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RESEARCH PAPER

The effect of fermentation process on increasing biodegradable organic waste reduction with Black Soldier Fly (BSF) larva bioconversion method

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Abstract. In 2021, Indonesia produced 64 million tons of waste, with market waste dominating at 22.7%. Organic waste processing can yield biogas, compost and can undergo the bioconversion process using Black Soldier Fly (BSF) larvae. BSF larvae lack cellulose-degrading enzymes. The addition of a fermentation process can increase growth and reduce waste. The research involved adding a fermentation process with two types of fermenters. Fermentation was carried out at different durations to determine to optimal fermentation period. The biodegradable waste utilised was vegetable and fruit waste from the market. As a control, organic waste without fermentation will also be utilized. The results indicated that fermentation had no significant effect on waste reduction. The reduction in fermented and non-fermented waste was 62.97% and 50.67%. Waste reduction is directly related to the larvae's ability to consume waste. Fermented waste treated with Trichoderma (10 days) had exhibited peak larval growth at 18 days of age, whereas non-fermented waste reached its peak growth at 25 days. The residue from fermented waste had a lower quantity but a higher the C/N ratio of 89.37, while non-fermented waste residue had a greater quantity with a C/N ratio of 62.11.

Keywords: bioconversion; biodegradable; Black Soldier Fly; fermentation; waste reduction

1. Introduction

The waste problem still persists in Indonesia. The high population density in urban areas results in elevated public consumption, thereby increasing waste generation (Salmanl et al., 2020). According to data from the Ministry of Environment and Forestry (KLHK) for 2021, Indonesia is estimated to produce 64 million tons of waste, with traditional market waste ranking second at 22.7%. Besides processing organic waste for use as biogas and compost, one way to process it is through bioconversion. Bioconversion is a process that utilizes microorganisms or insect larvae to consume and convert the nutrient content of organic waste into biomass (Muhayyat et al., 2016). Black soldier fly larvae, upon hatching, immediately consume the provided organic waste (Yuwono & Mentari, 2018). Vegetable and fruit waste contain cellulose and nutrients that can be transformed into new products. The bioconversion process of vegetable and fruit waste without prior fermentation by BSF larvae yields unsatisfactory results (Manurung

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et al., 2016). This low bioconversion efficiency is attributed to high cellulose content and and low protein content in vegetable and fruit waste, while BSF larvae lack cellulose-degrading enzymes (Kim et al., 2011). Cellulose enzymes are obtained from cellulolytic bacteria that symbiotically aid in BSF digestion. Therefore, it is essential to incorporate a fermentation process into waste treatment before feeding it to the BSF larvae. The utilization of cellulolytic bacteria as a producers of cellulase enzymes plays a crucial role in the bioconversion process, as these bacteria can decompose cellulose into glucose, serving as a carbon and energy source (Li et al., 2011).

In this study, an additional process was implemented in organic waste to increase waste reduction by BSF larvae. The organic waste will undergo anaerobical fermentation using two fermenters derived from *Trichoderma sp*. and *Lactobacillus sp*., each with a different fermentation duration. The organic waste used as a sample is sourced from the market. As a control to assess the impact of the sample type on the percentage of waste reduction and the growth rate of BSF larvae, organic waste without fermentation will also be employed. The final results aimed to determine the influence of fermenter type and fermentation duration on the percentage of market waste reduction by BSF larvae. The percentage of organic waste reduction will then be used to assess the effect of the fermentation process on reducing market organic waste in bioconversion processing with BSF.

2. Methodology

2.1. Study area

The research was a laboratory-scale experiment conducted at the Wonorejo nursery in Surabaya and at the Solid Waste and B3 Processing Laboratory within Department of Environmental Engineering at FTSPK-ITS. The research spanned a duration of 2 months (60 days), including a 21-day observation period of BSF and 13-day fermentation period.

2.2. Study of Literature

This research investigates waste composition, the consumption rate of Black Soldier Fly (BSF) larvae, the life cycle of BSF larvae, the impact of fermentation on market vegetable and fruit waste, larval growth supporting treatment, occurrence analysis, and the ensuring discussion. of the research. A literature review is conducted through scientific journals, textbooks, final project reports, and other reputable and legal sources.

