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Black Soldier Fly Larval Valorization Benefitting from Ex-Situ Fungal Fermentation in Reducing Coconut Endosperm Waste

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Abstract: Oftentimes, the employment of entomoremediation to reduce organic wastes encounters ubiquitous shortcomings, i.e., ineffectiveness to valorize recalcitrant organics in wastes. Considering the cost-favorability, a fermentation process can be employed to facilitate the degradation of biopolymers into smaller organics, easing the subsequent entomoremediation process. However, the efficacy of in situ fermentation was found impeded by the black soldier fly larvae (BSFL) in the current study to reduce coconut endosperm waste (CEW). Indeed, by changing into ex situ fermentation, in which the fungal *Rhizopus oligosporus* was permitted to execute fermentation on CEW prior to the larval feeding, the reduction of CEW was significantly enhanced. In this regard, the waste reduction index of CEW by BSFL was almost doubled as opposed to in situ fermentation, even with the inoculation of merely 0.5 wt % of *Rhizopus oligosporus*. Moreover, with only 0.02 wt % of fungal inoculation size to execute the ex situ fermentation on CEW, it could spur BSFL growth by about 50%. Finally, from the statistical correlation study using principal component analysis, the presence of *Rhizopus oligosporus* in a range of 0.5–1.0 wt % was regarded as optimum to ferment CEW via ex situ mode, prior to the valorization by BSFL in reducing the CEW.

Keywords: black soldier fly; ex situ fermentation; entomoremediation; coconut endosperm waste; valorization; *Rhizopus oligosporus*

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1. Introduction

Of late, *Hermetia illucens*, also known as black soldier fly, has been introduced in entomoremediation not only to treat phytoextraction-polluted biomass [1] but also to decontaminate the polluted soil in its larvae form [2]. The black soldier fly larvae (BSFL) have been widely employed as one of the waste management measures that is capable of reducing the mass of organic wastes, including animal manure, food waste and municipal

organic wastes [3–5]. The larval employment has also succeeded in overall bacteria reduction throughout entomoremediation [6]. The mature BSFL can be subsequently harvested, serving as a protein sources for animal feed. The larval biomass has also been extensively researched to replace common protein sources in aquaculture and animal farming [7–9]. The incorporation of harvested BSFL into the human diet in the form of textured protein may be possible, but this is limited by social stigma and regulatory laws. Thus, the forthcoming research studies are vitally important to sustain the human needs in the future [10].

Often, pre-treatment of organic wastes via anaerobic digestion and fermentation modes is needed prior to the introduction of BSFL to enhance the waste reduction performance [11,12]. Fermentation is described as biocatalyzing activities executed by microorganisms on available nutrients and the subsequent synthesis of secondary metabolites in completing other functional activities under the presence (aerobic) or absence (anaerobic) of dissolved oxygen [13]. Few studies have been carried out previously to determine the fermentation conditions required during BSFL-assisted bioconversion activities, such as inoculation of bacteria and fungus into the solid waste materials, to enhance the BSFL growth performance and waste reduction efficiency [14–16].

Rhizopus oligosporus, also known as tempeh starter, is mainly used in tempeh production in which it serves as a low-cost protein source packed with high nutrition [17]. The capability of *R. oligosporus* to produce several enzymes and synthesize intermediate metabolites has boosted the protein availability after the fungal fermentations [18]. In this study, the *R. oligosporus* was introduced into coconut endosperm waste (CEW) to execute fermentation processes through in situ and ex situ modes for larval feeding. By adopting these pre-treatment approaches, the presence of enzymes and intermediate metabolites may aid in BSFL nutrient assimilation to achieve better growth performance and effective waste reduction. The aim of this study was to determine the effect of different inoculum sizes of *R. oligosporus* on BSFL growth performance as well as efficiency of CEW reduction in both in situ and ex situ fungal fermentation modes. The comparative study could further refine the fermentation approach that could be adopted to spur the organic wastes reduction via entomoremediation by BSFL.

