 larvae were utilized in each reactor of this experiment. Weight analysis was carried out on the larval growth and the decomposed waste in the reactor. Three waste types and composition ratios were evaluated as seen in Table 1.

Table 1. Experiment Attributes including Waste Type and Composition Ratio

| Waste Composition Ratio | Waste Type                          | Frequency of Feedings | Repetition | Feeding rate in dry matter |
|-------------------------|-------------------------------------|-----------------------|------------|---------------------------|
| 100% Control            | IW (Immature Windrow Composting Material) | Day 1, 4, and 7       | 1          |                           |
| 100% Control            | RW (Residue Windrow Composting Material) | Day 1, 4, and 7       | 1          |                           |
| CM (Cow Manure)         | Day 1, 4, and 7                      | 1                     |            |                           |
| FW (Food Waste)         | Day 1, 4, and 7                      | 1                     |            |                           |
| 90:10                   | FW:IW                               | Day 1, 4, and 7       | 2          | 20 mg/larva.day           |
|                         | FW:RW                               | Day 1, 4, and 7       | 2          |                           |
|                         | FW:CM                               | Day 1, 4, and 7       | 2          |                           |
| 70:30                   | FW:IW                               | Day 1, 4, and 7       | 2          |                           |
|                         | FW:RW                               | Day 1, 4, and 7       | 2          |                           |
|                         | FW:CM                               | Day 1, 4, and 7       | 2          |                           |
| 60:40                   | FW:IW                               | Day 1, 4, and 7       | 2          |                           |
|                         | FW:RW                               | Day 1, 4, and 7       | 2          |                           |
|                         | FW:CM                               | Day 1, 4, and 7       | 2          |                           |

The parameters measured in this study were water content, C-organic, N-organic, C/N, pH, weight gain of larvae, percentage of waste reduction, and crude protein. More details on the measurement methods are elaborated in Table 2.

Table 2. Measurement Methods

| No | Parameters                  | Methods                              | Frequency of Measurement |
|----|-----------------------------|--------------------------------------|--------------------------|
| 1  | Percentage of waste reduction | Waste Reduction Index (WRI)          | Day 1 and 12             |
| 2  | Weight gain of larvae       | Weight calculation                   | Day 1, 4, 7, and 12      |
| 3  | Dry weight of larvae        | Dry weight calculation               | Day 1, 4, 7, and 12      |
| 4  | Larvae protein content      | Total nitrogen                       | Day 1 and 12             |
| 5  | Water content               | AOAC (Gravimetry)                   | Day 1, 4, 7, and 12      |
| 6  | pH                          | AOAC (pH meter)                      | Day 1, 4, 7, and 12      |
| 7  | Temperature                 | Thermometrics                        | Every day                |
| 8  | C-organic                   | AOAC (Gravimetry)                   | Day 1 and 12             |
| 9  | N-organic                   | Semi micro Kjeldahl                  | Day 1 and 12             |
| 10 | C/N                         | Ratio                                | Day 1 and 12             |
3. Results and Discussion

3.1 pH Conditions

The pH measurement was carried out before each feeding on the sample in the reactor. pH measurement was done to ensure that the pH condition of each composition was suitable for BSF larvae. Besides, pH analysis was also carried out to see the effect of BSF larvae on pH changes in waste that might occur. The pH measurement data can be seen in Figure 1.

Based on pH measurement data, each reactor had the same tendency, which was low at the beginning. On the seventh day until the last day the pH continued to increase. A decrease in pH indicated the process of organic matter degradation into organic acids [9]. It was the formation of organic acids during the composting process that caused the pH to decrease. The decomposition process also incorporated the formation of ammonia from the nitrogen content of organic matter. The formation of ammonia will increase the pH of the decomposing waste. According to a reference [10], an increase in pH in the decomposition process might be due to the change of organic acids into CO₂ and the presence of base cations resulting from mineralization of organic matter. The pH condition during the study did not affect the life phase of BSF larvae. On the final day, pH values in this decomposition ranged between 4.2-9.2. The decrease rate of larvae in this study was quite low (below 10%), there were even no deaths in several reactors. Young and mature BSF larvae have a high tolerance to extreme pH values, i.e. pH 0.7-13.7 [11].

