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# Integrating BSF larvae for the sustainable bioconversion of banana peels and sprout hulls waste

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Organic waste bioconversion, Black Soldier Fly Larvae, banana peels, sprout hulls waste, EM4.



**Abstract**

The management of organic waste presents a significant challenge in Indonesia, especially fruit and vegetable by-products. Black Soldier Fly (BSF, *Hermetia illucens*) larvae offer an effective solution due to their high potential for organic waste conversion. This study investigated the efficacy of BSF larvae in reducing and bioconverting organic waste composed of banana peels and sprout hulls, and examined the influence of EM4 fermentation on larval growth performance. Waste Reduction Index (WRI) and bioconversion rate (BCR) were calculated as percentages and analyzed descriptively, followed by the Kruskal–Wallis test. Furthermore, larval length and body weight were measured at 7, 11, 14, and 18 days of larval age and analyzed using Kruskal–Wallis with Dunn's post-hoc test. The results showed that EM4–fermented substrates containing a 1:3 ratio of banana peels to sprout hulls showed the highest waste reduction and bioconversion potential among all mixture treatments ($P < 0.05$). Although larval length and weight were lower than control substrate, no significant difference in larval length was detected, while individual larval weight was significantly higher compared to other organic waste combinations ($P < 0.001$). In this study, EM4 fermentation enhanced lignocellulosic degradation, improving substrate digestibility for BSF larvae and optimizing bioconversion efficiency, thus supporting more sustainable organic waste management strategies.

## Introduction

Waste management remains a major global concern affecting both developed and developing countries, particularly unprocessed organic waste. The Food and Agriculture Organization (2017) estimates that approximately 32% or 1.3 billion tons of organic waste are discarded annually worldwide. In Indonesia, the data from Sistem Informasi Pengelolaan Sampah Nasional – Kementerian Lingkungan Hidup dan Kehutanan (2023) confirmed that total national waste generation reached 17.7 million tons from 202 regencies and cities, with 66.81% (11.8 million tons) being properly managed and 33.19% (5.8 million tons) untreated.

Organic waste that is not properly managed can have serious impacts on environment, economy, and society (Seberini, 2020). In Indonesia, the major contributors of organic waste are residues from paddy production (44%), with fruits coming in second at 20% and vegetables at 16% (BAPPENAS, 2021). These

organic residues can significantly contribute to greenhouse gas emissions, primarily methane, which acceleratae global warming. Fruit and vegetable waste is thought to have a weekly global warming potential of 9.2 kg $CO_2$ (Von Massow et al., 2019), with organic waste accounting for approximately 40–45% of carbon dioxide emissions and 50–55% of methane emissions (Abdirahman et al., 2023).

Among all fruits, bananas are extensively produced reaching up to 9,245,427 tons annually (BPS, 2023). Nevertheless, 60% of banana biomass is considered waste (Acevedo et al., 2021), consisting of about 40% peel and 60% pulp. This implies that from a kilogram of bananas, around 400 g of peel are discarded as organic waste. Similarly, bean sprout processing also generates substantial waste in the form of sprout hulls, which are often discarded due to their low economic value and high perishability (Cahyanto et al., 2019). The potential sprout hulls waste in Jakarta alone is estimated at 8.35 tons per week (Saenab & Retnani, 2011), where each kilogram of mung beans could produce 2.98 kg of sprouts, generating about 112.98 g equivalent to 0.11% of sprout hulls waste.

Organic wastes which considered as a problem when untreated are actually could be a valuable source for bioconversion material. Black Soldier Fly (BSF; *Hermetia illucens*) larvae are recognized as an efficient biological agent for organic waste conversion as their ability to reduce organic waste by 65.5–78.9% per day (Diener et al., 2011). Sprout hulls waste are rich in crude fiber (50.89%), cellulose (45.1%), crude protein (12.09%), and contain considerable amounts of calcium, phosphorus, and energy (Pangestu et al., 2018). These characteristics make them a potential substrate for rearing BSF larve, while converting waste in valuable products into larvae biomass and offering sustainable solution for waste management. However, Meneguz et al. (2018) and Lalander et al. (2019) reported that the efficiency of this bioconversion agent depends on several factors including substrate composition and abiotic parameters that could affect larval development and bioconversion rate.

The use of certain substrates such as banana peels has negatively impacts Waste Reduction Index (WRI) values due to high lignin and hemicellulose content which are difficult to degrade (Indriyanti et al., 2023; Nyakeri et al., 2017; Kumar et al., 2018). BSF larvae lacks of specific enzymes to process lignin, thus rely on microbial activity to convert lignin into simpler sugars (Kim et al., 2011; Lee et al., 2014). Therefore, a pretreatment process such as biological delignification is necessary to enhance BSF substrate digestibility. Using microbial assistance to reduce lignin and hemicellulose content of organic waste, especially banana peels, could possibly enhance feed pretreatment as nutritious source for BSF larvae.

