Growth and Development Performance of *Hermetia illucens* L. (Diptera: Stratiomyidae) Larvae on Fermented Palm Kernel Meal (PKM) Substrate

Naomi Florenata Damanik¹, Ramadhani Eka Putra¹*, Ida Kinasih², Agus Dana Permana¹

¹School of Life Sciences and Technology, Bandung Institute of Technology, Bandung 40132, Indonesia
²Department of Biology, Faculty of Sciences and Technology, Universitas Islam Negeri Sunan Gunung Djati, Bandung, Indonesia

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ABSTRACT

Larvae of *Hermetia illucens* (Black Soldier Fly/BSF) are considered agents of bioconversion of organic waste, including by-products of agroindustrial waste. Palm kernel meal (PKM), a palm oil mill waste contains high lignocellulose, making it difficult for BSF larvae to digest in which pre treatment process is required. This study aims to analyze the growth and development performance of BSF larvae in fermented PKM waste using EM4 and molasses as the pretreatment process. Five (5) days old BSF larvae were reared in PKM waste, which was fermented with EM4 and molasses for 2, 3, and 4 days (F2, F3, and F4) and with water for four days (FA) while chicken feed (PA) applied as control. During this study, growth rate, average weight, developmental period, survival rate, and larval development period. Feed efficiency and feed reduction analyzed by efficiency of conversion of digested food (ECD), feed conversion ratio (FCR), waste reduction index (WRI) and substrate consumption rate (SCR). The growth and development performance of F2 group was the best among other treatment groups which is similar to larvae fed on chicken feed as a control. All treatments showed high larval survival rates (99.72-100.00%). On the other hand, the best reduction efficiency recorded in F3. Based on these result it can be concluded that pretreatment of PKM by EM4 and molasses is applicable to improve the quality of PKM as feeding material for BSF larvae.

1. Introduction

Organic waste management by macrophage has been emerging as a promising method to solve waste problem in municipal and industry. One of the most promising macrophage is black soldier fly (BSF) (*Hermetia illucens* L.). This insect originated from South American savanna (Liu et al. 2019) which undergoes a complete metamorphosis process (holometabola) which consists of the life stages of egg–larvae–prepupae–pupae–adult (Dzepe et al. 2020). This insect spend most of their adult phase to mating which limit their distribution to organic wastes pile while lacking interaction to fresh food. This behavior made them not considered as vector disease to human and animals (Banks et al. 2014; Oliveira et al. 2016; Liu et al. 2017). On the other hand, the larval phase of BSF insects is known as saprophagous (Diener et al. 2011; Liu et al. 2019; Scala et al. 2020). Black soldier fly larvae has capability to consume various types of organic wastes such as household organic waste, kitchen waste, and even agriculture and industry to animal waste and humans (Bondari and Sheppard 1987; Sheppard et al. 1994; Newton et al. 2005; Diener et al. 2011; Li et al. 2011; Zheng et al. 2012a, 2012b, Lalander et al. 2015; Nguyen et al. 2015; Manurung et al. 2016; Abdull et al. 2017a, 2017b; Jucker et al. 2017; Spranghers et al. 2017; Kinasih et al. 2018; Bava et al. 2019; Julita et al. 2019; Liu et al. 2019; Elsayed et al. 2020).

The ability of BSF larvae to consume various types of organic waste is due to the diverse and unique microbial communities inhibits the gut of larvae (Lee et al. 2014, 2016; De Smet et al. 2018). The final products of consumption are biomass that rich in protein and fat (Liu et al. 2019) which applicable as animal fed (St-Hilaire et al. 2007) and the residue which can also be used as good-quality compost (Liu et al. 2019).
Indonesia is known as one of the largest palm oil-producing countries in the world, according to BPS (2020), the production of palm oil and palm kernel in Indonesia can reach 44,759,147 and 8,951,829 tons per year. Based on palm kernel production, the by-product of 45% palm kernel meal (PKM) can become waste (Pasaribu 2018). Oil and palm kernel production waste in PKM is still used as an alternative feed for livestock because the nutritional content is still good. PKM contains protein (14–17%), lipid (9.1–10.5%) and fiber (12–18%), but there is also a lignin content of 13.6% (Sekoni et al. 2008; BPTP 2015). The protein and lipid content of PKM are important for the growth and development of H. illucens larvae (Julita et al. 2018; Gao et al. 2019; Liu et al. 2019). However, BSFL larvae does not have specific enzymes for digesting lignin material (Kim et al. 2011) and may highly depend on the microbial symbiont to convert lignocellulose into simple sugars or monosaccharides (Kim et al. 2011; Lee et al. 2014). Studies showed that by only depending on the internal symbiont, the larval developmental time is longer and size of final biomass is smaller than BSF larvae fed on feeding material low in lignin (Manurung et al. 2016; Supriyatna et al. 2016; Abduh et al. 2017a; Palma et al. 2019).

