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Impact of bokashi fermentation on life-history traits of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) larvae at an industrial scale

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RESEARCH ARTICLE

Abstract

Larvae of the black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) (BSFL) are increasingly used in a circular economy context for industrial production of protein, oil, and frass, while serving as a sustainable method for managing numerous types of organic waste. On both fronts, there are ongoing efforts to optimise feedstocks for increased larval performance, yields of protein and/or oil, and efficiency of volumetric reduction of waste. Fermentation of organic waste prior to providing it to BSFL can help accomplish both goals. A few studies have individually evaluated fermenting agents such as lactic acid bacteria, yeasts, and fungi, showing that they can improve BSFL digestion of biowaste. However, the potential of co-fermentation by multiple microbes to improve waste digestion by BSFL has not been well explored. Here we tested a type of anaerobic fermentation, known as bokashi, that simultaneously uses lactic acid bacteria, yeasts, and fungi, on a common nutritious industrial feedstock (brewery's spent grains) and on a nutritionally poor agricultural waste (unharvested oranges) on resulting life-history traits of BSFL. We show that bokashi-fermented substrates increased BSFL biomass and growth rate on both feeding substrates and dramatically reduced BSFL development duration on the nutritionally challenging oranges. Besides this, BSFL reared on fermented industrial feedstock reached the peak weight a day earlier, on average, than those feeding on the same unfermented substrate. Collectively, these effects would be beneficial for industrial BSF farming. We also highlight research areas to be tackled before bokashi fermentation can become widely adopted by the BSF farming sector.

Keywords: biowaste, brewery's spent grains, BSFL, lactic acid bacteria

1. Introduction

Larvae of the black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) (BSFL) are increasingly used worldwide in a circular economy framework to upcycle industrial, agricultural, and food waste. BSFL can divert these waste streams from landfills and transform them into protein (used for fishmeal and as feed for cattle, pigs, chickens and pets), lipids (used for feed and biodiesel production) and a residual (i.e. frass) that can be used as an organic soil amendment and fertiliser (Gasco *et al.*, 2020; Ojha *et al.*, 2021; Raksasat *et al.*, 2020; Ravi *et al.*, 2020). BSFL recycling now operates at an industrial scale, providing important recycling services to convert organic wastes into usable products (Van Huis, 2020). To expand the breadth of organic wastes that can be processed by BSFL, there is interest in developing tools to pre-process poor feedstocks and render them suitable for BSFL digestion. Pre-processing tools may also yield benefits when applied to suitable feedstocks, leading to enhancements of key production metrics such as accelerated larval growth rate, biomass produced, feed conversion, and protein and lipid accumulation (Peguero and Gold, 2021).

Microbial modification of feedstocks is one potentially cost-effective way to improve poor feedstocks and BSFL

production metrics. Several studies have shown that inoculating substrates with particular microbes improves BSFL development and performance in substrate usage. For example, Yu *et al.* (2011) tested three different strains of *Bacillus subtilis* and a strain of *B. natto* inoculated individually into chicken manure feedstock. Resulting fermentation significantly increased prepupal and pupal weights and, depending on the strain inoculated, also shortened development time. A similar study found that a strain of *B. subtilis* isolated from the BSFL digestive tract (BSF-CL) increased the substrate reduction rate and the weight of BSFL when used to ferment chicken manure feedstock (Xiao *et al*., 2018). Positive effects of BSF-CL held even when it was mixed with a more complex microbial community derived from BSF eggs (Mazza *et al*., 2020). Some studies revealed trade-offs, with a subset of production metrics improving and others remaining unchanged or reduced. Somroo *et al.* (2019) used *Lactobacillus buchneri* to ferment soybean curd residues and reported enhanced dry mass reduction, bioconversion rate, crude protein and fat production, but a reduction in the feed conversion rate relative to unfermented control and an artificial diet. Kooienga *et al.* (2020) tested three inoculants (*Arthrobacter* AK19, *Bifidobacterium breve*, and *Rhodococcus rhodochrous*), and reported an effect of rearing operation size on the magnitude of positive effects associated with *Arthrobacter* and *Rhodococcus*, with *B. breve* inoculation being negative across all scales. Other studies demonstrated that yeasts or fungi can improve digestion. Larvae grown on coconut endosperm waste inoculated with yeasts developed more rapidly and had increased biomass and lipid content (Wong *et al.*, 2020). And the fungus *Rhizopus oligosporus* improved digestibility of feedstocks when added simultaneously with larvae, or 72 h prior to larvae being introduced to feedstocks (Wong *et al.*, 2021).