Effective Microorganisms-4 (EM4) can facilitate this process due to the presence of lignin-degrading microbes such as *Actinomycetes*, *Streptomyces*, and *Lactobacillus*, which produce cellulase and laccase enzymes (Mulyani et al., 2024; Schroyen et al., 2015; Janusz et al., 2020). Fermentation using EM4 enhances nutrient availability, reduces fiber complexity, and generates compounds that improve BSF larval growth (Firdausy et al., 2020; Masrufah et al., 2020; Mirwandhono et al., 2022). By combining several organic waste, banana peels and sprout hulls, to be fermented with EM4 as pretreatment expetantly can increase BSF larvae digestibilty and convert it into improved organic waste reduction and higher quality of biomass.

Despite the growing use of BSF larvae for organic waste management, limited information is available on how microbial fermentation combined with mixed lignocellulosic substrates affects both waste reduction efficiency and larval growth performance. In particular, the synergistic effects of banana peel–sprout hull mixtures fermented with EM4 remain poorly understood. This study aimed to acknowledge the effects of EM4 fermentation on BSF feed substrate, to evaluate the ability of BSF larvae in reducing banana peels and sprout hulls waste, to assess and analyze feed substrate effects on larval development. To evaluate effects of feed substrate fermentation, several groups of experiments were left untreated and others fermented with EM4. The findings are expected to contribute to the development of sustainable organic waste management systems and provide as a reference for the establishment of BSF-based waste treatment facilities, particularly in residential areas.

## Materials and methods

### Preparation for Organic Waste Substrate

The larval feed substrate is composed from banana peels from local market in Manggarai and sprout hulls waste from local market in Pasar Gembrong Lama, Cempaka Putih, Central Jakarta. Both organic wastes were collected and placed in sterile plastic containers. The wastes were then thoroughly washed and chopped into small pieces, approximately 1–2 cm, using a mechanical shredder, following the method of

Dortmans et al. (2017). Water was added to assist homogenization at a feed-to-water ratio of 2:1 (w/v) in the blender to make feed substrate softer. The prepared substrate was then stored at –12°C until further use for BSF larval feed.

### Fermentation of Organic Waste Substrate Using EM4

Effective Microorganisms-4 (EM4, PT. Songgolangit Persada) was used to ferment feed substrate that has been prepared for the experiment. EM4 activation followed the method of Nurfitriani & Hutabarat (2014), where 500 mL of molasses was mixed with 1000 mL of water (ratio of 1:2), followed by the addition of 100 mL EM4. The activated EM4 solution was incubated 24 hours before use. A total of 8 mL of the activated EM4 was added to every 100 g of prewashed and homogenized organic waste substrate. The mixture was thoroughly stirred to ensure uniform distribution and transferred into 1300 mL airtight plastic jars (9 cm in diameter) for anaerobic fermentation. This process lasted for two days at ambient temperature, following the method of Sihombing & Mirwandhono (2022). Data of pH and temperature were measured on day 0 and day 2 using a calibrated digital three-in-one thermo-hygrometer and pH meter (YY-1030, Shenzhen Yago Technology).

### Hatching BSF Eggs

BSF used in this study originated from Organic Waste Management of BSF Maggot House, Cempaka Putih, Central Jakarta. Egg clutches were collected from oviposition traps made of wooden plates with narrow gaps, located inside a breeding cage. Approximately 1 g of BSF eggs was placed on a 17 × 5 × 11.5 cm rearing tray containing moist mixture of commercial chicken feed and water at a ratio of 1.5 L water to 1 kg feed. The larvae were fed on days 1 and 3 post-hatching. Seven-day-old larvae (7-day old larvae or shorten into 7-DOL) were used for the experiment. After reaching 7 days of age, larvae were separated from the residue using a fine mesh sieve, then counted, weighed, and transferred into experimental containers. All BSF from hatching until experiment day were reared under natural occurring ambient condition in the facility.

### Larval Rearing Conditions and Measurements

Larvae were transferred from the hatching trays to individual experimental containers. Experiments were conducted by using chicken feed (unfermented as K1 and fermented as K2) as controls and combining organic waste of banana peels to sprout hulls. Combination of organic waste varied from P1-P3 which were unfermented with composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively. This experiment was performed using validated procedures and repeated systematically as many as 4 replicates for each mixture treatment.

In this study, each treatment contained 100 larvae (approximately 4 g) of 7-DOL, placed in 750 mL thin-wall plastic containers with perforated lids for aeration under naturally occurring ambient conditions. Feeding was conducted on days 1, 5, and 8, with 50 g of organic substrate provided per feeding, totaling 150 g of feed per treatment. Environmental conditions, including substrate feed temperature, humidity, pH were monitored at each feeding interval using a digital three-in-one thermo-hygrometer and pH meter (YY-1030, Shenzhen Yago Technology).

As many as random 10 individuals were selected as representative samples to assess their developmental performance at the ages of 7-d, 11-d, 14-d and 18-d (pre-pupae) before being fed. For larval length measurements, larvae were photographed orthogonally with a ruler aside using a digital microscope, then images were analyzed using the ImageJ software (v.1.8.0) to estimate the length of the larvae in cm (from the mouth to the bottom of the last abdominal segment). For weight measurements, larvae were weighed using a digital scale with an accuracy of 0.001 g (Digital Carat Scale Mode DS-04B, Hanyu Electronic Technology). Mature larvae entering prepupal stage were transferred to a separate container and the residual substrate was collected, weighed, and recorded for waste reduction analysis.

