



## Review

# Biowaste treatment using black soldier fly larvae: Effect of substrate macronutrients on process performance

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## ABSTRACT

Black soldier fly larvae (BSFL) process is emerging as a promising alternative for the treatment of biowaste. Process performance (such as survival rate - SR and waste reduction efficiency - RE) depends on several factors (e.g. nutritional quality of the substrate) which need to be controlled. The nutritional quality of the substrate can be related to the overall concentration and relative abundance of dominant macronutrients, such as proteins (P), non-fibre carbohydrates (NFC), and lipids (L). Assessing how these substrate quality parameters influence the process performance is fundamental to determining the suitability of a given substrate to be treated by using BSFL and to optimise the process performance. The aim of this study was to gather, integrate, and elaborate published literature to present a comprehensive understanding of how the nutritional substrate quality, impact the process performance in terms of RE and SR. A systematic literature review was conducted by using the PRISMA methodology. The results were graphically elaborated to obtain a simple tool useful for a rapid prediction of the individual substrate suitability to BSFL process and for the evaluation of optimal mixture of different substrates to achieve desired outcomes. A good nutritional quality of substrate (when  $SR > 80\%$  and  $RE > 40\%$ ) can be generally identified based on the relative abundance of macronutrients ( $X_P$ ,  $X_{NFC}$ , and  $X_L$ ), when  $X_L < 0.6$ ,  $X_P > 0.05$ , and when  $X_P > 0.5$  if  $X_{NFC} > 0.2$ . Additionally, when  $X_P$  ranges between 0.05 and 0.15 a good quality substrate occurs if  $X_{NFC} > X_L$ .

## 1. Introduction

The use of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae (BSFL) is a promising alternative for the biological treatment of biowaste. The process effectively stabilizes putrescible organic waste by converting it into valuable resources, aligning with the principles of the circular economy. These resources include stabilized residues suitable for soil conditioning, larval biomass suitable as animal feed, or as a resource for the production of bio-lubricants, biodiesel, pharmaceuticals, etc. (Gold et al., 2020).

Process performance, both in terms of treatment efficiency (e.g. waste reduction efficiency) and biomass generation (e.g. survival rate, development time, weight, biomass composition), depends on several operation parameters which need to be controlled, such as, but not limited to, temperature (Harnden and Tomberlin, 2016), pH (Ma et al., 2018) and relative humidity (Cheng et al., 2017; Cammack and Tomberlin, 2017), nutritional quality of the feeding substrate (Gold et al., 2020), feeding rate (Diener et al., 2009), feeding frequency (Banks et al.,

2014a; Meneguz et al., 2018a) and larval density (Banks et al., 2014b). Among these parameters, optimizing the nutritional quality of the substrate for BSFL presents a greater challenge, as it strictly depends on the waste stream, although appropriate mixing of different waste streams may homogenize and increase the quality of the feed (Sari et al., 2022).

The nutritional quality of the substrate is related to the presence of dominant macronutrients such as proteins (P), non-fibre carbohydrates (NFC), and lipids (L), both in terms of overall macronutrient concentration presented as sum of NFC, P and L ( $\sum PCL$ , %) and relative abundance of macronutrients ( $X_P$ ,  $X_{NFC}$ , and  $X_L$ ) (Barragan-Fonseca et al., 2018a, 2018b; Broeckx et al., 2021a). Assessing how these substrate quality parameters influence the process performance would be fundamental to determine the suitability of a given substrate to be treated by using BSFL and to optimise the process performance. Many studies have investigated the influence of substrate quality on process performance by testing different overall macronutrient concentrations and macronutrient ratios, leading to generally agreed-upon conclusions, which can be summarised as follow (Cammack and Tomberlin, 2017;

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Barragán et al., 2018; Bellezza et al., 2022a, 2022b). Larvae biomass accounts for 32–58% of proteins and 15–39% of lipids, depending on substrate and feeding conditions (Gold et al., 2018). The rest are NFC and minerals. Whether BSFL can accumulate enough proteins and fats is essential for their well-being and for the process performance.