2.3. Determination of number sample

Two types of waste, namely vegetable waste and fruit waste, were used. The waste selected was fresh waste, aged 1-2 days after disposal. For vegetable waste, mustard greens and cabbage were utilized, while fruit waste included banana and papaya. There is a need to re-evaluate the recommended feeding rate for the combination of vegetable and fruit waste, which is 34 mg/larvae.day. The addition of waste based on fermenter variations and fermentation duration is presented in Table 1.

The selection of fermenter type is based on the capability of microorganisms in Trichoderma and SOC to break down cellulose tissue in vegetable and fruit waste. The duration chosen for use are 4, 7, 10, 13 days. This decision is made to encompass days both before and after the optimal fermentation period of the chosen fermenter.

Table 1. Research Variations.

No	Waste composition	Parameter	Fermentation duration (day)	Feeding rate (mg/larvae.day)
1	Vegetable:Fruit (50:50)	SOC (*Lactobacillus sp.*)	4 7 10 13	34
2	Vegetable:Fruit (50:50)	Trichoderma (*Trichoderma sp.*)	4 7 10 13	34

2.4. Research implementation

2.4.1 Preparation

The tools prepared tools encompass all the requirements for constructing reactors and conducting research. The BSF reactor measures 20 cm x 17.5 cm x 10.5 cm and is made of plastic. Small box containers are subsequently arranged on larger plastic tray containers (60 cm x 40 cm) to facilitaed their placement on shelves at the research site. The materials needed for the study include 5-days-old BSF larvae, molasses, market waste (mustard greens, cabbage, bananas, papaya), and fermenters (SOC and Trichoderma).

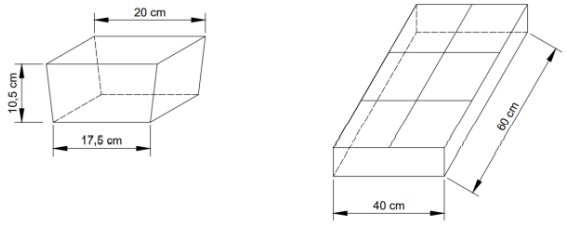

Figure 1. BSF reactor

2.4.2 Waste Fermentation

Garbage fermentation process is initiated after determining the weight of each waste source needed for feeding BSF larvae every seven days. Upon ascertain the weight and composition, the waste is placed into the fermentation reactor and sealed tightly. Fermentation commences with durations varying from 13 days, 10 days, 7 days, and 4 days.

Once the waste samples are placed into their respective reactors, a fermenter solution is prepared, comprising 80 ml of molasses, 20 ml of fermenter, and 200 ml of water. This solution is then sprayed onto the sample in each reactor (5 sprays). The reactor are securely sealed and left for the predetermined duration variation. If there is a significant change in temperature, a gas outlet is necessary.

2.5. Data Collection

The results from each reactor will be compared with those from the control reactor. Feeding is conducted every 7 days, and temperature and humidity measurements are performed daily (Popa & Green, 2012). The pH of the waste is measured at the beginning, after fermentation, and in the residue. Larval body mass is measured at the beginning, every 3rd and 6th day after the feeding rate, and at the conclusion of the research. Measurements every 3rd and 6th day after the feeding rate were conducted on 10% of living larvae population (Diener et al., 2011). The dry

weight of BSF larvae is measured at the beginning, midway, and at the end of the experiment. The measurements involve taking 5% of the BSF larvae, weighing them, and then subjecting them to a 105°C oven for 24 hours.

Throughout the research, it was uncertain whether all larvae would survive until the end, as some might die before reaching the prepupa stage. In such cases, the deceased larvae will be counted and recorded to calculate the survival rate among the larvae in the sample media used. Measurements of garbage dry weight are taken at the beginning, after fermentation, and at the conclusion of the experiment. The obtained residual data will be used to calculate the level of waste reduction achieved by BSF larvae during the study period (Diener, 2010). The measurement of carbon (C) and nitrogen (N) content in the waste is conducted on samples before fermentation, after fermentation, and at the end of the experiment. This procedure is performed to determine the C/N ratio of the waste at the end of the experiment. Assessing the C/N ratio of this waste residue is essential to gauge its potential for use as composting material.