2. Materials and Methods

2.1. Activation of Rhizopus Oligosporus and Spore Suspension Preparation

Prior to *Rhizopus oligosporus* activation, sterile potato dextrose broth (PDB) (Sigma Aldrich, St. Louis MO, USA) and potato dextrose agar (PDA) (Merck, Kenilworth, NJ, USA) were prepared by adding 6 g of dehydrated medium into 250 mL of sterile distilled water, and 39 g of dehydrated medium into 1 L of distilled water, respectively. Then, both the PDB and PDA were immediately autoclaved at 121 °C for 15 min. The 20 g of commercially-available *R. oligosporus* spores (Raprima Brand, Bandung, Indonesia) tempeh starter were activated in sterile PDB cultivation at a total volume of 250 mL. An incubator shaker (Lab Companion SI 600, Daejeon, Korea) set at 30 °C of incubating temperature and 180 rpm of rotating speed was used during the activation for a period of 48 h. Subsequently, 1 mL of activated *R. oligosporus* culture was aseptically transferred into the sterile PDA and spread by using an L-glass rod, until the liquid was fully absorbed by the agar, and incubated at 30 °C for 7 days or until the spores could be observed. The sterile distilled water was later added into the PDA, and a sterile inoculating loop was used to separate the spores from the agar medium. The distilled water containing the spores was collected and adjusted to approximately 1.0×10^6 spores/mL, which was determined by the cell counting method [18]. The spore suspension was finally stored at 4 °C until it was used as inoculant in coconut endosperm waste to execute the in situ and ex situ fermentation processes.

2.2. Growing Black Soldier Fly Larvae (BSFL)

Fresh BSFL egg clutches were procured locally from MLF Ingredient Sdn Bhd, Johor, Malaysia, transported to the laboratory and transferred into a sterile Petri dish containing a

wet filter paper to minimize the moisture loss during incubation. Then, the Petri dish was incubated at 27 °C until eclosion took place. The neonates were collected and reared with fresh coconut endosperm waste (CEW) until 6 days old prior to use in experiments [15]. Accordingly, the fresh CEW was bought from a local coconut milk store located in Seri Iskandar, Perak, Malaysia.

The moisture content of CEW was initially adjusted to 70% prior to *R. oligosporus* inoculation [15]. Each concentration of inoculum of *R. oligosporus* spore suspension, namely 0.02 wt %, 0.1 wt %, 0.5 wt %, 1.0 wt %, 1.5 wt %, 2.0 wt % and 2.5 wt %, was added into 10 g of fresh CEW to carry out fermentation processes via in situ and ex situ modes. The in situ mode was different from ex situ by having BSFL inoculating at the stage of fermentation, whilst the ex situ mode only had the BSFL inoculation after the completion of the fermentation process (72 h in this study). The larvae to CEW ratio was maintained at 20 BSFL over 10 g of CEW in a dry weight basis for every setup. A controlled setup with only 10 g of fresh CEW (dry weight) and inoculated by 20 BSFL was prepared for both in situ and ex situ fermentation modes. Each of the BSFL rearing setups used a 6 cm × 8 cm (diameter × height) ventilated transparent polyethylene container. Throughout the rearing period, the moisture content of CEW for all setups was maintained around 70%, and the BSFL rearing was discontinued when the larvae reached the fifth instar, indicated by the presence of a grayish cream body color and head size of more than 0.9 mm [19,20]. The larvae from every setup were harvested by hand picking, washed under running tap water, rinsed with distilled water, deactivated at −20 °C for five minutes and dried at 60 °C until a constant weight was obtained [15,21]. The remnant CEW was also collected and dried at 105 °C until a constant weight was achieved.

2.3. Measured Parameters from BSFL Growth

The total weight gained of BSFL was measured to determine the growth of BSFL from consuming the fermented CEW in completing the entomoremediation process. The growth of BSFL was also measured in terms of growth rate, signifying the average daily weight growth of BSFL.

$$\text{Total weight gained (g)} = \text{Final total BSFL dry weight (g)} - \text{Initial total BSFL dryweight (g)} \quad (1)$$

$$\text{Growth rate (g/day)} = \text{Total weight gained (g)}/\text{Rearing period (day)} \quad (2)$$

The degree of CEW reduction was determined by overall degradation, implying the total CEW weight decrement upon the BSFL entomoremediation on a dry weight basis. At the same time, the rate of CEW reduction was also calculated in terms of waste reduction index, denoting the rate of reduction of CEW throughout the rearing period.

$$\text{Overall degradation (\%)} = \text{Total CEW ingested (g)}/\text{Total CEW offered (g)} \times 100\% \quad (3)$$

$$\text{Waste reduction index (g/day)} = \text{Total CEW ingested (g)}/\text{Rearing period (day)} \quad (4)$$

2.4. Statistical Analysis

All parameters were conducted in triplicate for each treatment level. The Anderson–Darling normality test (α-level of 0.05) was then performed followed by one-way ANOVA (α-level of 0.05) incorporated with the least significant difference (LSD) test with ($p > 0.05$) using Minitab 17 Statistical Software (Minitab Inc., Pennsylvania, United States). The function of these statistical analyses was to verify the effect of different inoculum sizes of *R. oligosporus* on CEW reduction targeting on overall degradation and waste reduction index parameters.