Another approach has been developing to overcome this challenge by fermentation of feeding material. Microbe may alter the structure of feeding material made it more nutritious and easier to digest (Bianco et al. 2020). Studies showed the benefit of the fermentation of lignocellulose material to growth of BSF larvae (Gao et al. 2019; Liu et al. 2021; Permana et al. 2021, 2022). However, there is lack information on the effect of fermentation duration of lignocellulose material to growth dan development performance of BSF larvae. Furtherly, the study on the effect of fermentation of high protein feeding material to growth performance of BSF larvae also lacking. PKM is considered a material that rich in protein while also has significant lignocellulose which made it a suitable material to answer research questions. Furtherly, the result of this study may provides additional knowledge on the application of BSF larvae for agricultural wastes that rich in lignocellulose.

2. Materials and Methods

2.1. Study Site

This research was conducted in the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB), Toxicity Test Laboratory from January-August 2022.

2.2. Materials

Hermetia illucens’ eggs used in this study were mass-reared in the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB), Toxicity Test Laboratory. Eggs were hatching on chicken feed, and then the 5-day-old H. illucens larvae were taken and grown on the treatment of palm kernel meal substrates. Palm kernel meal (PKM) is obtained from a palm oil industrial factory in North Sumatra while chicken feed (Hi Pro – Vite 511®) purchased from local poultry shop. EM4 and molasses were obtained from local agricultural shop.

2.3. Pretreatments

Pretreatment was carried out by fermentation using livestock EM4 and molasses. The fermentation solution is made with a ratio of EM4: molasses: distilled water 1: 1: 1000 ml (according to the packaging for animal feed). The fermentation solution is mixed with the PKM substrate with a ratio of PKM substrate: fermentation solution, namely 1:2.2. Fermentation is carried out using tightly closed plastic and stored at room temperature in Toxicity Test Laboratory with fermentation duration of 2, 3 and 4 days, whereas, without fermentation EM4 and molasses are only made by mixing PKM substrate and water with the same ratio of 1:2.2 and stored in tightly closed plastic for four days. The ratio between PKM and water is essential because substrate moisture affects the growth and development of H. illucens larvae. The comparison of PKM and water used in this study refers to Caruso et al. (2014).

2.4. Treatment

BSF larvae were divided into 5 feeding treatment groups, which was PKM fermented with EM4 and molasses for two days (F2), three days (F3), four days (F4), and PKM soaked in water for four days (FA). Chicken feed (PA) was used as a control to compare PKM substrates. Each groups had five replications and 15 larvae for each replication. The larvae provided with feeding material with rate of 100 mg/larvae/day.

2.5. Growth and Development

The growth rate was analyzed by calculating the difference between the larvae’s final average weight and the larvae’s initial average weight divided by...
2.8. Data Analysis

The statistical analysis used was the Kruskal-Wallis test to analyze significant differences in growth rate, biomass growth, length, development period, and survival rate of ECD, FCR, WRI and SCR. The Kruskal-Wallis test was accomplished because the data did not meet the normality and homogeneity assumptions after the normality test using the Shapiro-Wilk test and the homogeneity test using the Levene test. Then, further analysis was done using Pairwise-Wilcoxon to find significant differences between treatments (Dzepe et al. 2020; Purba et al. 2021). Correlation analysis of ECD-FCR and WRI-SCR was also performed using the Spearman correlation test (Kawasaki et al. 2022; Ma et al. 2022). Each with a 95% confidence level and using the R Studio application version 4.2.0.