3. Result and Discussion

3.1. Moisture Content

Initial characteristics are presented in Table 2. The optimal water content for larvae food falls within the range of 60-90%. Monitoring and controlling water content are crucial as it can significantly impact the growth of BSF larvae (Kroes, 2012). Furthermore, the calculation of the wet weight requirement is performed to determine the waste mixture composition for each reactor. The necessary wet weight of the waste is 323 grams per feeding. During the fermentation process, there will be a reduction in the fermented waste. Therefore, it is necessary to increase the sample by 20% to prevent a shortage in sample weight after fermentation. The results of water content measurements for the sample are presented in Table 3.

Table 2. Waste sample moisture content

Waste sample	Moisture content (%)
Mustard	88.98
Cabbage	86.60
Pawpaw	84.79
Banana	74.87

Table 3. Sample moisture content

Sample name	Initial moisture content (%)	Moisture content after fermentation (%)	Residual moisture content (%)
TR 13	90.86 ± 0.43	92.76 ± 0.45	93.91 ± 0.94
TR 10	91.01 ± 0.02	93.34 ± 0.11	95.33 ± 1.66
TR 7	91.11 ± 1.21	92.62 ± 0.12	93.87 ± 2.20
TR 4	90.05 ± 0.15	92.80 ± 0.23	93.88 ± 0.95
SOC 13	90.75 ± 0.44	93.57 ± 0.12	94.85 ± 0.86
SOC 10	91.35 ± 0.10	93.83 ± 0.10	94.75 ± 0.73
SOC 7	90.91 ± 0.79	93.26 ± 0.17	95.16 ± 0.45
SOC 4	90.26 ± 0.19	93.34 ± 0.26	94.54 ± 0.96
Control	91.61 ± 0.39	91.61 ± 0.37	91.90 ± 0.43

The water content ranges from 90% to 92%. The water content increases after fermentation, a result of the microbial process that degrades the waste, leading to higher water content in the sample. This degradation process can also result in the formation of alcohol compounds (Sudarmadji et al., 1989).

3.2. pH

It is essential to measure the initial pH of the sample to assess on the growth of BSF larvae. The initial pH falls within the range 6.5 to 7.0, which is still suitable for organisms to thrive and is conducive to to fermentation (Tchobanoglous et al., 1993). The pH measurements of the sample results are revealed in Table 4.

Table 4. Sample pH.

Sample Name	Initial pH (%)	pH after fermentation (%)	Residual pH (%)
TR 13	6.5 – 7.0	5.5 – 6.5	6.5
TR 10	6.5 – 7.0	5.5 – 6.5	6.5
TR 7	6.5 – 7.0	6.0 – 6.5	6.5
TR 4	6.5 – 7.0	6.0 – 6.5	6.5
SOC 13	6.5 – 7.0	5.5 – 6.5	6.5
SOC 10	6.5 – 7.0	5.5 – 6.5	6.5
SOC 7	6.5 – 7.0	6.0 – 6.5	6.5
SOC 4	6.5 – 7.0	6.0 – 6.5	6.5
Control	6.5 – 7.0	6.5 – 7.0	6.5

During the fermentation process, the pH will decrease. This decline is a consequence of the activity of microorganisms in decomposing organic materials within the substrate into organic acids (Yuwono, 2006).

3.3. C/N Ratio

The initial C/N ratio will be compared with the residual C/N ratio. This comparison is conducted to determine the effect of waste decomposition by BSF larvae on C-organic and total N-content. The C content serves as an energy source for microorganisms, while the N content is utilized for protein synthesis (Selintung et al., 2013). The results of measuring the C/N ratio of the sample are presented in Table 5.

Table 5. C/N Ratio of Sample.

Sample name	Initial C/N (%)	C/N after fermentation (%)	Residual C/N (%)
TR 13	36.69	36.44	74.91
TR 10	36.63	31.12	89.37
TR 7	36.40	34.09	66.30
TR 4	36.28	37.12	64.40
SOC 13	37.32	33.44	71.64
SOC 10	37.01	36.36	68.30
SOC 7	35.76	31.38	84.79
SOC 4	36.08	40.14	80.94
Control	36.94	36.94	62.11

Anaerobic fermentation produces methane (CH_4), carbon dioxide (CO_2), and other compounds such as organic acids (Fahlevi et al., 2021). The fermentation process reduces C-Organic levels due to the formation of these compounds.