Proteins in diets are important for larval development and transformation to the pupa stage. Generally, larvae grown on biowaste higher in proteins have a higher weight, bioconversion rate, and larval protein content. However, protein excess in the diet can cause malnutrition, leading to side-effects such as higher mortality rate, lower yield, and substrate conversion (Tschirmer and Simon, 2015). This may be due to high ammonia, urea acid, and H<sub>2</sub>S concentrations, which are toxic to larvae (Almeida de et al., 2017). On the other hand, BSFL grown on biowaste lower in proteins (e.g. plant-based feed) have a lower weight and longer developmental time (Gold et al., 2018; Lalander et al., 2019; Ooninx et al., 2015).

Another significant component is lipid, which contain much more energy density but is not as digestible as protein. Adult flies require lipids as an energy reserve to survive. Since adult flies do not need to feed to reproduce, larvae must enrich themselves with fats for adult success (Jucker et al., 2017). However, larvae do not necessarily accumulate lipids from high-fat diets. They can also accumulate fat by initiating glycolysis from carbohydrates (Almeida de et al., 2017; Zhu et al., 2019). Indeed, high lipid content in larvae can be found when fed on substrates with high NFC content regardless of the lipid content in substrates (Ooninx et al., 2015; Meneguz et al., 2018b; Danieli et al., 2019). Unlike proteins and carbohydrates, larvae can survive and develop even without lipids. No negative effect on survival and waste reduction was observed when fed with a substrate without fats, such as bread (Lopes et al., 2020). On the other hand, too much fat in a diet may cause a detrimental effect on BSFL decreasing their survival (Nguyen et al., 2013; Kawasaki et al., 2022), growth (larval weight and length) (Nguyen et al., 2015) and prepupation (Kawasaki et al., 2022).

Carbohydrates are essential in the BSFL-rearing diet. Larvae utilize glucose, the monomer of carbohydrates, both as a building block for tissue and as a source of energy (Gold et al., 2018). Many studies observed lower larval survival when carbohydrates decreased (Nguyen et al., 2013; Kawasaki et al., 2022; Eggink et al., 2023). Generally, larvae fed on high-NFC have higher final weight and shorter development time (Bava et al., 2019). Ultimately excess or lack of any of the three mentioned macronutrients may negatively impact survival and performance (e.g. waste reduction), while a balance diet should be guaranteed, with  $NFC > P > L$ .

Although previous studies provide valuable insights as to how different macronutrient concentrations and their ratios impact the process, gaps persist in achieving a comprehensive understanding of how all possible scenarios, in terms of substrate quality, impact process performance. Limitations of the previous studies include a narrow range of: (i) overall macronutrient concentration ( $\sum PCL$ ), (ii) and individual macronutrient concentrations, and (iii) limited variations of macronutrient ratio. Many studies altered macronutrient concentrations but maintained balanced diets ( $NFC > P > L$ ) (Eggink et al., 2023; Rossi et al., 2023), thus excluding scenarios of unbalanced diets where proteins or lipids are in excess. Some studies focused on the influence of one macronutrient while keeping others constant; however, the range of the chosen macronutrient was narrow (e.g. Bellezza et al., 2022a). Isibika et al. (2021) covered the widest range of each macronutrient and their ratios.

The aim of this study was to gather, integrate, and elaborate all literature findings in order to reach a comprehensive understanding of how the nutritional substrate quality (in terms of  $\sum PCL$  and relative abundance of macronutrients) impacts the process performance, in terms of substrate reduction efficiency (RE) and larval survival rate (SR), focusing on biowaste treatment performance rather than resource recovery. A systematic literature review was conducted by using the PRISMA methodology (Liberati et al., 2009; PRISMA, 2023; Gurevitch

et al., 2018). After the descriptive analysis of all the gathered data, the relationship between the substrate quality and the process performance was investigated and graphically elaborated in order to obtain a simple tool useful for a rapid prediction of the individual substrate suitability to BSFL process and for the evaluation of optimal mixture of different substrates to achieve desired outcomes.

## 2. Materials and methods

### 2.1. Systematic review

A systematic literature review was conducted to identify literature sources investigating the use of BSF larvae for treating solid substrates, providing both the nutrient quality of substrates (in terms of proteins, non-fibre carbohydrates, and lipids content) and process performance (in terms of SR and/or RE).