### Data Analysis

The degradation potential of BSF larvae over a specific period was evaluated using the Waste Reduction Index (WRI), which indicates the efficiency and rate of substrate reduction (Amrul et al., 2022). Additionally, the Bioconversion Rate (BCR) was calculated to assess the proportion of nutrients in the substrate converted into larval weight, reflecting the nutritional efficiency of the feed (Rehman et al., 2019).

Both WRI and BCR were analyzed using Kruskal–Wallis test in RStudio version 4.4.1. Larval length and weight data were presented in average at the age of 7-, 11-, 14- and 18-DOL and were then statistically analyzed using Kruskal–Wallis test, followed by Dunn's post-hoc test with Bonferroni correction ($P < 0.001$) in RStudio version 4.4.1.

## Results and discussion

### 1. Fermentation Effect on Temperature, Humidity and pH of BSF Larval Feed Substrate

Fermentation is the process in which microorganisms break down complex organic compounds such as cellulose, hemicellulose and lignin into simpler molecules, thus reducing crude fiber content, improving nutritional value, and enhancing digestibility (Martina et al., 2025). According to Katongole et al. (2017), various yeasts, bacteria, and fungi applied to organic waste produce hydrolytic enzymes, such as cellulase and tannase, which degrade cellulose and tannins. In this study, fermentation of EM4 was conducted on treatments P4, P5, and P6. Treatment of P4 composed by 1:3 banana peels and sprout hulls waste, P5 composed by 1:1 banana peels and sprout hulls waste, while P6 had the highest composition of banana peels with ratio of 3:1 to sprout hulls waste. The visual characteristics of before and after fermentation substrates were presented in Figure 1, while the comparative values of temperature (°C), humidity (%), and pH before and after fermentation were shown in Figure 2a-c.

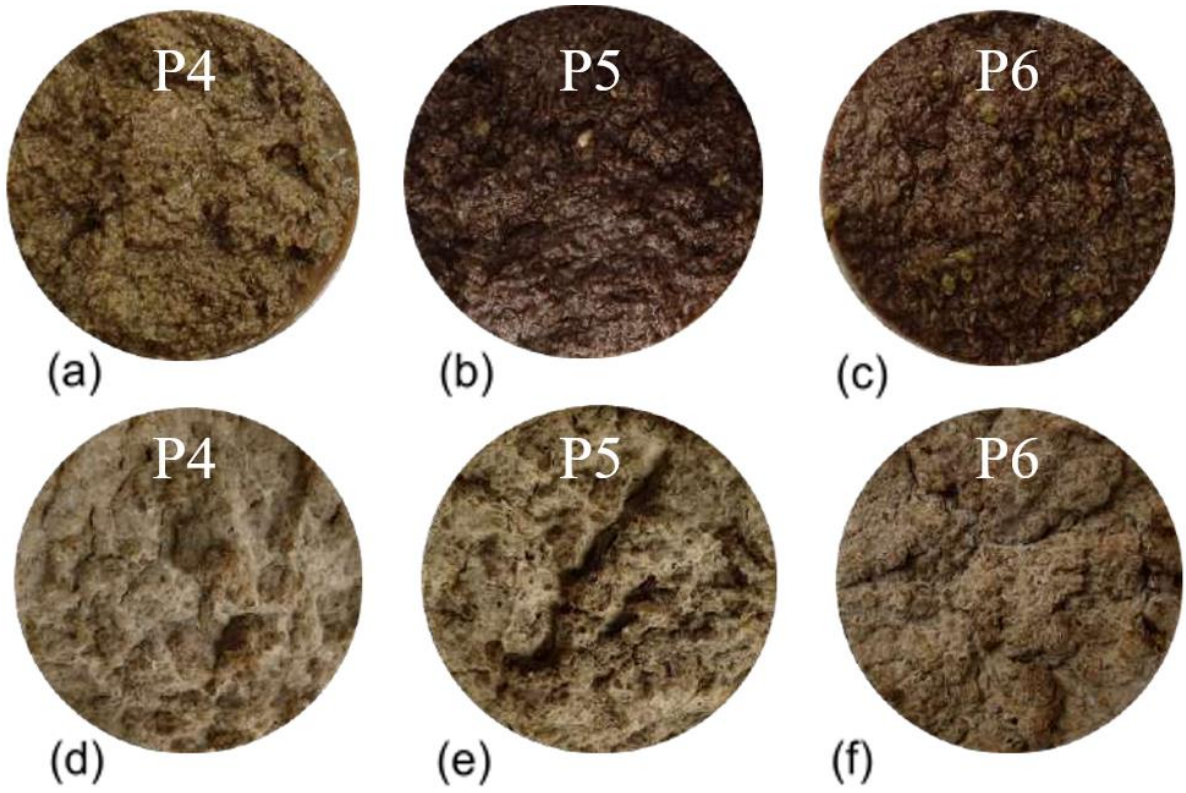


**Figure 1. Fermentation results of BSF larval feed before (a,b,c) and after fermentation (d,e,f)**

The efficiency of pre-treatment in lignin degradation strongly depends on the feed composition and operational conditions (Suhartini et al., 2024). Among treatments of P4, P5, and P6, temperature, humidity, and pH exhibited a noticeable increase. The noticable temperature increase was observed in P4 (0.21 ± 34 °C) and P5 (0.14 ± 34 °C), though overall variations were minor, ranging from 33.9 to 34.0 °C (Figure 2a). This temperature rise indicates active cellulolytic hydrolysis during fermentation, suggesting an increase in glucose levels derived from cellulose by cellulase enzymes present in EM4. Hamnasia et al., (2023) added that hydrolysis is an endothermic process that requires heat to convert cellulose into glucose. These processes consequently affected post-fermentation temperature of BSF feed substrates.