3.2 C/N Ratio After Decomposition

C/N ratio measurements were made at the initial and the final stage of the study, for comparison. The final C/N ratio results can be seen in Figure 2.
Figure 2. Initial and Final C/N Ratio

Figure 2 shows that the C/N ratio of each waste composition was decreased. During the process of decomposition, organic carbon content in waste would be reduced because it would be decomposed into CO₂, H₂O, and heat, while organic nitrogen would be relatively fixed. This was what caused the C/N value during the decomposition process to go down. The standard C/N ratio for compost was 10-20 \[12\]. The results of waste decomposition by BSF larvae in the various arrangements in this study did not all meet compost standards. Based on the data (Figure 2), the type of mixed waste FW:CM composition results from the decomposition of BSF larvae residues still did not meet compost standards. The C/N value in the mixture of food waste and cow manure was 22.8; 27.3; and 24.7. The FW:IW 90:10 reactor also had C/N above the compost standard of 22.8. The high C/N values in the FW:CM and FW:IW 90:10 reactors indicated that the residue from the decomposition of waste still did not reach optimal maturity. Organic Carbon content that was too high made the decomposition process slow \[13\]. The moderately short process (12 days) of decomposition by BSF larvae was one of the causes that the waste decomposition results were still not fully mature. Therefore, there is a need for further processing of the final residue to be utilized, one of which is to use the final residue of BSF larvae as a starter for the decomposition process of new BSF larvae. The utilization of residues from the decomposition of BSF larvae can also be varied with other composting methods. Statistical test results showed that the addition of BSF larvae did not play a significant role in reducing C/N (P-value 0.276 or P> 0.05).

3.3 Reduction Percentage
Based on the final residual that was left in the reactor, the reduction percentage can be found. Data on the percentage of waste reduction for each reactor treatment can be seen in Table 3.
Table 3. Reduction Percentage

| No | Waste Type Variation | Reduction Percentage (%) |
|----|----------------------|--------------------------|
|    | Control Reactor      |                          |
| 1  | CM (Cow Manure)      | 17.072                   |
| 2  | FW (Food Waste)      | 66.945                   |
| 3  | RW (Residue Windrow Composting Material) | 23.947 |
| 4  | IW (Immature Windrow Composting Material) | 39.665 |
|    | Mixture Waste Reactor |                          |
| 1  | FW:CM (90:10)        | 74.52 ±7.03              |
| 2  | FW:CM (70:30)        | 66.79 ±1.51              |
| 3  | FW:CM (60:40)        | 60.12 ±3.95              |
| 4  | FW:RW (90:10)        | 79.00 ±3.03              |
| 5  | FW:RW (70:30)        | 77.78 ±0.28              |
| 6  | FW:RW (60:40)        | 62.18 ±3.38              |
| 7  | FW:IW (90:10)        | 82.18 ±3.55              |
| 8  | FW:IW (70:30)        | 71.95 ±2.83              |
| 9  | FW:IW (60:40)        | 70.90 ±2.74              |

The highest reduction value in the control reactor was the BSF reactor of food waste at 66.95%, and the smallest reduction percentage was control residue windrow at 23.94%. The presence or absence of BSF larvae addition significantly influenced the value of waste reduction (P <0.05). The highest reduction for mixed waste types occurred at a ratio of 90:10 for all types of mixed waste. The highest reduction in a mixture of food waste with immature windrow composting material, residue windrow composting material, and cow manure was 82.18%, 79% and 74.52% respectively. In each type of composition, the greater the mixture of food waste, the greater reduction percentage was.

Based on the results of waste reduction by BSF larvae, the mass balance could be obtained. The calculation was done using dry weight. This mass balance measurement was divided into the percentage of final residue, larval weight percentage and metabolism percentage by the BSF larvae. The graph of mass formed can be seen in Figure 3.
Figure 3. Mass balance

The lowest residual percentage with the largest percentage of larval weight gain was in the composition of FW:IW 90:10. Reactor FW:IW 90:10 produced ± 18% of the total food supplied, ± 56% was converted into metabolic material, and ± 26% became larval weight.

This research shows that the addition of windrow composting material has a significant role in reducing organic waste and increasing larval body weight. Immature windrow composting material was the waste that underwent a fermentation process. Reference [14] explained that during the fermentation process there was a decrease in lignin by 51%, and a decrease in the hemicellulose content by 11%. The degradation process improved compost properties through the pre-digestion of polysaccharides into carbohydrates that were assimilated and associated to amino acids. The fermentation process on the waste sample types can increase the digestibility and appetite of BSF larvae [15]. The existence of the fermentation process is a way of processing through the decomposition of compounds from complex protein ingredients into simpler compounds so that it is easier to digest by BSF larvae. Windrow composting material also functions as a bulking agent. The addition of bulking agent to the process of decomposition of organic waste by BSF larvae is effective and practical for preventing odors, adjusting humidity, equilibrating nitrification and increasing porosity for oxygen [16]. Statistical test results show that variations in food types and ratio of waste composition have a significant influence on the percentage of waste reduction. P value for the waste type was 0.018 and P value for waste composition ratio was 0.011 (P value <0.05), indicating that the type of waste and the ratio of waste have a significant influence on the percentage of waste reduction by BSF larvae. Proper waste composition and comparison will provide a higher reduction. It can also show that the type of waste given to BSF larvae determines the larva's appetite to reduce organic waste, as well as the ratio of waste given.