3. Results

3.1. Growth

Larvae had the highest growth rate in chicken feed. However, the growth rate between the four treatments of fermented PKM and pretreated with water was not significantly different and lower than chicken feed as a control. Nevertheless, among PKM treatments applied in this study, 2-day fermented PKM (F2) had a higher growth rate (Figure 2).

3.2. Development

The development performance of BSF larvae was analyzed by the survival rates and development time to reach the prepupal phase. Larvae performed the shortest time to develop into prepupae in chicken feed (PA) and were not significantly different from 2-day (F2) and 3-day (F3) fermented PKM (Table 1). On the other hand, larvae fed in PKM pretreated with water (FA) had a longer larval developmental time than all treatments and control. However, all treatments showed high larval survival rates and were not significantly different from chicken feed (PA).

3.3. Efficiency of Conversion

BSF larvae had high conversion efficiency (ECD) in chicken feed (PA). They were significantly different with 4-day fermented PKM and PKM pretreated with water. There is no significant difference among PKM treatments. However, the results showed that conversion efficiency tends to be low in 4-day fermented PKM and PKM pretreated with water compared to chicken feed (Figure 3).
Mass loss through metabolism (grams)

Total feed (gram) → H. illucens larvae → Prepupal biomass (gram) → Residue (gram)

Figure 1. Main concept of mass balance in Hermetia illucens larvae

Figure 2. The growth rate of BSF larvae fed on fermented palm kernel meal (PKM). Graph bars followed by different letter had the value significantly different among others (P<0.05)

Table 1. Development time of H. illucens larvae on fermented PKM and chicken feed

<table>
<thead>
<tr>
<th>Feed</th>
<th>Larval development time (days)</th>
<th>Larval survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-days fermented PKM</td>
<td>17.00±0.00a</td>
<td>99.88±0.44a</td>
</tr>
<tr>
<td>3-days fermented PKM</td>
<td>17.60±1.34a</td>
<td>99.87±0.48a</td>
</tr>
<tr>
<td>4-days fermented PKM</td>
<td>20.60±1.34a</td>
<td>99.72±1.09a</td>
</tr>
<tr>
<td>4-days water soaked PKM</td>
<td>23.60±1.34a</td>
<td>99.80±0.76c</td>
</tr>
<tr>
<td>Chicken feed</td>
<td>15.80±1.64a</td>
<td>100.00±0.00a</td>
</tr>
</tbody>
</table>

Larval development time and survival rate values followed by different letter on the same column vary significantly (P<0.05)

On the other hand, the food conversion ratio (FCR) was used to measure the ratio of feed converted to biomass. The more efficient larvae convert feed to biomass, usually followed by less feed needed to consume. The result showed that larvae fed on chicken feed (PA) also had the lowest FCR. On the other hand, larvae fed on PKM pretreated with water showed a higher ratio among all PKM treatments and were significantly different (Figure 3).

Spearman’s correlation test a strong negative correlation (p-value < 0.05; R > 0.70), which means there were feed influences in the larval digestibility efficiency (Figure 4). This result indicated that higher FCR will followed by low ECD.

3.4. Efficiency of Reduction

The efficiency of reduction performance was analyzed by waste reduction index (WRI) and substrate consumption rates (SCR). The highest waste reduction index was achieved by chicken feed (PA) and significantly different with fermented PKM and PKM pretreated with water (p-value > 0.05), which also showed the highest substrate consumption rates.

Figure 3. The efficiency of conversion of digested food (ECD) (left) and food conversion ratio (FCR) of BSF larvae fed on fermented palm kernel meal (PKM) and commercial chicken feed. Graph bars followed by different letter had the value significantly different among others (P<0.05)
Among the PKM treatments, 3-day fermented PKM showed the highest substrate consumption rate, though the substrate consumption rate was not significantly different with PKM soaked in water (Figure 5). Compared to control, the substrate consumption rate of treatment significantly lower.

Spearman’s correlation was also analyzed to confirm the relationship between WRI and SCR (Figure 6). It showed a medium positive correlation between WRI and SCR ($R = 0.79$), which means the index reduction has a correlation with substrate consumption rates that shows the higher the ability of larvae to eat the feed given will affect a higher reduction index.