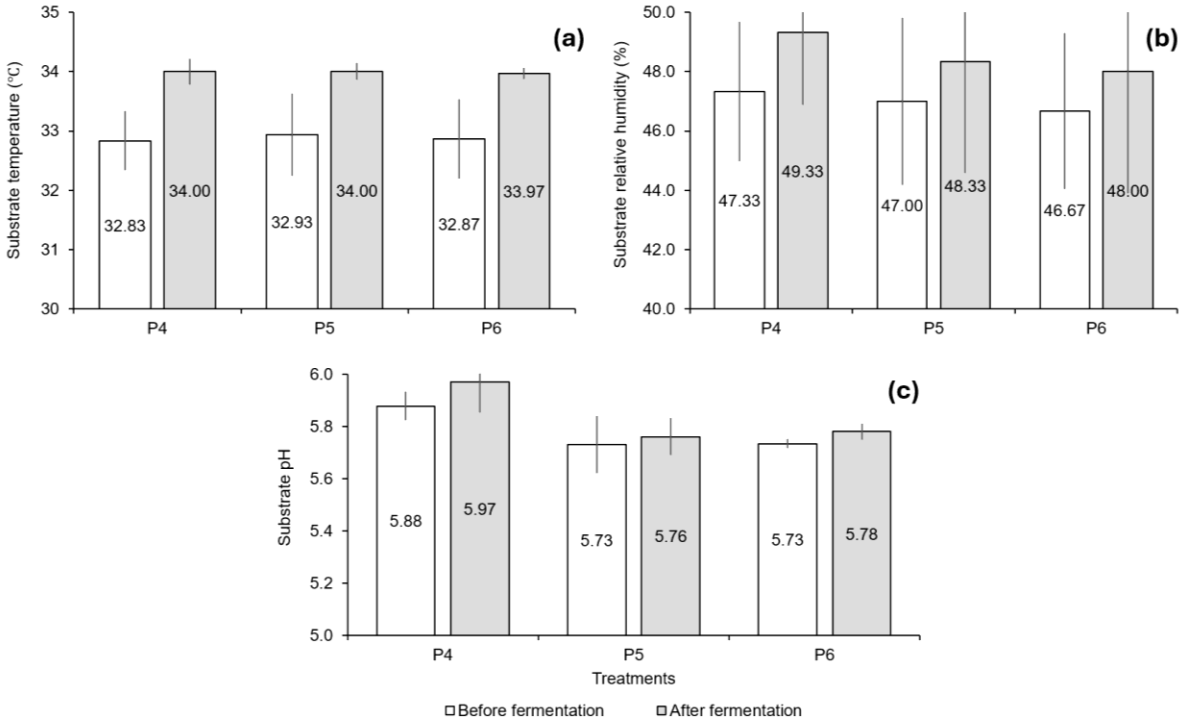


**Figure 2. Comparison of before and after: (a) temperature (°C), (b) relative humidity (%), and (c) pH in the fermentation process of organic waste from banana peels and sprout hulls. Note: P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively.**

Relative humidity also increased with the highest rise observed in P4 (from 47.33% to 49.33%) (Figure 2b).This high rise might caused by the higher moisture in sprout hulls waste, approximately 65–70% (Mulyati et al., 2020; Saenab, 2010). The increase in water content was resulted from vapor formation during microbial metabolic activity throughout fermentation (Hariyono et al., 2021). Within 48 h, pH were increased in all treatments, with the highest in P4 (5.97 ± 0.116) (Figure 2c). This rise was due to the presence of lactic acid bacteria (LAB) in EM4, such as *Lactobacillus* spp., which metabolize sugars as carbon sources, producing lactic acid and other organic compounds that influence pH dynamics (Yuktika et al., 2017). Saputri & Suhandoyo (2024) also added that protein in organic waste would be broken down by EM4 and released ammonia which can increase pH level. However, the results could vary as each treatment had different composition of organic waste as food source for the EM4.

## 2. Comparison of Waste Reduction Index (WRI) and Bioconversion Rate (BCR)

The Waste Reduction Index (WRI) reflects the capacity of BSF larvae to consume organic waste over time; a higher WRI indicates a greater waste reduction efficiency (Hakim et al., 2017). While BCR represents the proportion of organic substrate converted into larval weight, primarily protein and lipid fractions (Siddiqui et al., 2022a, b). Variations in WRI and BCR values among treatments were illustrated in Figure 3a-b.