The selection of literature sources has been performed by means of a step-by-step method procedure following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009; PRISMA, 2023; Gurevitch et al., 2018). The flow diagram of the stepwise screening procedure is depicted in Fig. 1. Literature sources were identified using the following databases: Web of Science, PubMed, and Scopus. The research had no publishing date limit. Documents identification was carried out by searching a keyword chain in the Titles, Abstracts, or Keywords, which was obtained by combining the following three categories (combined using ‘AND’):

- Keywords on the insect of interest: “Black Soldier Fly” OR “*Hermetia illucens*”.
- Keywords on substrate macronutrient analysis: Protein\* OR Lipid\* OR “Ether extract” OR fat\* AND NOT “Fatty Acid” OR Carbohydrate\*.
- Keywords on process performance metrics: Survival\* OR Mortality\* OR “Waste reduction”.

Documents had to meet the following criteria to be included in the review: (i) the document type is an article, (ii) the language of the article is English, and (iii) an electronic version of the article is available. Documents such as Review Articles, Proceeding Papers, Book Chapters, Letters, etc., were excluded. Articles cited by those identified, but not found by the identification step of PRISMA procedure, were further included as external sources. Identified articles underwent screening, and those with abstracts exceeding the topic of interest were excluded (e.g. quality of larval biomass). The next phase of full-text reading excluded articles according to the following criteria: (i) absence or incalculable data regarding protein, lipids, and non-fibre carbohydrates content in feeding substrate, (ii) absence of both survival rate and waste reduction, (iii) presence of contaminants or toxic compounds in substrates, (iv) unusual results justified by the authors as being affected by experimental errors or other human-related issues.

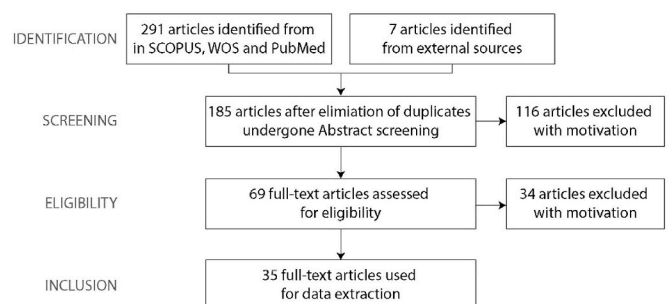


Fig. 1. Flow diagram of the step-by-step procedure followed in the systematic critical review for identification of articles (List of selected articles is provided in Supplementary Material).

2.2. Data collection and elaboration

The analysis of the substrates nutritional quality was performed considering the following parameters:

- The overall macronutrients concentration per substrate dry weight ( $\sum PCL$ ; %)
- The single macronutrients concentrations per substrate dry weight (P, NFC, L; %) and their relative abundance ( $X_P$ ,  $X_{NFC}$ , and  $X_L$ ), calculated according to the following formula:

$$X_i = \frac{I}{\sum PCL}$$

where:

i = the single macronutrient (P = protein, NFC = carbohydrates and L = lipids)

I = the single macronutrient concentration per substrate dry weight (P, NFC, L; %)

$\sum PCL$  = the overall macronutrients concentration per substrate dry weight (%)

The analysis of the process performance was performed considering the following parameters:

- Survival rate (SR, %)
- Reduction Efficiency (RE, %), (typically defined as waste reduction) calculated according to the following formula:

$$RE(\%) = \frac{S_{in} - S_{out}}{S_{in}} \cdot 100$$

where:

$S_{in}$  = total mass of dry substrate at the beginning of the process

$S_{out}$  = total mass of dry substrate at the end of the process

Data on substrate quality and process performance were collected from 35 papers (83% of which is ranked Q1), resulting in 177 substrates, including both biowaste and artificial substrates. SR and RE data were provided for 147 and 113 substrates respectively.

A descriptive analysis was firstly conducted on all collected data by considering both the overall substrates and the six substrate categories: (i) meat and fish waste, (ii) manure, (iii) grain waste, (iv) food waste, (v) vegetables and fruits, and (vi) artificial substrates. The artificial substrates included all optimal diets for larval breeding (e.g. chicken feed and Gainesville diet), specific mixtures of various substrates and substrates not fitting to any of the mentioned categories (e.g. insects, sludge, paper pulp). Significant differences between the mean concentrations calculated for the different categories were checked by one-way ANOVA performing Tukey pairwise comparisons ( $p \leq 0.05$ ); results are provided in supplementary material.