3. Results and Discussion

3.1. Larval Growths

At the end of entomoremediation, the 20 mature BSFL from each setup were harvested and measured in terms of overall larval growths. As presented in Table 1, a negligible effect arising from the in situ fermentation treatment mode could be concluded as the

total weight gained, and the growth rate of BSFL was merely fluctuating in narrow ranges, namely 0.65–0.76 g and 0.055–0.065 g/day, respectively. These results vindicated that the inoculation of *R. oligosporus* in CEW to perform in situ fermentation was infeasible to aid the BSFL growth, since its impact was negligible.

Table 1. Growth performances of black soldier fly larvae (BSFL) fed with in situ fermented coconut endosperm waste (CEW). Values are mean ± standard deviation (n = 3).

R. oligosporus Inoculum Size (wt %)	Total Weight Gained (g)	Growth Rate (g/day)
0 (Control)	0.71 ± 0.06	0.059 ± 0.005
0.02	0.77 ± 0.05	0.065 ± 0.004
0.1	0.76 ± 0.01	0.063 ± 0.001
0.5	0.73 ± 0.03	0.061 ± 0.002
1.0	0.71 ± 0.04	0.059 ± 0.003
1.5	0.76 ± 0.04	0.063 ± 0.004
2.0	0.65 ± 0.01	0.055 ± 0.001
2.5	0.75 ± 0.02	0.063 ± 0.002

On the other hand, the ex situ fermentation executed by a similar fungus on CEW enhanced the BSFL growth upon feeding, as demonstrated in Table 2. Under the ex situ fermentation treatment mode, the total weight gained of BSFL was significantly increased from 0.70 g under the control to 1.08 g when merely inoculated with 0.02 wt % of fungal inoculum size. With further increases of *R. oligosporus* inoculum concentration from 0.5 wt % to 2.5 wt %, the total weight gained of BSFL fluctuated between 0.95 and 1.13 g. Moreover, the growth rate of BSFL under the control was only 0.058 g/day, and this value was increased to 0.09 g/day at only 0.02 wt % of *R. oligosporus* inoculum. Nevertheless, the growth rates were later maintained around 0.08–0.094 g/day with the increase of *R. oligosporus* inoculum concentration. These findings showed that although the various *R. oligosporus* inoculum concentrations did not exert a positive impact on BSFL growth substantially, especially from the in situ fermentation treatment mode, the differences in larval growth performances between in situ and ex situ fermentations were still very significant. Therefore, the choice of fermentation mode plays a vital role in promoting BSFL growth, since the total weight gained and growth rate of BSFL could be, respectively, different by approximately 26–63% compared to that between the in situ and ex situ fermentation treatment modes. Compared to a previous study by Wong et al. [15], the BSFL had slightly lower total weight gain but higher growth rate under ex situ condition.

Table 2. Growth performances of BSFL fed with ex situ fermented CEW. Values are mean ± standard deviation (n = 3).

R. oligosporus Inoculum Size (wt %)	Total Weight Gained (g)	Growth Rate (g/day)
0 (Control)	0.70 ± 0.06	0.058 ± 0.005
0.02	1.08 ± 0.10	0.090 ± 0.009
0.1	1.02 ± 0.11	0.085 ± 0.009
0.5	1.13 ± 0.11	0.094 ± 0.009
1.0	1.04 ± 0.03	0.087 ± 0.003
1.5	0.95 ± 0.04	0.079 ± 0.003
2.0	1.06 ± 0.07	0.089 ± 0.006
2.5	0.97 ± 0.25	0.081 ± 0.021