The studies cited above demonstrate that microbes can be used to improve BSFL production through inoculation into feedstocks. However, the efficacy of microbial mixtures, especially those that combine phylogenetically diverse taxa, such as bacteria, yeasts, and fungi, has not been tested yet. Within this area of testing mixtures, it is also beneficial to evaluate products that are readily available and proven to be effective in accelerating waste recycling in other applications. Here, we determined changes in production metrics of BSFL reared on variable quality substrates treated with bokashi inoculum, which is used to initiate the process of bokashi composting. Bokashi composting is an anaerobic fermentation under acidic conditions facilitated by lactic acid bacteria, purple non-sulphur bacteria, yeasts and Actinomycetes. Bokashi composting of organic waste produces, in a matter of weeks compared to the months necessary for traditional aerobic composting or for vermicomposting, a nutrient-rich organic fertiliser that promotes growth and pathogen/pest defence capabilities in crop plants (Olle and Williams, 2013; Pagliaccia *et al*., 2020). Digestion of food waste by bokashi fermentation and BSFL has been tested side-by-side (Alattar *et al*., 2016) but never in a complementary fashion. We hypothesised that bokashi fermentation of feedstocks will improve BSFL production metrics, with relative benefits being more apparent on feedstocks that are normally more difficult to digest. To test this hypothesis, we evaluated the effects of bokashi inoculum addition on digestion of a nutritionally rich feedstock (brewer's spent grains) and a nutritionally poor feedstock (citrus fruit waste from agricultural production) relative to a standard diet control.

2. Material and methods

Diet preparation

We selected two organic waste streams as targets for assessment with and without bokashi fermentation: brewery's spent grains, a common and nutritious industrial feedstock, donated by a local brewery, and unharvested oranges, a nutritionally challenging substrate, collected at an orange orchard within the Agricultural Operations fields at UC Riverside. To generate the fermented treatments, we mixed both substrates, separately, with a bokashi starter (premium bokashi bran, Bokashi Living, Vancouver, Canada) in 20-l bokashi composters (kitchen composter, Bokashi Living, Vancouver, Canada) by alternating layers of substrates of about 2.5 cm at a ratio of 15 mg of bran per layer, as per manufacturer's recommendation. Every other day, we drained the leachate (liquid) that accumulated below a grate holding up the substrate above a reservoir at the bottom of the bin. Leachate exited through a valve at the base of the composter. We allowed substrates to ferment for four weeks at room temperature, after which we stored stable digestates at 4±1 °C until use. Fermented and unfermented oranges were ground to a size equivalent to spent grains (6-8 mm) and drained of excess juice prior to use in experiments. In all experiments with substrates, we included the standard Gainesville diet as a control, with this diet made by mixing 50% wheat bran (Baker's Authority, Maspeth, NY, USA), 30% alfalfa meal (Walt's Organic Fertilizer Co., Seattle, WA, USA), 20% corn meal (Yummmico, Inc., Hialeah, FL, USA) and hydrating it to 70%.