3.4 Physico-chemical Characteristics of Decomposition Results

The final result of the waste reduction process by BSF larvae consisted of harvested BSF larvae and the decomposition residue in the form of organic material. The results of the larval decomposition residue were compared with SNI Standard: 19-7030-2004 regarding compost specifications of domestic organic waste in Indonesia. This was done to see the potential utilization of residues from BSF larvae decomposition into compost. Data on the recapitulation of physical-chemical characteristics of the final decomposition of each reactor treatment can be seen in Table 4.
Table 4. Physico-Chemical Characteristics of Decomposition Results

| Waste Type Variation  | pH     | Moisture Content (%) | C (%) | N (%) | Ratio C/N | Color          |
|-----------------------|--------|----------------------|-------|-------|-----------|----------------|
| FW:CM (90:10)         | 4.4    | 50.74                | 22.29 | 0.976 | 22.84     | Greenish brown |
| FW:CM (70:30)         | 5.3    | 68.91                | 24.14 | 0.883 | 27.34     | Greenish brown |
| FW:CM (60:40)         | 5.8    | 64.46                | 24.23 | 0.979 | 24.75     | Greenish brown |
| FW:RW (90:10)         | 5.0    | 55.05                | 18.91 | 0.953 | 19.84     | Black          |
| FW:RW (70:30)         | 7.5    | 59.98                | 16.64 | 1.199 | 13.88     | Black          |
| FW:RW (60:40)         | 9.2    | 53.16                | 14.85 | 0.960 | 15.48     | Black          |
| FW:IW (90:10)         | 4.9    | 34.11                | 24.15 | 0.969 | 24.93     | Blackish brown |
| FW:IW (70:30)         | 6.9    | 38.99                | 18.90 | 0.978 | 19.32     | Black          |
| FW:IW (60:40)         | 8.4    | 56.42                | 16.74 | 0.999 | 16.77     | Black          |
| Standard SNI-19-7030-2004 | 6.8-7.49 | ≤50 | 9.8-32 | > 0.4 | 10-20 | Black |

The results of comparisons with SNI 19-7030-2004 show that not all the residual of BSF larvae decomposition meet compost standards. Residue from the decomposition of waste by BSF larvae was still not fully mature. The C/N ratio of the results of waste decomposition was still quite high due to the short decomposition time of twelve days. Residues from the decomposition of BSF larvae that can potentially be used as compost are in the type of FW:IW and FW:RW ratio of 70:30.

The FW:IW and FW:RW ratio of 60:40 also met the standard C/N ratio for compost, but the residual pH was still too high. The pH value for FW:IW 60:40 was 8.4 and FW:RW 60:40 was 9.2. The increase in pH during the waste decomposition process occurred due to the decomposition of nitrogen. Nitrogen in organic matter decomposes into NH₃, and NH₃ binds with water to form alkaline NH₄OH [17]. pH values that are too alkaline can be overcome by adding sulfur. The addition of sulfur can stimulate bacteria in the soil to convert sulfur to sulfuric acid, thereby reducing pH [18].

3.5 Change in Weight of BSF larvae

BSF larvae weight measurements were performed at each feeding. These measurements were carried out to determine the effect of the type of waste and the ratio of the given waste composition. In this study, BSF larvae weight was measured in both wet and dry weight conditions. Larvae dry weight measurements were performed to determine the water content in the larval body and larvae weight gain. Results of larval weight gain from the first day to the end can be seen in Figure 4 and Table 6.
The rate of BSF larvae weight gain was quite rapid after the 7th day with larval age of 13 days. From the weight gain of the larvae, we acquired the weight gain of BSF larvae per day. The weight gain value of BSF larvae can be seen in Table 5.