The relationship between the substrate quality (in terms of  $\sum PCL$  and relative abundance) and the process performance was finally investigated. Complete dataset and references, together with statistical analysis, can be found in Supplementary material.

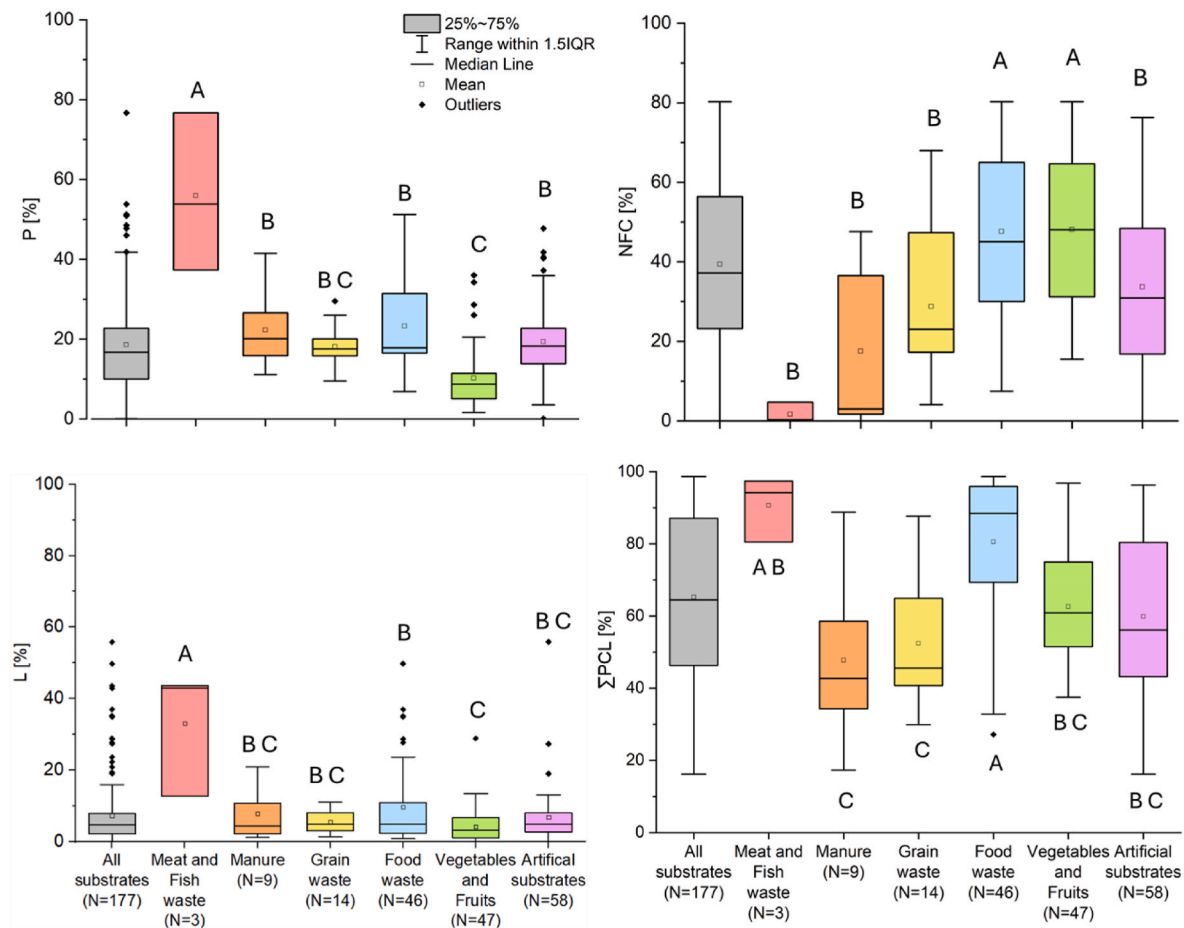


Fig. 2. Box plots of the concentrations of the overall macronutrients ( $\sum PCL$ ; %) and of each macronutrient (P, NFC, L; %) per substrate dry weight gathered for each substrate category. In each chart, average value of box plots with the same letter do not vary significantly ( $P < 0.05$ ). P = protein; NFC = non-fibre carbohydrates; L = lipids. (N = number of substrates).