Measurement of black soldier fly larvae growth rate and development duration on substrates

We conducted the experiment in a room with controlled temperature (27±1 °C) and humidity (70±5%), and 12:12 L:D photoperiod. Three replicates of the experiment were carried out in succession (i.e. five plastic boxes, one per treatment, were handled at any time). Except for the fermented substrates, which were taken from the same cold-stored fermentation batch, spent grains and oranges

were donated and collected the day before the experiment, respectively, given the impossibility of cold storing them for an extended period. Gainesville diet was prepared on the same day the experiment was set up. We placed eight kilograms of each substrate in a 60×40×15 cm plastic crate (Sterilite, Townsend, MA, USA) and emptied a cup of 10,000 5-day-old larvae (5-DOL) (Evo Conversion Systems, LLC, College Station, TX, USA) on the centre of the substrate, letting the larvae self-disperse. We measured moisture daily using a soil tester (RCYAGO, Shenzhen, China P.R.) and added water as needed if humidity dropped below 70%. Starting moisture level was approximately 85% for spent grains, 80% for fermented spent grains, 90% for oranges, and 85% for fermented oranges. We aerated substrates by hand daily by thoroughly shuffling the diet for about one minute with a scooping motion. At the end of daily manual aeration, we randomly selected 50 larvae from each crate, rinsed to remove feeding substrates, pat-dried and weighed using a precision balance (U.S. Solid, Cleveland, OH, USA) then returned larvae to the crates after data collection. We weighed 50 larvae (Yang and Tomberlin, 2020) daily until at least 5% of BSFL became prepupae (Bosch *et al.*, 2020). We estimated prepupal percentage by collecting three samples of 100 BSFL each as soon as prepupae were observed in the crate and counting the number of prepupae in each sample. We used the data collected during the experiment to calculate three life-history traits: (1) development duration, here defined as the number of days from the 5-DOL stage to the prepupal stage; (2) daily weight fluctuations for the first 10 days of the experiment (minimum number of days for which larval weights were available for all treatments, due to large differences across substrates in time to complete development); (3) growth rate, defined as (larva average final body weight (mg) – larval average initial body weight (mg)) / development duration (days).

Statistical analyses

We analysed data on development duration and growth rate by two-way ANOVA using the *lm* function. We evaluated model residuals for normality and homoscedasticity (Crawley, 2012) and performed multiple comparisons of the means using the Tukey HSD test. To analyse larval weights, we used the R package *nlme* and *lmer* function to perform a two-way mixed-model ANOVA with rearing substrates, time and their interaction as explanatory variables, experimental units (the plastic boxes) as a random factor and daily weights as the response variable. We checked model residuals graphically to assess normality as above. As biological replicates were carried out at different times, in all analyses we included replicate as a factor to assess if temporal differences influenced the results.

3. Results

For development duration data, the replicate factor was marginally significant (ANOVA, F=4.66, df=2, P=0.045), so it was kept in the model. This significance was likely due to a shorter development duration for BSFL reared on oranges on replicate 1 (24 days vs 31 and 32 days for replicate 2 and 3, respectively). Rearing substrate had a significant effect on development duration (ANOVA, F=67.90, df=4, P<0.001); however, the Tukey HSD revealed that this was driven exclusively by BSFL reared on oranges. Feeding on bokashi-fermented oranges reduced the development duration of BSFL by 59% when compared to BSFL reared on unfermented oranges (12 vs 29 days in average, respectively) (Table 1).

In the analysis of growth rate data, the replicate factor did not have a significant effect (ANOVA, F=2.84, df=2, P=0.117), so it was removed from the final model. Rearing substrate had a significant effect on BSFL growth rate (ANOVA, $F_{4,10}$=32.1, P<0.001), with the two bokashi treatments again having a major impact (Table 1). BSFL reared on fermented spent grains had the highest growth rate, nearly identical as that of BSFL reared on the control diet, and significantly higher than that of BSFL reared on unfermented spent grains. Similarly, BSFL reared on bokashi fermented oranges had a growth rate more than double that of BSFL reared on unfermented oranges (Table 1).