3.2. Reduction of Fermented Organic Wastes

The BSFL were employed as a bioagent to reduce the CEW treated via either in situ or ex situ fermentation by *R. oligosporus* inoculation. The degree of organic waste reductions was measured in terms of overall degradation and waste reduction index. Whilst exploiting the fungal in situ fermentation treatment mode, the overall degradation of CEW was maintained around 30% ($p > 0.05$) (Figure 1). Compared with ex situ fermentation, the overall degradation values were increased considerably. Starting at 28% under the control, ex situ fermentation by merely 0.02 wt % of *R. oligosporus* inoculation improved the overall degradation to 39% ($p < 0.05$). The overall degradation achieved its peak at 48% when the CEW was inoculated with 0.5 wt % and 1.0 wt % of fungus. As shown in Figure 1, the best inoculum sizes from the LSD test were 0.5 wt % and 1.0 wt %, which had similar impacts on the overall degradation of CEW. The high inoculum concentrations, namely 1.5–2.5 wt %, should be avoided if scaling up due to costs, even though the results had similar impacts with 0.5 wt % and 1.0 wt %.

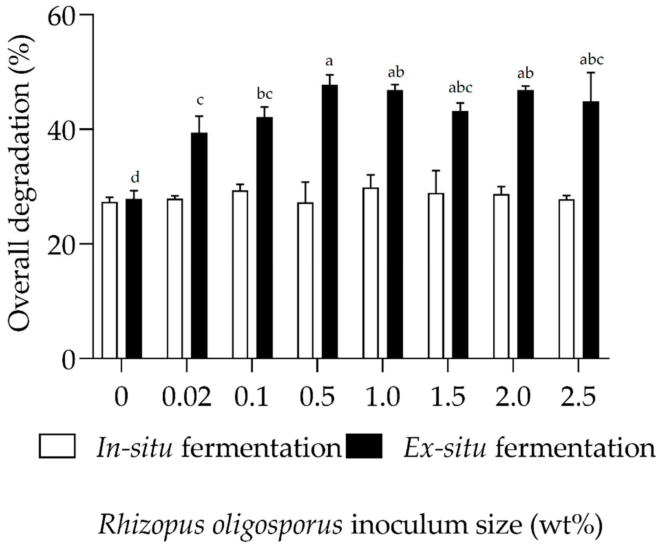

Figure 1. Overall degradation of CEW by BSFL at various inoculum sizes of *R. oligosporus* performing in situ and ex situ fermentations. Mean values with different superscript characters signify the significant differences ($p < 0.05$).

The values decreased slightly with further increases of fungal inoculum concentrations. In comparison between both fermentation modes, there was a large gap of CEW reduction by BSFL. The choice of ex situ fermentation engendered the higher overall degradation of CEW by BSFL, ranging from 42% to 75%, as opposed to in situ fermentation. This was also in conformity with the BSFL growth performance in which the comparison between the fermentation modes carried more impact on CEW reduction than did the inoculum sizes of *R. oligosporus* within the individual fermentation mode. The unfortunate results of employing the fungal in situ fermentation treatment mode could be due to the fermentation process being executed in tandem with the BSFL entomoremediation process. From the current study, it was also propounded that the in situ fermentation proffered opportunity to the BSFL to ingest part of the introduced *R. oligosporus* cells together with CEW at the early stage of fermentation. The presence of a remnant population of *R. oligosporus* to carry out enzymatic activities was inefficient, derailing the fermentation process. Thereby, the 0.5–1.0 wt % range was considered sufficient for ex situ fermentation to attain a significant overall degradation of CEW via subsequent BSFL valorization.

In comparison with works from Wong et al. [15], the overall degradation of CEW was enhanced with the inoculation of *R. oligosporus* by around 10% as opposed to the bacterial fermentation. From the study by Mohd-Noor et al. [22], the results showed that the overall

degradation of CEW after self-fermentation could only achieve around 32% to 36%. These results were about the same percentages (around 30%) as reported for in situ fermentation in the current study. However, looking into ex situ fermentation, the performance of overall degradation was strongly enhanced as compared with in situ fermentation. On the other hand, as opposed to the study by Wong et al. [23], the overall degradation of CEW fermented by yeast through in situ fermentation was able to achieve about 48% to 53% at different yeast concentrations. The differences found among self-fermentation as well as bacteria-, yeast- and fungi-assisted fermentations could be explained by the different metabolism activities of each bioagent, and hence more studies on the impact of other bioagents are deeply needed in the future.