### 3. Results and discussion

#### 3.1. Descriptive analysis of data overall and by substrate categories

The nutritional quality of all substrates and each substrate category was analysed in terms of the concentrations of overall macronutrients ( $\sum$ PCL; %) and of the individual macronutrient (P, NFC, L; %) (Fig. 2), as well as in terms of relative abundance of proteins ( $X_P$ ), carbohydrates ( $X_{NFC}$ ), and lipids ( $X_L$ ) (Fig. 3).

Overall, the substrates exhibited a narrow range of both macronutrients' concentrations and ratios. Most substrates (75%) had  $\sum$ PCL concentrations exceeding 50%. Only four substrates had  $\sum$ PCL below 20%, with diluted chicken feed showing the lowest at 16% (e.g. Barragán et al., 2018)). More than 70% of substrates were well-balanced in macronutrients ( $NFC > P > L$ ), with CH concentrations higher than 40% and P and L concentrations typically below 25% and 10%, respectively (Fig. 2). Consequently,  $X_{NFC}$  ranged from 0.4 to 0.9,  $X_P$  from 0.1 to 0.4, and  $X_L$  from 0 to 0.2 (Fig. 3). Substrates with low overall macronutrient concentration or imbalanced macronutrients (with excess lipids or proteins) were limited.

Considering the different substrate categories, most substrates fall into the food waste, vegetable and fruit, and artificial substrates categories, with totals of 46, 47 and 58 substrates, respectively. In contrast, only three substrates were meat and fish waste, nine were manure and 14 were grain waste. The distribution of substrates among these categories reflects their nutritional quality and suitability for larval processing. In fact, the most numerous categories, such as food waste, vegetables and fruits, and artificial substrates, are characterized by the best nutritional quality, with the highest  $\sum$ PCL concentrations and well-balanced macronutrient profiles. On the other hand, Meat and fish, despite being high in  $\sum$ PCL, had the highest P and L content, while manure had the lowest  $\sum$ PCL concentrations.

The process performance for each substrate category was analysed in terms of survival (SR, %) and reduction efficiency (RE, %) rates (Fig. 4). In general, reflecting the nutritional quality of the substrate, RE increased with  $\sum$ PCL and individual increase of NFC, and decrease of P and L. The lowest RE values were observed in manure, with the highest values in food waste and vegetable and fruit categories. The results are consistent with Eggink et al. (Sari et al., 2022), and Bava et al. (2019), whose observed a higher final larval weight and shorter development time when fed on high-NFC diets, as larvae consume more food to accumulate enough proteins before the pupa stage. Meat and fish,

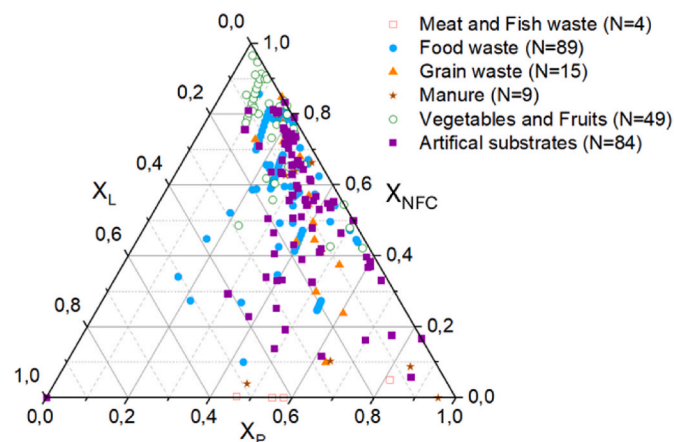


Fig. 3. The relative abundance of proteins ( $X_P$ ), carbohydrates ( $X_{NFC}$ ), and lipids ( $X_L$ ) for all substrates, grouped into six substrate categories.  $X_P$ ,  $X_{NFC}$ , and  $X_L$  were calculated as ratio between the macronutrient concentration per substrate dry weight (i.e. P, NFC and L, %) and overall macronutrients concentration per substrate dry weight ( $\sum$ PCL, %). P = protein; NFC = non-fibre carbohydrates; L = lipids. (N = number of substrates).

despite having the highest  $\sum$ PCL, showed the lowest RE due to the nutrient imbalance, with the highest P and L contents.