Table 1. Development duration from 5-day-old-larvae stage to prepupal stage and growth rate of black soldier fly larvae reared on different substrates.[1]

Rearing substrate	Development duration (days)	Growth rate (mg/day)
Gainesville diet	10.7±0.3a	11.2±0.5ab
Brewery's spent grains	11.0±0.6a	8.5±0.7ac
Bokashi fermented spent grains	10.7±0.3a	11.8±0.4b
Oranges	29.0±2.5b	3.1±0.5d
Bokashi fermented oranges	12.0±1.2a	7.4±0.9c

[1] Different letters indicate significant differences (P<0.001) at the Tukey HSD test. Values given as mean ± standard error.

In the analysis of daily weight dynamics, the replicate factor was not significant (ANOVA, F=0.80, df=2, *P*=0.48), so it was removed from the final model. All other factors had a significant effect on BSFL weight (ANOVA, F=39.72, df=4, *P*<0.001 for substrate, F=181.95, df=10, *P*<0.001 for time and F=9.53, df=40, *P*<0.001 for their interaction). Day had a significant effect, with the exclusion of T0 (initial weights of 5-DOL) and day 1, after which differences in weights by rearing substrates started to emerge (Figure 1). The most noteworthy differences concerned the bokashi-fermented substrates. BSFL reared on fermented spent grains reached their peak weight a day earlier (day 6) than those reared on unfermented spent grains (day 7), with a 21% average increase in weight (140.5±8.4 vs 110.7±11.6 mg). Also, weights of BSFL reared on fermented spent grains were comparable to those of BSFL reared on the control diet at any time point. The impact of bokashi fermentation was even more remarkable on oranges. BSFL reared on fermented oranges had a growth curve that closely resembled that of BSFL reared on unfermented spent grain, a popular and nutritious feedstock for industrial farming of BSF. Remarkably, BSFL reached peak weight on fermented orange substrate 17 days earlier than BSFL reared on unfermented oranges (day 8 vs day 25), and just one day later than BSFL reared on unfermented spent grains

4. Discussion and conclusions

We determined that bokashi fermentation improves the quality of both substrates examined as a feeding substrate for BSFL. Although brewery's spent grains represent a nutritious feeding substrate, to the point that it is possibly the most used feedstock for industrial farming of BSF, bokashi fermentation enhanced its quality. BSFL reared on fermented brewery spent grains had a high growth rate and an average increase of 21% in the peak weight, which is when BSFL are harvested to maximise industrial production of protein. Peak weight was also reached a day earlier than when BSFL were fed unfermented spent grains. Faster time to peak weight is beneficial for industrial BSF farming because more production cycles can be completed in a given period of time.

The positive effect of bokashi fermentation was even more remarkable on oranges. BSFL fed on fermented oranges were comparable to those fed on unfermented spent grains in terms of growth rate and larval biomass. Development duration was reduced by more than double compared to BSFL fed on unfermented oranges and was no different than that achieved on the other typical nutritious substrates. This finding suggests that bokashi fermentation could be critical in expanding the application of BSF farming to the agricultural sector, a still largely unexplored and overlooked domain. For example, bokashi fermentation could be used to efficiently convert agricultural residues, which may be poor or unsuitable substrates, into more homogeneous, nutritious feedstocks. However, there are several areas that still require more research before this technique can be widely adopted by the BSF-farming community, mainly scalability, optimisation of microbial combinations, and studying impacts of fermentation on microbial communities in resulting frass. Future research should also elucidate the mechanisms behind the positive effects of bokashi fermentation on BSFL. We speculate that pH might play a role (Raksasat *et al.*, 2022), as well as