In the vegetable - fruit sample, the C/N ratio was initially high. The reduction in C content in the residue is 1/5 of the C content after fermentation, while the N content in the residue decreased by 1/2 of the N content after fermentation. Consequently, the final ratio exhibits an increase in the ratio of C/N, indicating that BSF larvae absorb more N content. N levels are absorbed by BSF larvae to form cells and proteins (Wahyono et al., 2019). Additional treatment is needed for the residue to be used as organic compost. This involves, firstly, reducing the water content in the sample by up to 50%, and secondly, adding organic compounds or compost with a high N content.

3.4. Larvae Weight Gain

The weight growth of the larvae is calculated by taking 10% of the number of larvae, representing the growth rate of the larvae as a percentage. Data collection on larval growth was conducted every three days after feeding. The results of measuring the C/N ratio of the sample are presented in Figure 2.

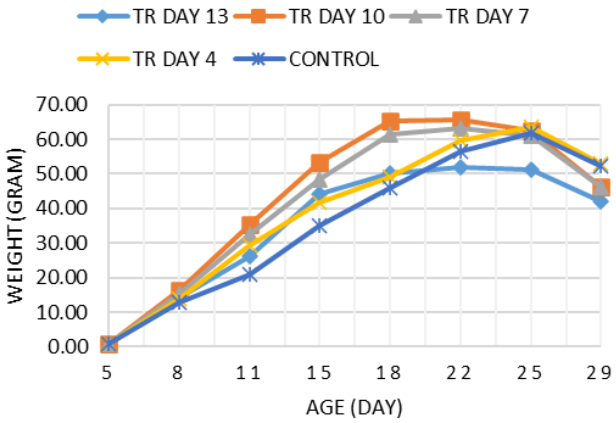

Figure 2. Larvae growth in Trichoderma fermenter

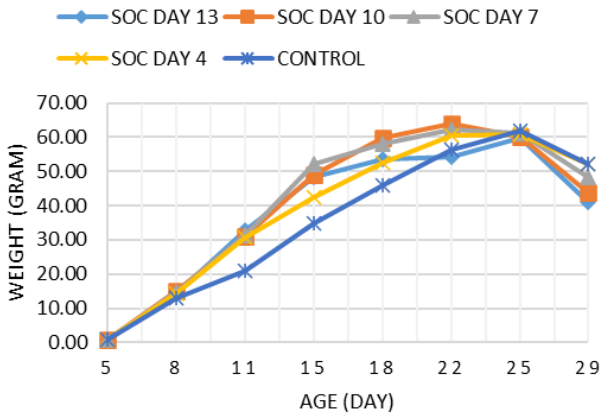

Figure 3. Larvaeg in SOC fermenter

Fermentation in waste plays an important role in increasing the growth of BSF larvae. When the eggs hatch, the larvae immediately start consuming the surrounding substrate. Larvae weight continues to increase until the prepupa phase. During this phase, the larvae do not engage in feeding activities, leading to a slight decrease in the body weight of the prepupae of BSF larvae (Wahyuni et al., 2020).

In the samples that underwent fermentation, the larvae weight tended to be higher at the ages of 18 days and 22 days, while the samples without fermentation exhibited a growth peak at 25 days of age. This pattern aligns with the acceleration development of prepupae in the fermented samples. Planning waste treatment using with the BSF larvae bioconversion method can benefit from the addiotionof fermentation process to expedite a larval growth and development. This also shorten the harvesting period of the larvae, contributing to increased waste reduction because the larvae aged 3-18 days are actively absorbing nutrients from the waste. Additional feeding after the larvae reach >18 days of age becomes inefficient.

3.5. Waste reduction

Waste reduction during fermentation with Trichoderma and SOC was calculated by subtracting the initial dry weight from the residual dry weight of each reactor.

Table 6. Waste Reduction by BSF.