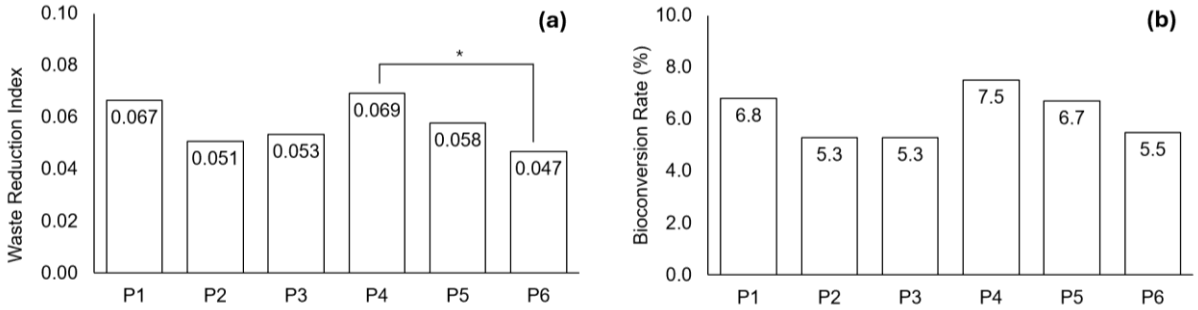


**Figure 3. Comparison of (a) waste reduction index and (b) bioconversion rate values for each treatment.**
Note that P1-P3 were unfermented with varied composition of banana peels and sprout hulls f 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls of 1:3, 1:1 and 3:1 respectively.

Kruskal–Wallis analysis revealed a significant difference in WRI among treatments ($P < 0.001$), with P4 showing a significantly higher value than P6. However, no significant differences ($P > 0.001$) were shown in BCR among treatments (Table 3). The P4 treatment exhibited the highest WRI (0.069) and BCR (7.5%), demonstrating the strong positive correlation between waste reduction and bioconversion efficiency (Figure 3a and 3b). Isibika et al. (2021) also reported that feed substrate with 100% banana peels achieved BCR of 5.8% compared to substrate with smaller banana peels composition, highlighting the advantage of mixed substrates. However, bioconversion performance of BSF may vary due to differences in substrate type, larval density and condition of rearing temperature and humidity (Cheng et al., 2017; van Rozen et al., 2023). In Figure 3, P1 (unfermented) and P4 (fermented) treatment with higher sprout hulls content achived better reduction and conversion rate, thus, adding these could possibly serve improved feed substrate to be converted by BSF larvae.

The slightly higher WRI observed in fermented treatments (Figure 3, P4-P6) can be attributed to microbial pre-digestion by EM4, which softens the feed substrate and enhances nutrient availability. Acceleration of photosynthetic bacteria in EM4 could facilitate organic decomposition and amino acid synthesis, improving palatability and nutrient assimilation in BSF larvae. Moreover, Actinomycetes during fermentation could produce antimicrobial compounds for enhancing larval feed quality (Rofi et al., 2021). Compared to previous findings by Permana et al. (2022), the higher WRI obtained in this study suggested that EM4-assisted fermentation and mixed organic substrates enable BSF larvae to consume a greater proportion of organic material while minimizing residual waste.

The significant difference in waste reduction was only seen between P4 and P6 treatment ($P<0.05$) showing the highest WRI in Figure 3a. The strong positive correlation between WRI and BCR indicated that increased substrate degradation was directly associated with enhanced nutrient conversion into larval biomass. The superior performance of the mixed and fermented substrate was also reported by Isibika et al. (2021), suggesting the synergistic effect of substrate diversity on larval growth and waste conversion efficiency.

### 3. Effects of EM4-Optimized Feed on BSF Larval Length and Weight Performance

Larval growth in terms of length and weight was recorded at 7, 11, 14, and 18 days of larval age. The mean larval length at 18 DOL ranged between 1.30 and 2.02 cm (Figure 4). According to Kim et al. (2010), BSF larvae can reach lengths of up to 20 mm, widths of approximately 6 mm, and experience a substantial increase in biomass from the third to the sixth instar, supporting the observed growth patterns in this study. The largest length at 18 DOL was observed in treatment K2 ($2.02 \pm 0.098$ cm; fermented chicken feed), while the shortest was in P6 ($1.30 \pm 0.086$ cm; 3:1 banana peels and sprout hulls waste).