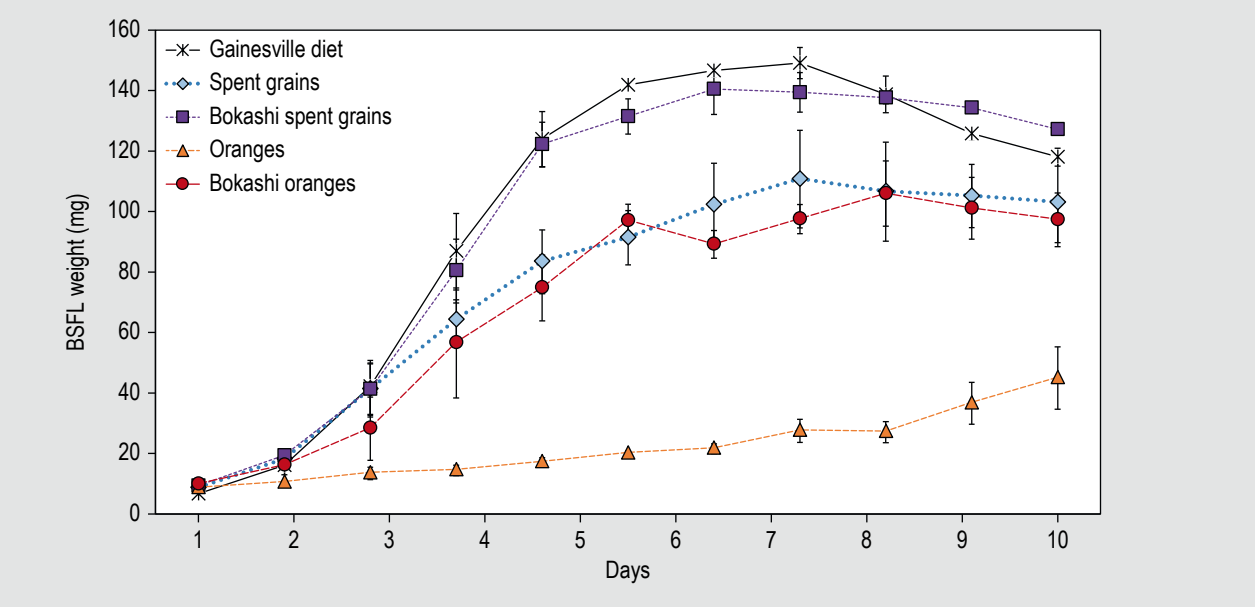

Figure 1. Average daily weight (mean ± standard error) of black soldier fly larvae (BSFL) reared on different substrates for the first 10 days of the experiment.

antimicrobial metabolites known to be produced by lactic acid bacteria (Hadj Saadoun *et al.*, 2020).

Tackling these knowledge gaps will require research in several areas, including the economics of inputs required for use of bokashi in BSFL production. For example, the commercial inoculum used in this study is a proprietary mixture of fermenting agents known as 'effective microorganisms' (Higa and Parr, 1994). Availability of an already commercialised product for initiating fermentation is helpful, but the product is currently available in small, household appropriate volumes. Purchasing inoculum in these volumes could be too costly for the high quantities of waste handled by industrial BSF facilities. There is also the fundamental question of whether the 'effective microorganisms' mixture is the optimal choice for BSFL production.

More research will be needed to optimise bokashi mixtures for BSFL applications, which could be integrated with work on the overall costs/returns and logistics of bokashi fermentation in rearing operations. There is room for improvement in standardizing fermentation conditions, including ratio of inoculum to waste, control of abiotic conditions, and duration of the anaerobic process (Alattar *et al.*, 2012). The smart bin engineered by Lew *et al.* (2021) represents a first step in the right direction. Fermentation of bio waste as part of a circular economy is also rooted in traditional agricultural practices (Nene, 2018) and partnerships to illuminate indigenous knowledge of fermentation could advance wider use of this technique in sustainable waste management, including BSFL production. In this context, it will also be important to consider how fermentation augments output of BSFL production, such as the residual generated. Such materials, often referred to as BSFL frass, can be used as a fertiliser and soil amendment and is being explored for its potential to enrich microbial communities that confer health benefits to plants. Based on prior work showing benefits of bokashi fermentation on plant rhizosphere health (Pagliaccia *et al.*, 2020), it is likely that incorporation of bokashi fermentation into BSFL rearing will further enhance properties of frass and progress toward circular economy goals.