Moving to the waste reduction index in valorizing CEW, there was an inconsequential impact exerted by *R. oligosporus* in executing in situ fermentation, regardless of inoculum concentration (Figure 2). The values fluctuated slightly, in the range of 0.22–0.25 g/day, with increasing fungal inoculum concentrations ($p > 0.05$). Meanwhile, the waste reduction index from BSFL was significantly ameliorated when the CEW was fermented via the ex situ treatment mode. The values were observed to increase from 0.23 g/day for control to 0.33 g/day at 0.02 wt % and 0.35 g/day at 0.1 wt % of inoculum concentrations ($p < 0.05$). The highest waste reduction index was recorded at the inoculum size range of 0.5 wt %, 1.0 wt % and 2.0 wt %, i.e., approximately 0.40 g/day. Except at low *R. oligosporus* inoculum concentrations of 0.02 wt % and 0.1 wt %, the waste reduction index values were increasing over 50% under the ex situ fermentation as opposed to in situ fermentation. Likewise, the waste reduction index shared a similar trend with other measured parameters, whereby the *R. oligosporus* performing in situ fermentation on CEW had little impact on the BSFL valorization process regardless of fungal inoculum sizes. On the other hand, the reduction of CEW by BSFL benefitted from the execution of fungal ex situ fermentation. As compared with Wong et al. [15], the WRI of CEW with bacterial fermentation was only about 0.2–0.25 g/day. The inoculation with *R. oligosporus* increased the WRI value by nearly one-fold at the maximum point. A study by Mohd-Noor [22] showed that through self-fermentation, the WRI could attain the highest WRI of merely 0.024 g/day after a two week self-fermentation period. In addition, the yeast-assisted in situ fermentation of CEW showed the WRI increased from 0.31 g/day to 0.40 g/day [23], which was quite similar to the WRI results obtained by the *R. oligosporus*-assisted ex situ fermentation in the current study. The comparative study may propound that the use of yeast could be a better choice than bacteria or *R. oligosporus*-assisted fermentation. Better yet, other desired parameters such as lipid or protein production from harvested BSFL should be taken into consideration. As stated previously, different microorganisms have varied impacts on CEW in terms of overall degradation and WRI due to different metabolic activities.

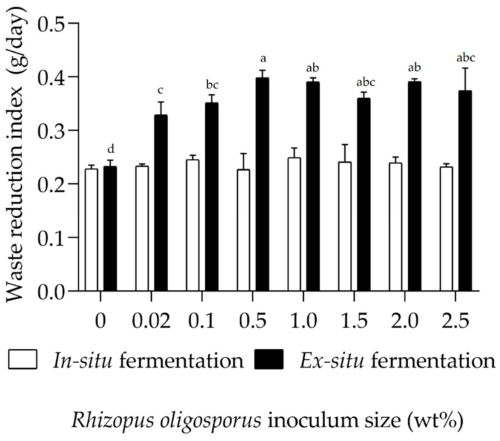

Figure 2. Waste reduction index of CEW by BSFL at various inoculum concentrations of *R. oligosporus* performing in situ and ex situ fermentations. Mean values with different superscript characters signify the significant differences ($p < 0.05$).

However, this study delving into the interaction of BSFL and inoculated exo-microbes such as *R. oligosporus* to execute ex situ fermentation was intended to unveil the larval–microbial interactive mechanisms, which may hold insights for economical valorization of organic wastes.

Principal component analysis was subsequently used to determine the statistical correlation of measured variables against the *R. oligosporus* inoculum sizes under ex situ fermentation treatment mode. As shown in Figure 3, the use of low inoculum sizes of *R. oligosporus* favored the growth of BSFL. Increasing the inoculum sizes of *R. oligosporus* to 0.5 wt % and 1.0 wt % in executing the ex situ fermentation enhanced the reduction of CEW via valorization by BSFL. These statistical correlations eased the decision making in employing BSFL to reduce ex situ fermented CEW. The results suggested that the low inoculum sizes of *R. oligosporus* could be adopted to yield heavier mature BSFL. In reducing more CEW, the mid-range of *R. oligosporus* inoculum concentrations could be employed to execute ex situ fermentation prior to the valorization by BSFL.