Table 5. Weight of BSF larvae

| Waste Type | Initial dry weight (mg) | Final dry weight DW (mg) | Dry weight of larvae (mg/larva.day) | Initial total weight (mg) | Final total weight (mg) | Total weight of larvae (mg/larva.day) |
|------------|--------------------------|--------------------------|-------------------------------------|---------------------------|-------------------------|--------------------------------------|
| CM         | 0.7                      | 8.97                     | 0.75                                | 2.2                       | 42.8                    | 3.69                                 |
| FW         | 0.5                      | 51.06                    | 4.59                                | 1.8                       | 134.8                   | 12.09                                |
| IW         | 0.4                      | 3.31                     | 0.27                                | 1.3                       | 8.6                     | 0.67                                 |
| FW:CM (90:10) | 0.5                  | 58.07                    | 5.23                                | 1.8                       | 192.1                   | 17.30                                |
| FW:CM (70:30) | 0.6                | 59.18                    | 5.33                                | 2.0                       | 178.4                   | 16.04                                |
| FW:CM (60:40) | 0.5                  | 52.50                    | 4.72                                | 1.8                       | 173.0                   | 15.56                                |
| FW:RW (90:10) | 1.1                   | 70.92                    | 6.35                                | 3.6                       | 198.9                   | 17.76                                |
| FW:RW (70:30) | 1.1                   | 53.11                    | 4.73                                | 3.5                       | 173.2                   | 15.43                                |
| FW:RW (60:40) | 1.1                   | 49.68                    | 4.41                                | 3.8                       | 159.1                   | 14.12                                |
| Waste Type          | Initial dry weight (mg) | Final dry weight DW (mg) | Dry weight of larvae (mg/larva.day) | Initial total weight (mg) | Final total weight (mg) | Total weight of larvae (mg/larva.day) |
|---------------------|-------------------------|--------------------------|------------------------------------|---------------------------|------------------------|--------------------------------------|
| FW:IW (90:10)       | 0.6                     | 65.92                    | 5.94                               | 1.9                       | 291.2                  | 26.30                                |
| FW:IW (70:30)       | 0.6                     | 64.69                    | 5.83                               | 1.9                       | 194.5                  | 17.51                                |
| FW:IW (60:40)       | 0.6                     | 58.30                    | 5.25                               | 1.9                       | 190.7                  | 17.17                                |

Based on the larvae weight calculation data, it can be inferred that the greater the reduction of waste, the greater the wet weight of the converted larvae would be. The biggest waste reduction was at the FW:IW 90:10 reactor at 82.18% with the mass of larvae formed at the end being 291.2 mg/larvae. The protein and carbohydrate content influenced larval growth. The carbohydrate value of kitchen waste is 56.79 g and protein 20.41 g, while that of livestock manure carbohydrate is 47.61 and protein is 22.66. The greater the composition of the food waste, the greater the larval weight gain was.

The dry weight of larvae increased in the FW:IW ratio of 60:40 had a value not much different from the food waste control, so there was no need for additional windrow waste mixture. However, seeing the final residual results, not adding materials with high N content would result in the final C/N ratio from waste decomposition being still too high. Also, the presence of a mixture of composition and waste ratio influences larval growth. The weight of BSF larvae in mixed waste reactors was much greater than pure waste without mixing.

Statistical test results show that variations in food types affected the growth of larvae, but the ratio of waste composition did not affect the growth of BSF larvae. The P value for food types was 0.008 and the P value for the ratio of waste composition was 0.122. The type of waste given had a significant influence on larval weight gain. This can be related to the nutritional content of feed ingredients influencing the weight gain of BSF larvae. In contrast, waste ratio did not significantly influence the weight gain of BSF larvae.

### 3.6 Protein Content of BSF Larvae

At the end of the study, larvae protein content was measured. Protein measurements were carried out for the type of reactor that has the largest percentage of waste reduction in each type of waste composition. Data from protein measurements can be seen in Table 6.

| Waste Type          | Protein (%) |
|---------------------|-------------|
| FW:CM (90:10)       | 48.747      |
| FW:RW (90:10)       | 63.107      |
| FW:IW (90:10)       | 54.592      |

The highest protein content was in the type of FW:IW (90:10), i.e., 54.59%. The high protein content of these larvae could have been caused by the greater dry weight converted, in addition to the nutrition of larval food materials that would also affect the larval protein value. The protein content of BSF larvae in this study was quite high. The level of protein requirement for carnivorous fish is 40-50% and omnivore is 25-35% [21]. The value of BSF larvae protein content in the composition and
ratio selected in this study meet the levels of fish protein requirements, so that BSF larvae are suitable as a substitute for fish food.

4 Conclusions
The reduction of organic waste by BSF larvae was calculated based on the waste residue that remained after 12 days of bioconversion process. The highest reduction occurred in the FW: IW (90:10) with a percentage of 82.18%, FW:RW with a percentage of 79%, and then FW:CM (90:10) with a percentage of 74.52%.

The type of organic waste and the ratio of the composition of waste affected the level of waste reduction by BSF larvae (P <0.05). The higher the reduction percentage, the higher the growth rate of larvae was. The highest weight increase of BSF larvae was observed at a mixture of food waste and immature windrow compost (FW:IW) with ratio of 90:10, i.e. 291.2 mg/larva (wet weight), containing 54.6% of protein content. Then second highest was at a mixture of food waste and residue windrow compost (FW:RW) with ratio of 90:10, i.e. 198.9 mg/larva (wet weight), containing 63.1% of protein content. Following this, a mixture of FW:CM with ratio of 90:10 had an increase of 192.1 mg/larva (wet weight of BSF larvae) containing 48.75% of protein content.

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