### 3.5. Mass Balance

In summary, all the performances of growth and development of BSF larvae fed with fermented PKM, soaked in water and chicken feed for mass production can be shown in mass balance. Mass balance as a proportion of feed used for biomass, metabolism and the residue were analyzed (Figure 7). The results showed that the range of the proportion of feed for biomass in PKM treatments (fermented and soaked in water) was 7.93–12.08%. Moreover, the proportion of chicken feed for biomass was 17.95%. The proportion of metabolism in PKM treatments was 20.09–27.16%, and in chicken feed was 25.73%. Using this mass balance, the proportion of residue in PKM treatments was...
62.15–69.77%. Instead, in chicken feed, it was 56.32%. The proportions were various, and it can be influenced by the type of feed given by different pretreatment.

4. Discussion

4.1. Growth

The results showed that BSF larvae fed with chicken feed (PA) had a higher growth rate than palm kernel meal (PKM). It could be related to nutrient content of feeding material, especially protein and carbohydrate. The chicken feed contains 21–23% protein and a maximum crude fiber of 5% (Simanjuntak 2018) while fresh PKM contains 21–23% crude fiber and 14–19.24% crude protein (Ezieshi and Olomu 2007; Sekoni et al. 2008; BPTP 2015). Studies showed that low ratio of protein and carbohydrate improve the growth rate (Barragan-Fonseca et al. 2019; Putra et al. 2020; Eggink et al. 2023). Chicken feed has high carbohydrate to provide energy needed either for biomass production and egg production (Al-Kendi and Al-Gubary 2022).

On the other hand, PKM generally consists of three forms of crude fiber: hemicellulose, cellulose, and lignin and the lignin content is about 12.0–13.6% (Sekoni et al. 2008; Caruso et al. 2014), whereas chicken feed does not contain lignin. High fiber tends to affect low digestibility in larvae. This condition could trigger the compensatory growth (Supriyatna et al. 2016), which makes the larvae allocate the energy obtained to maintain its viability to continue its development, by reducing the proportion of energy to biomass.

Even though it has been given pretreatment with fermentation using commercially available EM4 and molasses, the growth rate is similar with the pretreated without EM4 and molasses. There are three possible hypotheses: (1) the concentration of EM4 in the livestock used in this study was too low. Study by Firdausy et al. (2022) reported the improvement of growth rate of BSF when organic waste pretreated by EM4 fermentation. Hence, for further research could be design to find the best amount of EM4 application; (2) the duration of fermentation. The longer the fermentation is likely to be ineffective because it may reduce other nutrient content, too, such as protein (Mulyono et al. 2019). However, it is good for converting crude fiber consists of hemicellulose, cellulose, and lignin into simple sugars (Caruso et al. 2014); (3) types of microbes. The result on the growth rate of PKM fermented by commercial EM4 and naturally occurring microbes was not significant different. This could indicated that microbes of commercial EM4 relatively unsuitable for fermentation of PKM. Exploring the naturally occurring microbes in the future research could solve this problem.

Compare to other study on other lignocellulose feeding materials, BSF larva growth rate recorded in this study was higher than some materials (e.g. coconut testa, banana peel, spent coffee ground) (Table...
2). This result may showed the benefit fermentation to provides necessary nutrition for BSF larvae.

Another possible explanation of this finding is the availability of the protein on the feeding material. For example, coconut Testa had 7% protein and 17% crude fiber (Appaiah et al. 2014; Putra et al. 2020), banana peel had 1% protein and 30% crude fiber (Anhwange et al. 2009; Putra et al. 2020) and spent coffee ground had 19.93% protein and 18.8% crude fiber (Permana et al. 2018). All of these materials has lower protein content than PKM (14.0–19.24%) and brewery by-products (20.05%) which produced better growth rate than previous materials. This protein could be used by BSF larvae as material for improve their grow rate as omnivore insect, like BSF larvae, more likely to regulate protein than carbohydrate (Wang et al. 2018). By this, this insect more likely to suffer growth deficiency when received insufficient amount of protein (Simpson and Raubenheimer 2005).