Sample name	Initial weight (gram)	Residual weight (gram)	Reduction (%)
TR 13	70.19	30.29	56.85
TR 10	64.55	23.90	62.97
TR 7	71.53	32.40	54.71
TR 4	69.79	30.66	56.07
SOC 13	62.32	27.30	56.20
SOC 10	59.83	26.86	55.10
SOC 7	65.32	25.32	61.23
SOC 4	64.58	28.59	55.74
Control	81.32	40.12	50.67

The reduction value in the samples with fermentation was greater than in the samples without fermentation. The highest reduction value was observed in samples fermented with Trichoderma for 10 days, reaching 62.97%. Meanwhile, the lowest reduction value was found in the sample without fermentation, which was 50.67%. The lower residual yield in the unfermented market waste sample was due to its lower water content. Consequently, the dry weight was greater than that of the other samples. Bioconversion using BSF larvae is limited by the high cellulose content in the waste, while BSF larvae lack cellulose-degrading enzymes (Kim et al., 2011). The addition of fermentation can increase waste degradation by BSF larvae through the assistance of cellulose degrading enzymes produced by microorganisms in the fermenter used.

The level of waste reduction was then assessed using an ANOVA test to determine whether the addition of fermentation to the waste had an effect on increasing the reduction of biodegradable waste through the BSF larvae bioconversion method. The ANOVA test compared

the reduction of each reactor. One Way ANOVA analysis was used to determine the significance of differences in the effect of the fermentation duration and type of fermenter on waste reduction (Susanto, 2002). The ANOVA test was performed using a 95% confidence level, with a significance value of less than 0.05 ($P < 0.05$). The results of the ANOVA test showed that the addition of fermentation did not have a significant effect on increasing waste reduction, as P value was 0.929 ($P > 0.05$), signifying no significant impact. The observed increase in reduction was minimal, at 12.30%.

3.6. Survival Rate

The survival rate refers to the number of BSF larvae that endure throughout the process. The highest SR value (89.7%) was recorded in samples with Trichoderma fermentation for 10 days, while the lowest SR (84.5%) was observed in samples with Trichoderma fermentation for 13 days. Samples with a combination of vegetable and fruit waste exhibited an average survival rate of 87.9%.

Table 7. BSF larvae survival rates.

Sample name	Survival rate (%)
TR 13	84.5 ± 6.3
TR 10	89.7 ± 1.1
TR 7	88.9 ± 6.7
TR 4	86.8 ± 7.4
SOC 13	87.4 ± 6.0
SOC 10	87.9 ± 6.7
SOC 7	88.4 ± 0.7
SOC 4	88.4 ± 4.5
Control	88.9 ± 6.0

Feed media with high water content can lead to anaerobic conditions. According to Saragi's research in 2015, the anaerobic decomposition of organic matter produces NH_3 (ammonia) and CH_4 (methane), which can inhibit feed consumption and affect larval growth. The composition of feed waste, moisture content, and air temperature are factors that can influence larval weight and survival rate. The survival rate (SR) value provides a pattern of change as well as the pattern of larval growth. This pattern emerges because the feeding characteristics of the larvae vary according to the variations. Larvae that struggle to adapt or experience stress after being removed from the hatching medium may not survive. The survival rate of BSF larvae can be impacted by the water content in the feed since larvae generally prefer drier environments (Katayane et al., 2014), (Hakim, 2017), the nutritional quality of the feed (Hem & Saurin, 2011), and the temperature during the experiment (Zhang et al., n.d.).

4. Conclusion

The fermentation process applied to market waste for bioconversion processing with BSF larvae had no significant effect ($P = 0.929 > 0.05$) on increasing reduction. The largest reduction value was observed in market waste fermented with Trichodherma for 13 days, reaching 62.97%, while the market waste without fermentation exhibited a reduction of 50.67%. The fermentation treatment of market waste for bioconversion processing with BSF larvae did affects the growth of BSF larvae. Market waste fermented with Trichoderma for 10 days resulted in BSF larvae growth

at 18 days of age, whereas in non-fermented waste, BSF larvae growth peaked at 25 days. This implies that the process of harvesting larvae can be expedited in fermented waste. Fermentation also impacts the residue. In terms of quantity, samples fermented using Trichoderma for 10 days had lower residual weight compared to samples without fermentation. In terms of quality, the fermented sample exhibited a higher C/N ratio of 89.37, while the C/N ratio of the unfermented sample was 62.11.

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