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Conflict of interest

The authors declare no conflict of interest.

References

Alattar, M.A., Alattar, F.N. and Popa, R., 2016. Effects of microaerobic fermentation and black soldier fly larvae food scrap processing residues on the growth of corn plants (*Zea mays*). Plant Science Today 3: 57-62. https://doi.org/10.14719/pst.2016.3.1.179

Alattar, M.A., Green, T.R., Henry, J., Gulca, V., Tizazu, M., Bergstrom, R. and Popa, R., 2012. Effect of microaerobic fermentation in preprocessing fibrous lignocellulosic materials. Applied Biochemistry and Biotechnology 167: 909-917. https://doi.org/10.1007/s12010-012-9717-5

Bosch, G., Oonincx, D.G.A.B., Jordan, H.R., Zhang, J., Van Loon, J.J.A., Van Huis, A. and Tomberlin, J.K., 2020. Standardisation of quantitative resource conversion studies with black soldier fly larvae. Journal of Insects as Food and Feed 6: 95-109. https://doi.org/10.3920/JIFF2019.0004

Crawley, M.J., 2012. The R book. 2nd edition. John Wiley & Sons, Chichester, United Kingdom, 1080 pp.

Gasco, L., Biancarosa, I. and Liland, N.S., 2020. From waste to feed: a review of recent knowledge on insects as producers of protein and fat for animal feeds. Current Opinion in Green and Sustainable Chemistry 23: 67-79. https://doi.org/10.1016/j.cogsc.2020.03.003

Hadj Saadoun, J., Luparelli, A.V., Caligiani, A., Macavei, L.I., Maistrello, L., Neviani, E., Galaverna, G., Sforza, S. and Lazzi, C., 2020. Antimicrobial biomasses from lactic acid fermentation of black soldier fly prepupae and related by-products. Microorganisms: 8: 1785. https://doi.org/10.3390/microorganisms8111785

Higa, T. and Parr, J.F., 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment. International Nature Farming Research Center, Atami, Japan.

Kooienga, E.M., Baugher, C., Currin, M., Tomberlin, J.K. and Jordan, H.R., 2020. Effects of bacterial supplementation on black soldier fly growth and development at benchtop and industrial scale. Frontiers in Microbiology 11: 587979. https://doi.org/10.3389/fmicb.2020.587979

Lew, P.S., Nik Ibrahim, N.N.L., Kamarudin, S., Thamrin, N.M. and Misnan, M.F., 2021. Optimization of Bokashi composting process using effective microorganisms-1 in smart composting bin. Sensors 21: 2847. https://doi.org/10.3390/s21082847

Mazza, L., Xiao, X., Ur Rehman, K., Cai, M., Zhang, D., Fasulo, S., Tomberlin, J.K., Zheng, L., Soomro, A.A., Yu, Z. and Zhang, J., 2020. Management of chicken manure using black soldier fly (Diptera: Stratiomyidae) larvae assisted by companion bacteria. Waste Management. 102: 312-318. https://doi.org/10.1016/j.wasman.2019.10.055

Nene, Y.L., 2018. The concept and formulation of Kunapajala, the world's oldest fermented liquid organic manure. Asian Agrihistory 22: 8-14. https://10.17485/aah/2018/v22i1/18292.