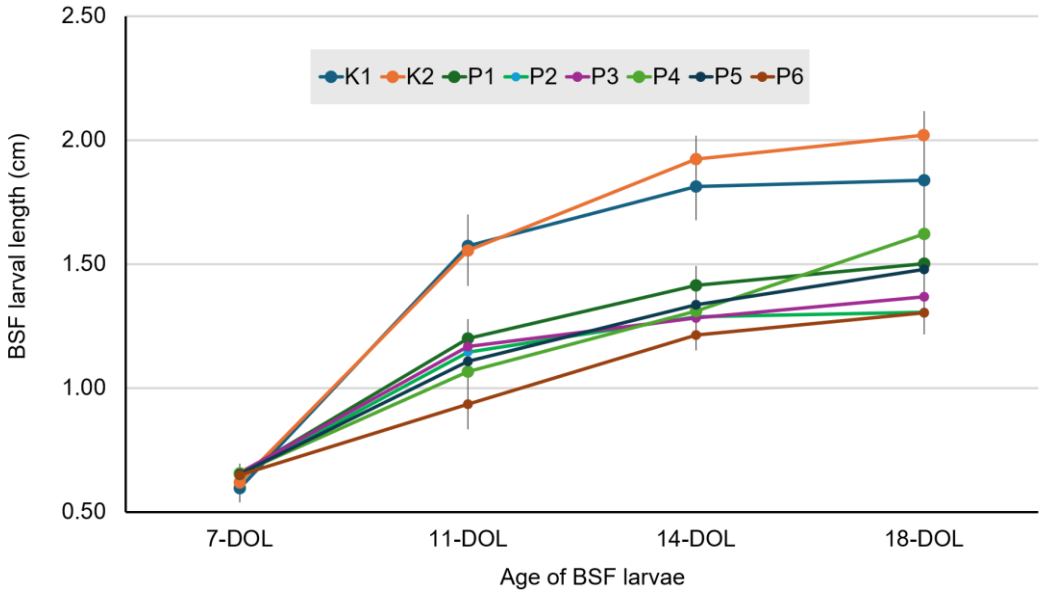


**Figure 4. BSF larval length growth (mean ± SD) at 7-DOL, 11-DOL, 14-DOL, and 18-DOL.**
Note that K1 (unfermented) and K2 (EM4 fermented) were control treatment using chicken feed; P1-P3 were unfermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively. DOL means Day Old Larvae.

At early developmental stages (7 DOL), larval length was relatively had uniform length among treatments as larvae were originated from the same age of hatching cohort (Figure 5), therefore the uniformity in larval length was expected and indicated minimal variation at early developmental stages. At 11-DOL, the highest mean larval length was recorded in treatment K1 (1.57±0.123 cm), which was significantly different from all other treatments (Table 1; $P<0.001$). In contrast, no significant differences were detected among treatments P1 through P6 at this stage, suggesting comparable performance under these regimes during mid-larval development (Figure 4; Table 1).


**Figure 5. Development process of BSF larvae 7-DOL, 11-DOL, 14-DOL and 18-DOL with (a) = K1, (b) = K2, (c) = P1, (d) = P2, (e) = P3, (f) = P4, (g) = P5 and (h) = P6.**
Note that K1 (unfermented) and K2 (EM4 fermented) were control treatment using chicken feed; P1-P3 were unfermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively.

At 14-DOL, treatment K2 resulted in the highest larval length (1.92 ± 0.095 cm), which was significantly greater than all other treatments (Table 1; $P<0.001$). This was followed by treatment K1 (1.81

± 0.137), while the lowest larval length at this stage was observed in treatment P6 (1.21 ± 0.064 cm), which was significantly lower than K1, K2, and P1 (Table 1). Significant differences among treatments were also shown at 18-DOL (Figure 4; Table 1). The longest larvae were obtained under treatment K2 (2.02 ± 0.098 cm), which was not statistically different from K1 (1.84 ± 0.115 cm) and P4 (1.62 ± 0.172 cm) (Table 1), indicating comparable growth performance among these treatments in this developmental stage.

**Table 1. Mean values (±SD) of BSF Larval Length from 7- to 18-DOL**

| Treatments | Mean of Length (cm) | | | |
|---|---|---|---|---|
| | 7-DOL | 11-DOL | 14-DOL | 18-DOL |
| K1 | 0.060±0.058$^{a}$ | 1.57±0.123$^{a}$ | 1.81±0.137$^{a}$ | 1.84±0.115$^{ab}$ |
| K2 | 0.062±0.061$^{a}$ | 1.56±0.145$^{b}$ | 1.92±0.095$^{b}$ | 2.02±0.098$^{a}$ |
| P1 | 0.065±0.034$^{a}$ | 1.20±0.079$^{c}$ | 1.41±0.079$^{ac}$ | 1.50±0.094$^{ce}$ |
| P2 | 0.065±0.031$^{a}$ | 1.14±0.095$^{c}$ | 1.29±0.093$^{cd}$ | 1.31±0.076$^{de}$ |
| P3 | 0.066±0.033$^{a}$ | 1.17±0.080$^{c}$ | 1.28±0.111$^{cd}$ | 1.37±0.092$^{cde}$ |
| P4 | 0.066±0.032$^{a}$ | 1.07±0.078$^{c}$ | 1.31±0.078$^{cd}$ | 1.62±0.172$^{bc}$ |
| P5 | 0.065±0.029$^{a}$ | 1.11±0.083$^{c}$ | 1.34±0.091$^{cd}$ | 1.48±0.071$^{ce}$ |
| P6 | 0.065±0.047$^{a}$ | 0.93±0.100$^{c}$ | 1.21±0.064$^{d}$ | 1.30±0.086$^{de}$ |

Note: * K1 (unfermented) and K2 (EM4 fermented) were control treatment using chicken feed; P1-P3 were unfermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively.

** Mean values with superscript notation differences indicated significant differences ($P$<0.001) after Bonferroni Correction based on Dunn's Test after Kruskal-Wallis Test

*** DOL means Day Old Larvae

Larval growth performance varied significantly among treatments, with the highest mean length observed in the fermented chicken feed group (K2), followed by K1 and P4. The improved growth in K2 suggested that nutrient-rich and readily digestible substrates promote optimal larval development. At the early stage (7 DOL), larval length was relatively similar among the treatments, showing similar trend during initial rearing responses. From 11 DOL onward, distinct growth started to appear, reflecting the cumulative influence of substrate composition and nutrient availability on larval length development (Figure 4, Table 1). The comparable final larval lengths (18-DOL) obtained in treatment P4 and the chicken feed controls demonstrated that the fermented mixed organic waste substrate (EM4 fermented 1:3 banana peels and sprout hulls waste) could sustain larval growth at levels equivalent to those achieved with commercial feed.