High SR values (averaging above 80%) were achieved by all substrate categories except for Meat and fish waste (Fig. 4). This lower SR may be attributed to the higher concentrations of P and L in Meat and fish waste compared to other categories (Gold et al., 2020; Nguyen et al., 2013; Kawasaki et al., 2022). Consequently, SR is more influenced by nutrient balance than by  $\sum$ PCL concentrations, consistently with previous studies (e.g. Gold et al., 2020; Bellezza et al., 2022b; Bava et al., 2019).

#### 3.2. Influence of substrate nutritional quality on process performance parameters

The relationships between process performance parameters (SR and RE) and nutritional quality are presented using scatter and triangular charts. Axis in the triangular charts represent the relative abundance of macronutrients, while colour represents the percentage of SR or RE, with red indicating the highest (100%) and dark blue the lowest (0%). The variation of SR in relation to  $\sum$ PCL and the relative abundance of macronutrients (i.e.  $X_P$ ,  $X_{NFC}$ , and  $X_L$ ) is illustrated in Fig. 5. When considering the relationship between SR and  $\sum$ PCL (Fig. 5A), the data show that for 89% of the substrates considered, survival rates exceeded 80% regardless of macronutrient concentrations, confirming that SR is independent of  $\sum$ PCL levels. Conversely, SR is influenced by the relative abundance of macronutrients. Fig. 5B illustrates this relationship in a triangular chart, showing the variation of SR with changes in  $X_P$ ,  $X_{NFC}$ , and  $X_L$ . SR decreases (SR < 80%) with excess lipids ( $X_L > 0.6$ ), lack of proteins ( $X_P \leq 0.05$ ), and excess proteins ( $X_P > 0.5$ ) with low carbohydrates ( $X_{NFC} < 0.15$ ). Moreover, SR is impaired when  $X_P$  ranges between 0.05 and 0.15, and  $X_{NFC} < X_L$ . The positive influence of carbohydrates on larval growth and survival, already confirmed by several studies (Kawasaki et al., 2022; Bava et al., 2019), is particularly important in case of P excess or deficiency.

The variation of RE in relation to  $\sum$ PCL and the relative abundance of macronutrients (i.e.  $X_P$ ,  $X_{NFC}$ , and  $X_L$ ) is illustrated in Fig. 6. Unlike SR, RE shows a slightly dependence on the  $\sum$ PCL (Fig. 6A). Fig. 6A shows two distinct clusters of data: one for  $\sum$ PCL < 60% and one for  $\sum$ PCL > 60%, with the average RE for  $\sum$ PCL > 60% being higher than that for  $\sum$ PCL < 60%. Consequently, the relationship between RE and the relative abundance of macronutrients was analysed separately, by means of two triangular charts, for  $\sum$ PCL < 60% and  $\sum$ PCL > 60% (Fig. 6B). For  $\sum$ PCL < 60%, RE rates decrease (RE < 40%), similar to SR, in cases of excess lipids ( $X_L > 0.65$ ), lack of proteins ( $X_P \leq 0.05$ ), and excess proteins ( $X_P > 0.55$ ) with low carbohydrates ( $X_{NFC} < 0.2$ ). A few exceptions are visible as blue spots in the RE > 40% area, corresponding to pig manure, characterised by low bioavailability of nutrients (Naseri et al., 2023), and spent coffee grains with autolyzed brewer's yeast, classified by authors as too dehydrated substrates for larvae (Sideris et al., 2021). For  $\sum$ PCL > 60%, data show that RE is generally > 40% and it increases further when  $X_P > 0.6$  and  $X_{NFC} > X_L$ . However, the available data exclude poor-quality substrates with excess lipids and proteins and lack of proteins.

