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EDITORIAL

Enhancing reproducibility in black soldier fly research

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Abstract

Over the past decade, there has been a steady increase in research focused on insects as food and feed, integrated with waste management and fertiliser production, with notable attention given to the black soldier fly (*Hermetia illucens*, BSF). Extensive knowledge has been developed regarding waste bioconversion and characterisation of the products generated (larval biomass and frass). However, the diverse range of rearing methods for BSF larvae (BSFL) has led to equally diverse outcomes. This confusion can lead to new research in the field of BSF being conducted using sub-optimal BSFL treatment conditions. In this editorial we propose that calibration procedures within distinct research areas should be considered when planning new BSFL experiments.

Keywords

Hermetia illucens – calibrating rearing conditions – verifying experimental outcomes – standardisation protocol

1 Introduction

Global interest in understanding and optimising the conversion of organic waste streams using BSFL is increasing, in line with the increasing demand for insects as protein (van Huis, 2020). Many knowledge gaps have been filled during the technology's development over the past 10 years and there is now more understanding of waste bioconversion dynamics with BSFL, larval growth and quality, and other relevant factors (Gold *et al.*, 2020; Seyedalmoosavi *et al.*, 2022). BSF conversion technology is now reaching a wider audience and new sectors, resulting in even more detailed data collection. Interest in extraction of bioactive substances from larvae and frass for use in oil refineries, antibiotics, fertiliser products and biodiesel production, among many other examples, has prompted much research on BSFL conversion (Siddiqui *et al.*, 2024). Research is also being conducted on associated impacts of treatments (e.g. pathogen inactivation) and products (e.g. larvae as feed, frass as fertiliser) (Hoffmans *et al.*, 2024). Another area of research is plant nutrition, examining potential benefits of frass for agriculture by optimising its fertilising capacity, extracting bioactive substances, and developing tailored products (Lopes *et al.*, 2022). Other sectors aim at extracting chitin from BSFL for multiple uses (Soetemans *et al.*, 2020), or extracting fat, fatty acids, and peptides for production of biodiesel, antimicrobial substances, and other compounds (Mohan *et al.*, 2023; Xia *et al.*, 2021). There are numerous other potentially relevant and beneficial topics that have not yet been explored. These new sectors embracing BSFL bioconversion in their scope will greatly contribute to further development of this technology. However, for researchers that are new to BSF research, selecting a methodology that aligns with the purpose of the study

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can be challenging. This can lead to imprecise selection of the BSFL rearing methodology, which can pose challenges in reproducing, comparing, or utilising the obtained results in future research.

2 The need for calibration of laboratory methodologies

Reading through a vast body of literature and extracting the precise information needed for establishing a well-designed BSFL rearing process is already difficult and will become more challenging as the number and variety of BSF-related publications continue to grow. Researchers new to the field of BSF research are likely to have limited knowledge about practical aspects of BSF conversion as they start planning their experiments and data collection. Misinterpretations during the initial set-up can result in data that under- or over-estimate the impact of the BSFL conversion process on target parameters, and the results may therefore not accurately reflect the true potential of this technology. Factors to consider include the rearing substrate, which is well known to have a significant impact on process efficiency and biomass conversion efficiency (Lalander *et al.*, 2019), and the larval composition (Chia *et al.*, 2020). Evidence also indicates that process parameters such as larval density, substrate feed depth, and larval feed dose influence the efficiency of the process (Lopes *et al.*, 2023). Larval density can also impact the composition of the larval biomass (Barragan-Fonseca *et al.*, 2018), while the number of feeding occasions can impact the pathogen reduction potential (Lopes *et al.*, 2020). Another concern is that some experiments are conducted at too small a scale (typically involving 10-200 larvae) with low larval density (<2 larvae cm^{-2}), potentially missing the dynamics of larval movements observed when a larger number of larvae aggregate (Yang and Tomberlin, 2020). In addition, the feed rate of the larvae may be much higher than is feasible for them to consume (>0.4 g total solids (TS) $larva^{-1}$), resulting in the substrate under investigation not being properly consumed (Lopes *et al.*, 2023). Alternatively, a much too small feed dose could be employed (<0.05 g TS $larva^{-1}$), rendering the BSFL conversion conditions used in the experiment unrealistic. There is thus an urgent need for the BSF research community to establish concise methodologies and protocols. This would aid both established researchers and emerging sectors in conducting experiments that generate reliable, reproducible, and comparable data.