In larval length response, P4 represented a promising alternative for rearing substrate, offering lower cost and greater accessibility while maintaining comparable growth performance with controls. The superior performance of P4 is likely driven by the combined effects of substrate composition and microbial pre-treatment. The higher proportion of sprout hull waste in P4 diluted the lignocellulosic fraction and produced a softer substrate texture, thereby enhancing palatability and ingestion efficiency in BSF larvae, which preferentially consume low-fiber and nutrient-accessible substrates (Rofi, 2021). Not only the mixture factors, but EM4-mediated fermentation also enhances substrate softness and nutrient bioavailability (Suciati, 2017), thereby facilitating more efficient ingestion and nutrient assimilation for BSF larvae.

In contrast, banana peel–dominant substrates such as P6 contained higher levels of lignin, cellulose, and hemicellulose, which limited enzymatic accessibility and nutrient assimilation (Sukowati et al., 2014). Although EM4 fermentation partially improved substrate digestibility, the high lignocellulosic load likely exceeded the capacity of microbial pre-digestion to sufficiently reduce substrate complexity, resulting in constrained larval growth. These results indicated that EM4 fermentation is most effective when applied to substrates with moderate fiber content, highlighting the importance of balancing substrate composition and microbial pre-treatment to optimize BSF larval growth and bioconversion efficiency.

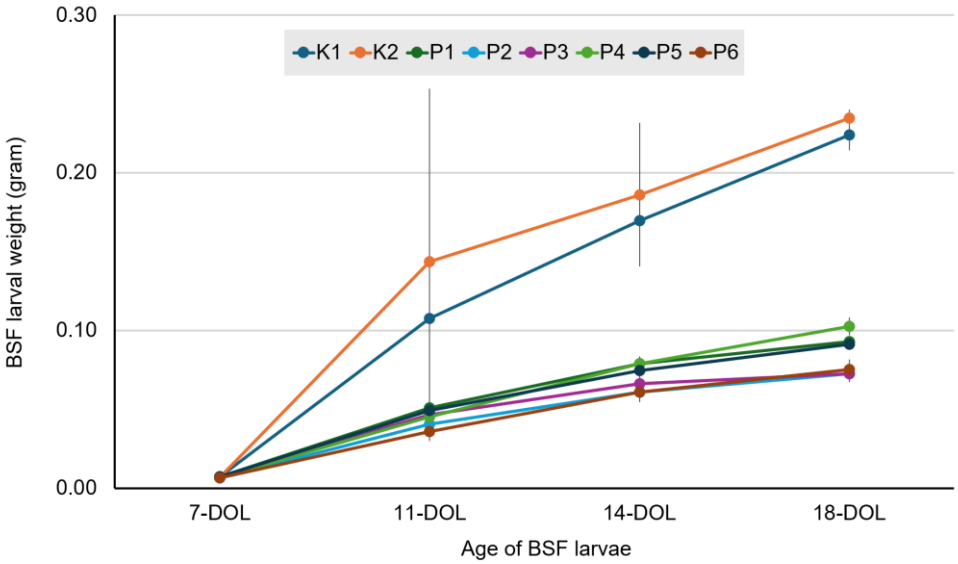


**Figure 6. BSF larval weight growth (mean±SD) from 7-DOL, 11-DOL, 14-DOL and 18-DOL.**
Note that K1 (unfermented) and K2 (EM4 fermented) were control treatment using chicken feed; P1-P3 were unfermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively. DOL means Day Old Larvae.

Larval weight followed a similar trend with larval length across treatments. At 18-DOL, weights ranged from 0.073 to 0.235 g (Figure 6), with the highest weight consistently recorded in K2 and the lowest in P6 treatment (Table 2). The consistent increase in K2 treatment indicating superior nutritional availability in this substrate. This aligned with Permana et al. (2022), who found that fermented chicken feed produced heavier larvae than banana peel-based diets. Among mixed organic waste treatments, P4 treatment obtained the greatest mean larval weight (0.103 ± 0.006 g) at the final larval stage, significantly outperforming other mixed feed treatments (Table 2; $P<0.001$). This confirmed that the microorganisms in EM4 facilitates the partial hydrolysis of complex organic compounds, thereby increasing nutrient and supporting larval growth (Suciati, 2017). Biological delignification mediated by EM4-associated microorganisms—such as *Streptomyces* spp., which produce cellulase, and *Lactobacillus* spp., which produce laccase—facilitates lignin degradation and improves feed quality (Schroyen et al., 2015; Janusz et al., 2020; Mulyani et al., 2024). Consequently, the enhanced enzymatic breakdown of lignocellulosic components in the P4 treatment is likely contributed to improved substrate conversion efficiency and increased larval weight.