### 4.2. Development

The result increasing developmental time with increasing fermentation time. It could related to decreasing nutritional content of substrate. According to Gustika (2022, unpublished data), the percentage of carbohydrates in fresh PKM decreased from 62.85% to 24.32% while protein content decreased from 16.05% to 7.22% after fermentation for seven days. It is aligned with Myers et al. (2008) and Miranda et al. (2021) that the quality and quantity of feed influence the development time of larvae such as feed rate, moisture, and nutrients (carbohydrate, protein, fat and fiber). Significant increasing of development time related to physiological requirement of protein for development as larvae required specific amount of accumulated protein to continue their development into next phase (Oonincx et al. 2015; Barragan-Fonseca et al. 2018; Gold et al. 2018; Lalande et al. 2019). On the other hand, fermentation duration did not have significant negative impact to mortality of larva which indicated the level of nutrition of PKM fullfilled minimum requirement for larva.

In comparison with the previous study used lignocellulose substrate, 2-day fermented PKM larval development time was shorter than most of result from various studies (Table 3). However, it is slightly longer than fermented soybean husk (Permana et al. 2022) and brewery's spent grain (Liu et al. 2018). Longer developmental time could be related to higher protein content and availability of carbohydrate (from digestible carbohydrates) from substrate as both soybean husk and brewery's spent grain relatively lack of lignin compare to PKM. During feeding process some of the hemicellulose could digested by microbes and increased the carbohydrate content providing energy source for larva. This condition could altered the protein : carbohydrate ratio to suitable level for larva development which tend to by low protein and high carbohydrate (Barragan-Fonseca et al. 2019, 2020). On the other hand, the result on development period confirmed the growth rate plasticity oft BSF larvae which can consume varied nutrient compositions but will take longer to develop when fed in poor-quality substrates (Dmitriew 2011; Banks et al. 2014).

### 4.3. Efficiency of Conversion

The efficiency of conversion of digested feed (ECD) is essential as information for the utilization

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**Table 2. Comparative data on the growth rate of H. illucens larvae on fermented PKM with various lignocellulose substrates**

<table>
<thead>
<tr>
<th>Feed</th>
<th>Growth rate (mg/day)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut Testa</td>
<td>0.07</td>
<td>(Putra et al. 2020)</td>
</tr>
<tr>
<td>Banana peel</td>
<td>0.08</td>
<td>(Putra et al. 2020)</td>
</tr>
<tr>
<td>Spent coffee ground</td>
<td>1.41</td>
<td>(Permana et al. 2018)</td>
</tr>
<tr>
<td>4-days fermented PKM</td>
<td>1.94</td>
<td>This study</td>
</tr>
<tr>
<td>2-days fermented PKM</td>
<td>2.75</td>
<td>This study</td>
</tr>
<tr>
<td>Winery by-product</td>
<td>6.00</td>
<td>(Meneguz et al. 2018)</td>
</tr>
<tr>
<td>Brewery by-product</td>
<td>14.00</td>
<td>(Meneguz et al. 2018)</td>
</tr>
</tbody>
</table>

**Table 3. Comparative data on the development time of BSF larvae on fermented PKM with various lignocellulose substrates**

<table>
<thead>
<tr>
<th>Feed</th>
<th>Development time (days)</th>
<th>Survival rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewery's spent grain</td>
<td>14.97</td>
<td>98.00</td>
<td>(Liu et al. 2018)</td>
</tr>
<tr>
<td>Fermented soybean husk</td>
<td>15.00</td>
<td>98.80</td>
<td>(Permana et al. 2022)</td>
</tr>
<tr>
<td>2-days fermented PKM</td>
<td>17.00</td>
<td>99.88</td>
<td>This study</td>
</tr>
<tr>
<td>Fermented banana peel</td>
<td>19.20</td>
<td>98.30</td>
<td>(Permana et al. 2022)</td>
</tr>
<tr>
<td>4-days fermented PKM</td>
<td>23.60</td>
<td>99.80</td>
<td>This study</td>
</tr>
<tr>
<td>Fermented maize waste</td>
<td>24.20</td>
<td>93.00</td>
<td>(Gao et al. 2019)</td>
</tr>
<tr>
<td>7-days fermented PKM</td>
<td>28.00</td>
<td>96.00</td>
<td>(Gustika 2022; unpublished data)</td>
</tr>
<tr>
<td>Fermented barley waste</td>
<td>31.00</td>
<td>98.67</td>
<td>(Permana et al. 2021)</td>
</tr>
<tr>
<td>Rice straw</td>
<td>38.20-54.20</td>
<td>51.21-98.27</td>
<td>(Manurung et al. 2016)</td>
</tr>
</tbody>
</table>
of H. illucens larvae on a production scale because it is related to the biomass (yield) of larvae and the nutritional content produced so that it can be reused as alternative feed. The ECD value determines feed conversion efficiency used to consider the quality and quantity of organic substrates used for the growth of BSF larvae (Supriyatna et al. 2016; Pliantiangtam et al. 2021; Permana et al. 2022).