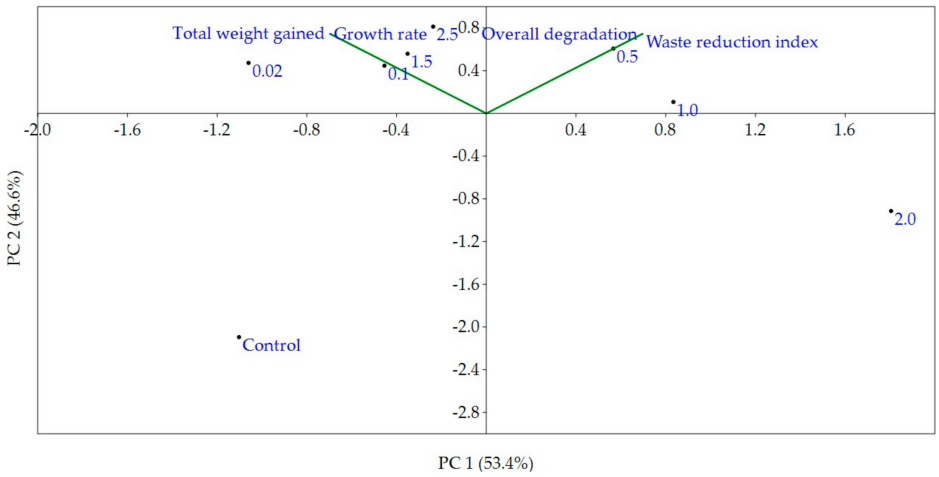

Figure 3. Principal component analysis of measurement variables under ex situ fermentation treatment mode (PC 1: eigenvalue: 2.1355; variability: 53.38%; cumulative: 53.4%; PC 2: eigenvalue: 1.8664; variability: 46.61%; cumulative: 99.99%).

4. Conclusions

In comparison with in situ fermentation, the introduction of ex situ fermentation greatly promoted the growth of BSFL and improved the reduction of CEW via larval valorization. It was propounded that the use of in situ fermentation permitted the BSFL to ingest fresh *R. oligosporus*, retarding the fungal enzymatic activities to break down the biopolymers in CEW. In the case of ex situ fermentation, the *R. oligosporus* inoculating CEW had enough time to break the biopolymers and subsequently ease the assimilation process by BSFL whilst reducing the CEW. From the statistical correlation study using principal component analysis, the presence of *R. oligosporus* in a range of 0.5–1.0 wt % was regarded as optimum to ferment CEW via the ex situ mode, prior to the valorization by BSFL.