3 Guidelines and their verifications

The need for common guidelines has been recognised by the BSF research community and initial attempts to establish consensual guidelines and protocols to standardise BSF conversion experiments at laboratory and industrial scale have been made (Bosch *et al.*, 2020; Deruytter *et al.*, 2023). However, Bosch *et al.* (2020) concluded that the use of a universal standard diet as a reference point for BSFL conversion is made more difficult by a lack of basic knowledge and reference values. On the other hand, Deruytter *et al.* (2023) found that the use of a common guideline for BSFL conversion on the industrial scale, despite using the same substrate, may lead to high variation in the results. This was attributed to variations in abiotic and biotic factors (ventilation, temperature, genetics) across the different locations. Therefore, establishing a universal, ready-to-go BSF experiment manual may not be straightforward, and it is crucial to acknowledge that thresholds can be fluid and that it can be difficult to determine how much deviation is acceptable.

4 The way forward

Keeping negative/positive controls is common practice in many research disciplines, and should be standard in all research (Torday and Baluška, 2019). One way forward could be for BSF laboratories to come together and create a guideline on how to calibrate BSFL conversion trials across laboratories, taking biotic and abiotic factors into account. When experiments are conducted by researchers who have not yet acquired an adequate level of knowledge in BSFL bioconversion, large variations in the data can occur (own observations, unpublished data). Standardising the evaluation and calibration of BSF conversion parameters could make the obtained results more 'valid, reliable and replicable', in the words of Bosch *et al.* (2020). Due to the difference in scale from laboratory to industrial level (Yakti *et al.*, 2022), we suggest separating the calibration of a laboratory experiment from an industrial process.

Using a positive control treatment with a control substrate would ensure that experimental conditions in laboratories new to BSF research are within the expected range for BSFL conversion. The expected range can be determined by collecting positive control treatment results from different BSF laboratories and establishing acceptable values across laboratories based on this range. As a start, a positive control treatment for BSFL

conversion could involve an agreed control substrate (Gainesville diet or various chicken feeds) and predefined process parameters (larval density, feed dose, number of feeding events, time of harvest) and an evaluation of process efficiency metrics (e.g. material reduction, bioconversion efficiency, larval survival, and larval yield). Once the guideline, efficiency metrics and acceptable ranges are established, they can be utilised by new laboratories wanting to conduct research on, e.g. the microbiome of BSFL. If laboratories new to BSF research face challenges in aligning the process efficiency of the positive control treatment within the established acceptable range, they would be encouraged to reassess their experimental set-up until they fall within this acceptable range. We suggest incorporating this positive control treatment in the training of new personnel, as a method to introduce them to BSFL bioconversion. In addition, we recommend including the results for the positive control treatment in publications. In a second step, the calibration could be extended to include commonly used substrates around the world, such as local food wastes, gradually extending over the entire range of substrates currently used in BSFL rearing.

5 Conclusion

Standardised, peer-reviewed, and reproducible methodologies are not yet widely available to the broader BSF research community and have proved challenging to apply universally. Establishing positive control treatments to calibrate a laboratory to an expected outcome, would make the results generated in BSFL research more reliable, reproducible, and comparable. In this editorial, we aim to inspire collaboration among BSF colleagues around the world for the development of methodology guidelines, to reach consensus on the selection of process efficiency metrics and explore the acceptable range of efficiency values in control treatments. This effort seeks to assist both newcomers and established BSF researchers in navigating the diverse fields of BSF research.

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