**Table 2. Mean values (±SD) of BSF Larval Weight from 7- to 18-DOL**

| Treatments | Mean of Weight (gram) | | | |
|---|---|---|---|---|
| | 7-DOL | 11-DOL | 14-DOL | 18-DOL |
| K1 | 0.0073±0.0016$^{ab}$ | 0.108±0.017$^{a}$ | 0.170±0.018$^{a}$ | 0.224±0.010$^{a}$ |
| K2 | 0.0070±0.0012$^{ab}$ | 0.144±0.110$^{b}$ | 0.186±0.046$^{a}$ | 0.235±0.005$^{a}$ |
| P1 | 0.0073±0.0004$^{b}$ | 0.051±0.007$^{ce}$ | 0.079±0.005$^{b}$ | 0.093±0.003$^{b}$ |
| P2 | 0.0065±0.0001$^{ac}$ | 0.040±0.004$^{cd}$ | 0.061±0.003$^{c}$ | 0.073±0.005$^{c}$ |
| P3 | 0.0071±0.0001$^{b}$ | 0.046±0.002$^{c}$ | 0.066±0.003$^{c}$ | 0.073±0.003$^{c}$ |
| P4 | 0.0068±0.0004$^{bc}$ | 0.045±0.005$^{c}$ | 0.079±0.002$^{b}$ | 0.103±0.006$^{d}$ |
| P5 | 0.0072±0.00004$^{b}$ | 0.049±0.003$^{c}$ | 0.074±0.005$^{b}$ | 0.091±0.003$^{b}$ |
| P6 | 0.0065±0.0004$^{ac}$ | 0.036±0.006$^{d}$ | 0.061±0.006$^{c}$ | 0.075±0.006$^{c}$ |

Note: * K1 (unfermented) and K2 (EM4 fermented) were control treatment using chicken feed; P1-P3 were unfermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively; while P4-P6 were EM4-fermented with varied composition of banana peels and sprout hulls waste of 1:3, 1:1 and 3:1 respectively.

** Mean values with superscript notation differences indicated significant differences ($P<0.001$) after Bonferroni Correction based on Dunn's Test after Kruskal-Wallis Test

*** DOL means Day Old Larvae

The comparatively lower performance observed in banana peel-dominant treatments (P3 and P6) shown in Figure 6 and Table 2 could be attributed to their high lignocellulosic content, consisting primarily of hemicellulose (23.2%), cellulose (14.56%), and lignin (21.29%) (Sukowati et al., 2014). Lignin forms complex and rigid associations with cellulose and hemicellulose, creating a stucture that might restrict enzymatic hydrolisis and decrease substrate bioavailability. This structural resistance might microbial degradation and enzymatic access in the larval gut, thus constraining nutrient release and assimilation for their growth. Consequently, the absence of delignification and pretreatment of feed substrate could impact the digestibility and energy conversion efficiency of BSF larvae (Permatasari et al., 2014). This aligned with Isibika et al (2019) and Raksasat et al. (2020) who reported that this high lignocellulosic compounds in banana peel-dominant treatment can negatively affected BSF and produced smaller larval size and weight. Although BSF larvae possess gut-associated microbiota capable of degrading simple polysaccharides, their ability is limited for lignocellulosic compounds thus resulting inefficient depolymerization and low fermentable sugars. Consequently, the metabolic energy for BSF larval growth and biomass is largely reduced. Collectively, these constrains impacted lower growth performance –reflected in reduced larval weight and length– observed when BSF larvae were reared on substrates dominated by high lignocellulosic content.

Overall, larval length and weight accumulation were strongly influenced by substrate quality and digestibility, with EM4-fermented substrates supporting superior growth performance at the final larval stage. In contrast, banana peel-dominant diets limited larval weight gain due to their high lignocellulosic content and restricted enzymatic accessibility, underscoring the importance of microbial pre-treatment to enhance nutrient availability and efficiency using BSF as bioconversion agent. The EM4-fermented mixed organic waste substrate with ratio of 1:3 banana peels to sprout hulls waste sustained larval growth comparable to commercial feed, indicating an accessible alternative for rearing substrate without compromising growth performance and supporting bioconversion facilty using BSF for waste management.

## Conclusion

The present study demonstrated that BSF larvae could effectively convert mixed organic waste of banana peels and sprout hulls waste into valuable biomass, with fermentation using EM4 significantly enhancing waste reduction and bioconversion efficiency. However, its efficiency was affected by the composition used in mixed organic waste. Among all treatments, the fermented substrate with 1:3 banana peel to sprout hulls waste had the highest WRI and BCR value, as well as improved larval growth performance which was comparable to the commercial feed. The use of EM4 could promote substrate pre-digestion by microbial enzymatic activity, facilitating better nutrient assimilation for BSF larvae. These findings highlighted the effect of mixed organic waste and the potential of EM4-assisted bioconversion for improving sustainable and efficient strategy in managing organic waste while producing high-quality BSF larval biomass.

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review and redactional correction. Elsa Lisanti: critical review and data analysis. Ratna Komala: critical review and redactional correction. Weri Herlin: literature review.

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**Data availability:** The raw data supporting the conclusions of this article, including regular measurement of substrate temperature, humidity, and pH during fermentation as well as larval rearing condition, measurement of larval length and weight, measurement of total waste used and waste residue by BSF larvae. The data will be made available by the authors, without undue reservation, to any qualified researcher.

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