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References

1. Bulak, P.; Polakowski, C.; Nowak, K.; Waśko, A.; Wiącek, D.; Bieganowski, A. Hermetia illucens as a new and promising species for use in entomoremediation. *Sci. Total Environ.* **2018**, *633*, 912–919. [CrossRef]
2. Ewuim, S.C. Entomoremediation-a novel in-situ bioremediation approach. *Anim. Res. Int.* **2013**, *10*, 1681–1684.
3. Zhou, F.; Tomberlin, J.K.; Zheng, L.; Yu, Z.; Zhang, J. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. *J. Med. Entomol.* **2013**, *50*, 1224–1230. [CrossRef]
4. Diener, S.; Solano, N.M.S.; Gutiérrez, F.R.; Zurbrügg, C.; Tockner, K. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valoriz.* **2011**, *2*, 357–363. [CrossRef]
5. Nguyen, T.T.; Tomberlin, J.K.; Vanlaerhoven, S. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environ. Entomol.* **2015**, *44*, 406–410. [CrossRef] [PubMed]
6. Lalander, C.H.; Fidjeland, J.; Diener, S.; Eriksson, S.; Vinnerås, B. High waste-to-biomass conversion and efficient salmonella spp. Reduction using black soldier fly for waste recycling. *Agron. Sustain. Dev.* **2015**, *35*, 261–271. [CrossRef]
7. Schiavone, A.; Cullere, M.; De Marco, M.; Meneguz, M.; Biasato, I.; Bergagna, S.; Dezzutto, D.; Gai, F.; Dabbou, S.; Gasco, L. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Ital. J. Anim. Sci.* **2017**, *16*, 93–100. [CrossRef]
8. Rana, K.S.; Salam, M.; Hashem, S.; Islam, M.A. Development of black soldier fly larvae production technique as an alternate fish feed. *Int. J. Res. Fish Aquac.* **2015**, *5*, 41–47.
9. Cullere, M.; Tasoniero, G.; Giaccone, V.; Miotti-Scapin, R.; Claeys, E.; De Smet, S.; Dalle Zotte, A. Black soldier fly as dietary protein source for broiler quails: Apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal* **2016**, *10*, 1923–1930. [CrossRef] [PubMed]
10. Wang, Y.-S.; Shelomi, M. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods* **2017**, *6*, 91. [CrossRef]
11. Elsayed, M.; Ran, Y.; Ai, P.; Azab, M.; Mansour, A.; Jin, K.; Zhang, Y.; Abomohra, A.E.-F. Innovative integrated approach of biofuel production from agricultural wastes by anaerobic digestion and black soldier fly larvae. *J. Clean. Prod.* **2020**, 121495. [CrossRef]
12. Li, W.; Li, Q.; Zheng, L.; Wang, Y.; Zhang, J.; Yu, Z.; Zhang, Y. Potential biodiesel and biogas production from corncob by anaerobic fermentation and black soldier fly. *Bioresour. Technol.* **2015**, *194*, 276–282. [CrossRef] [PubMed]
13. Chen, H. *Modern Solid State Fermentation*; Springer: Dordrecht, The Netherlands, 2013.
14. Gao, Z.; Wang, W.; Lu, X.; Zhu, F.; Liu, W.; Wang, X.; Lei, C. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. *J. Clean. Prod.* **2019**, *230*, 974–980. [CrossRef]
15. Wong, C.Y.; Lim, J.W.; Chong, F.K.; Lam, M.K.; Uemura, Y.; Tan, W.N.; Bashir, M.J.K.; Lam, S.M.; Sin, J.C.; Lam, S.S. Valorization of exo-microbial fermented coconut endosperm waste by black soldier fly larvae for simultaneous biodiesel and protein productions. *Environ. Res.* **2020**, *185*, 109458. [CrossRef]
16. Mazza, L.; Xiao, X.; Ur Rehman, K.; Cai, M.; Zhang, D.; Fasulo, S.; Tomberlin, J.K.; Zheng, L.; Soomro, A.A.; Yu, Z. Management of chicken manure using black soldier fly (Diptera: Stratiomyidae) larvae assisted by companion bacteria. *Waste Manag.* **2020**, *102*, 312–318. [CrossRef]
17. Handoyo, T.; Morita, N. Structural and functional properties of fermented soybean (tempeh) by using *Rhizopus oligosporus*. *Int. J. Food Prop.* **2006**, *9*, 347–355. [CrossRef]
18. Nelofer, R.; Nadeem, M.; Irfan, M.; Syed, Q. Nutritional enhancement of barley in solid state fermentation by *Rhizopus oligosporus* ml-10. *Nutr. Food Sci. Int. J.* **2018**, *6*, 555700. [CrossRef]
19. Diclaro, J.; Kaufman, P.E. Black soldier fly *hermetia illucens* linnaeus (Insecta: Diptera: Stratiomyidae). *EENY* **2009**, *461*, 1–3.
20. Kim, W.-T.; Bae, S.-W.; Park, H.-C.; Park, K.-H.; Lee, S.-B.; Choi, Y.-C.; Han, S.-M.; Koh, Y.-H. The larval age and mouth morphology of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Int. J. Ind. Entomol.* **2010**, *21*, 185–187.
21. Li, Q.; Zheng, L.; Qiu, N.; Cai, H.; Tomberlin, J.K.; Yu, Z. Bioconversion of daiy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Manag.* **2011**, *31*, 1316–1320. [CrossRef]

22. Mohd-Noor, S.N.; Wong, C.Y.; Lim, J.W.; Uemura, Y.; Lam, M.K.; Ramli, A.; Bashir, M.J.; Tham, L. Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. *Renew. Energy* **2017**, *111*, 646–654. [CrossRef]
23. Wong, C.Y.; Mohd Aris, M.N.; Daud, H.; Lam, M.K.; Yong, C.S.; Abu Hasan, H.; Chong, S.; Show, P.L.; Hajoeningtijas, O.D.; Ho, Y.C.; et al. In-Situ Yeast Fermentation to Enhance Bioconversion of Coconut Endosperm Waste into Larval Biomass of Hermetia illucens: Statistical Augmentation of Larval Lipid Content. *Sustainability* **2020**, *12*, 1558. [CrossRef]