Ojha, S., Buβ, S., Psarianos, M., Rossi, G. and Schlüter, O.K., 2021. Edible insect processing pathways and implementation of emerging technologies. Journal of Insects as Food and Feed 7: 877-900. https://doi.org/10.3920/JIFF2020.0121

Olle, M. and Williams, I.H., 2013. Effective microorganisms and their influence on vegetable production – a review. Journal of Horticultural Science and Biotechnology 88: 380-386. https://doi.org/10.1080/14620316.2013.11512979

Pagliaccia, D., Bodaghi, S., Chen, X., Stevenson, D., Deyett, E., De Francesco, A., Borneman, J., Ruegger, P., Peacock, B., Ellstrand, N., Rolshausen, P.E., Popa, R., Ying, S. and Vidalakis, G., 2020. Two food waste by-products selectively stimulate beneficial resident citrus host-associated microbes in a zero-runoff indoor plant production system. Frontiers in Sustainable Food Systems 4: 593568. https://doi.org/10.3389/fsufs.2020.593568

Peguero, D.A., Gold, M., Vandeweyer, D., Zurbrügg, C. and Mathys, A., 2021. A review of pretreatment methods to improve agri-food waste bioconversion by black soldier fly larvae. Frontiers in Sustainable Food Systems 5: 745894. https://doi.org/10.3389/fsufs.2021.745894

Raksasat, R., Lim, J.W., Kiatkittipong, W., Kiatkittipong, K., Ho, Y.C., Lam, M.K., Font-Palma, C., Mohd Zaid, H.F. and Cheng, C.K., 2020. A review of organic waste enrichment for inducing palatability of black soldier fly larvae: wastes to valuable resources. Environmental Pollution 267: 115488. https://doi.org/0.1016/j.envpol.2020.115488

Raksasat, R., Abdelfattah, E.A., Liew, C.S., Rawindran, H., Kiatkittipong, K., Mohamad, M., Mohd Zaid, H.F., Jumbri, K., Lam, M.K. and Lim, J.W., 2022. Enriched sewage sludge from anaerobic pre-treatment in spurring valorization potential of black soldier fly larvae. Environmental Research, 113447. https://doi.org/0.1016/j.envres.2022.113447

Ravi, H.K., Degrou, A., Costil, J., Trespeuch, C., Chemat, F. and Vian, M.A., 2020. Larvae mediated valorization of industrial, agriculture and food wastes: biorefinery concept through bioconversion, processes, procedures, and products. Processes 8: 857. https://doi.org/10.3390/pr8070857

Somroo, A.A., Ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. and Zhang, J., 2019. Influence of *Lactobacillus buchneri* on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*) for food and feedstock production. Waste Management 86: 114-122. https://doi.org/10.1016/j.wasman.2019.01.022

Van Huis, A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. Journal of Insects as Food and Feed 6: 27-44. https://doi.org/10.3920/JIFF2019.0017

Wong, C.Y., Kiatkittipong, K., Kiatkittipong, W. and Lim, J.W., 2021. *Rhizopus oligosporus*-assisted valorization of coconut endosperm waste by black soldier fly larvae for simultaneous protein and lipid to biodiesel production. Processes 9: 299. https://doi.org/10.3390/pr9020299

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., Goh, P.S., Kausarian, H., Pan, G.-T. and Lim, J.W., 2020. *In-situ* yeast fermentation to enhance bioconversion of coconut endosperm waste into larval biomass of *Hermetia illucens*: statistical augmentation of larval lipid content. Sustainability 12: 1558. https://doi.org/10.3390/su12041558

Xiao, X., Mazza, L., Yu, Y., Cai, M., Zheng, L., Tomberlin, J.K., Yu, J., van Huis, A., Yu, Z., Fasulo, S. and Zhang, J., 2018. Efficient co-conversion process of chicken manure into protein feed and organic fertilizer by *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae and functional bacteria. Journal of Environmental Management 217: 668-676. https://doi.org/10.1016/j.jenvman.2018.03.122

Yang, F. and Tomberlin, J.K., 2020. Comparing selected life-history traits of black soldier fly (Diptera: Stratiomyidae) larvae produced in industrial and bench-top-sized containers. Journal of Insect Science 20: 1-6. https://doi.org/10.1093/jisesa/ieaa113

Yu, G., Cheng, P., Chen, Y., Li, Y., Yang, Z., Chen, Y. and Tomberlin, J.K., 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environmental Entomology 40: 30-35. https://doi.org/10.1603/EN10126