The result showed short fermentation duration improved the digestibility of PKM close to digestibility of commercial chicken feed. This value significantly better than application of naturally occurring microbes (FA treatment). It seem that commercial E4 include microbes with ability to digest fiber This is presumably due to pretreatment by EM4 and molasses may decrease the crude fiber of PKM and increase the larvae's digestibility (Pamintuan et al. 2020; Permana et al. 2021). Thus, the 2-day fermented PKM can be considered for BSF larvae growth substrate.

Furthermore, feed conversion efficiency can also be seen from the FCR (food conversion ratio) to determine the feed ratio the larvae can convert into biomass. The more efficiently the larvae use feed for processing bioconversion, the smaller the FCR (Broeckx et al. 2021). This study also confirmed the negative correlation between ECD dan FCR. Low FCR indicated that the substrate was unsuitable for feeding material (Nyakeri 2017) and this furtherly explained lower growth rate of larvae for substrate with low FCR. However, in this study the correlation between both value was not strong which indicated that consumed feed not all converted into biomass especially on the low quality feeding material.

4.4. Efficiency of Reduction

This study showed correlation between substrate digestibility, growth rate, dan the rate of waste reduction. Just as nutritional composition affects the digestibility of larvae, so does the waste reduction ability of larvae. In this study, we found similar WRI among treatment groups However, the substrate consumption was not significantly different from that of PKM soaked in water. The similarity indicated that fermentation may not significantly change the palatability of PKM. In addition, the lowest consumption rate obtained at 4-day fermented PKM could related to lower nutrition content and increased acidity of the substrate due to additional acid produced by fermentation (Lim et al. 2019). Based on this result, if the purpose of application of BSF larvae is to reduce the waste then the 3-day fermented PKM can be considered.

There was negative correlation between WRI and SCR yet the correlation was not strong. It could be caused that not all reduction caused by consumption by larvae. Thus, there is possibility that eventhough the FCR is high, however the substrate physical condition may not change much or converted into other material that not significantly lighter than previous material.

4.5. Mass Balance

The relatively high proportion of biomass indicates that the substrate utilized for the formation of biomass and high conversion efficiency. BSF larvae consumed the chicken feed more likely to convert it into biomass than treatment groups. High consumption rate also correlated to the amount of residues which were the lowest.

Among treatment groups, BSF larvae feed on the PKM soaked in the water for 4 days (FA) utilized most of the substrate for metabolism. There are several hypothesis to explain this result

• Higher metabolism could related to the effort of larvae to maintaining homeostasis through energy generating. Study showed that unsuitable substrate could lead to increasing allocation of food for energy (Barragan-Fonseca et al. 2018; Laganaro et al. 2021).
• Change in substrate conditions such as acidity, water content, and bulk density. Keeping PKM inside the water could alter the physical properties of substrate by increasing the water content and bulk density and effecting the BSFL larvae (Bekker et al. 2021; Yakti et al. 2023). High water content and bulk density reduce the aeration for larvae which lead to increasing effort for homeostasis (Palma et al. 2018; Lalander et al. 2020). In more extreme case, some larvae could be drown (Lalander et al. 2020).

In conclusion, short fermentation duration of PKM by commercial EM4 could be considered as pretreatment from BSF larvae substrate to improve the digestibility of substrate. Fermentation may alters the substrate nutrient content and physical properties which influence the growth performance of BSF larvae. Further study should carried out to find the most optimum concentration of EM4 for pretreatment of PKM for BSF larvae substrate.
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