#### 3.3. Identification of good nutritional quality substrate and limitations

The identification of good nutritional quality substrates (GQS) suitable for larvae treatment was performed based on the relative abundance of macronutrients, by fixing the following thresholds for process performance parameters: SR = 80% and RE = 40%. The area satisfying both SR > 80% and RE > 40% on all triangular charts (Figs. 5B and 6B) identifies the possible combinations of  $X_P$ ,  $X_{NFC}$ ,  $X_L$  for a substrate to be considered of good nutritional quality for larvae treatment, for any  $\sum$ PCL (Fig. 7A). According to Fig. 7A, GQS (SR > 80% and RE > 40%) occurs with  $X_L < 0.6$ ,  $X_P > 0.05$ , and when  $X_P > 0.5$  if  $X_{NFC} > 0.2$ . Additionally, when  $X_P$  ranges between 0.05 and 0.15 a GQS occurs if

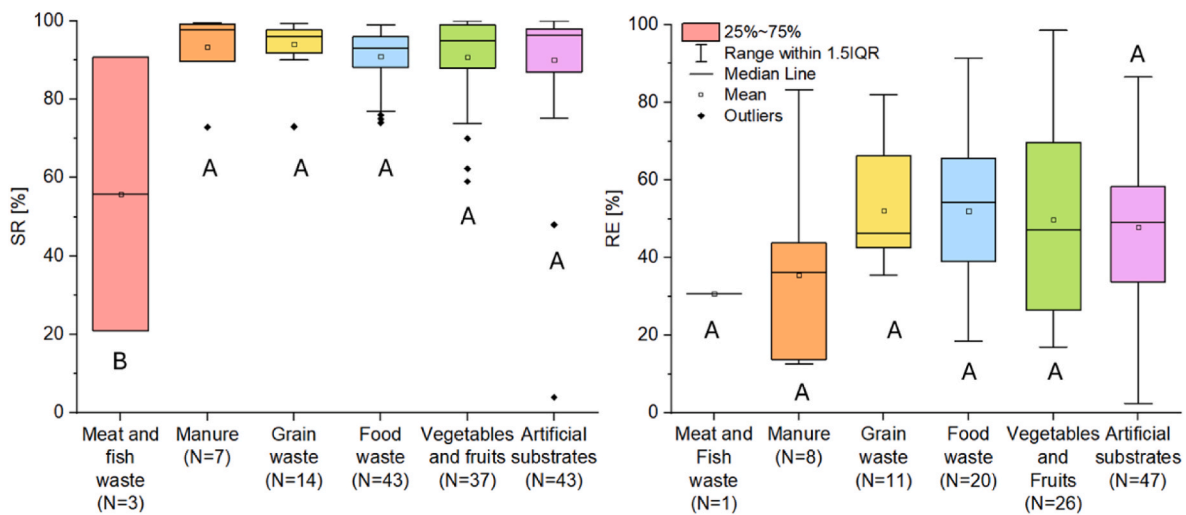


Fig. 4. Box plot of the survival (SR, %) and reduction efficiency (RE, %) rates gathered for each substrate category. In each chart, average value of box plots with the same letter do not vary significantly ( $P < 0.05$ ). (N = number of substrates).

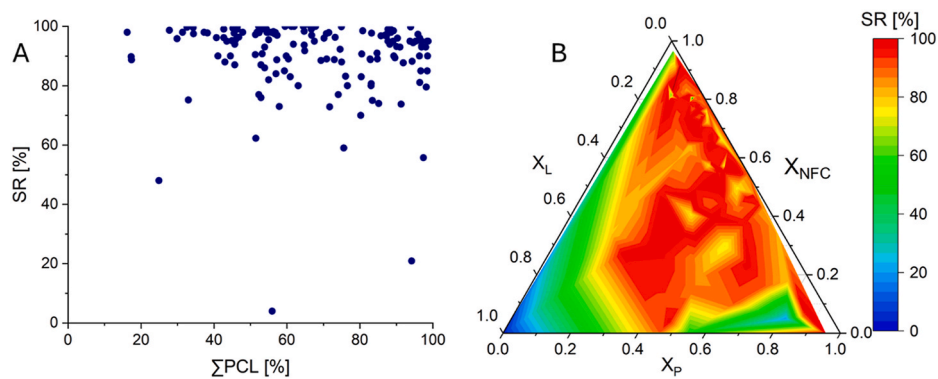


Fig. 5. Variation of the survival rate (SR) in relation to: (A) overall macronutrients concentration ( $\Sigma PCL$ ) and (B) relative abundance of macronutrients (i.e.  $X_p$ ,  $X_{NFC}$ , and  $X_L$ ). P = protein; NFC = non-fibre carbohydrates; L = lipids. Number of considered substrates: 147.

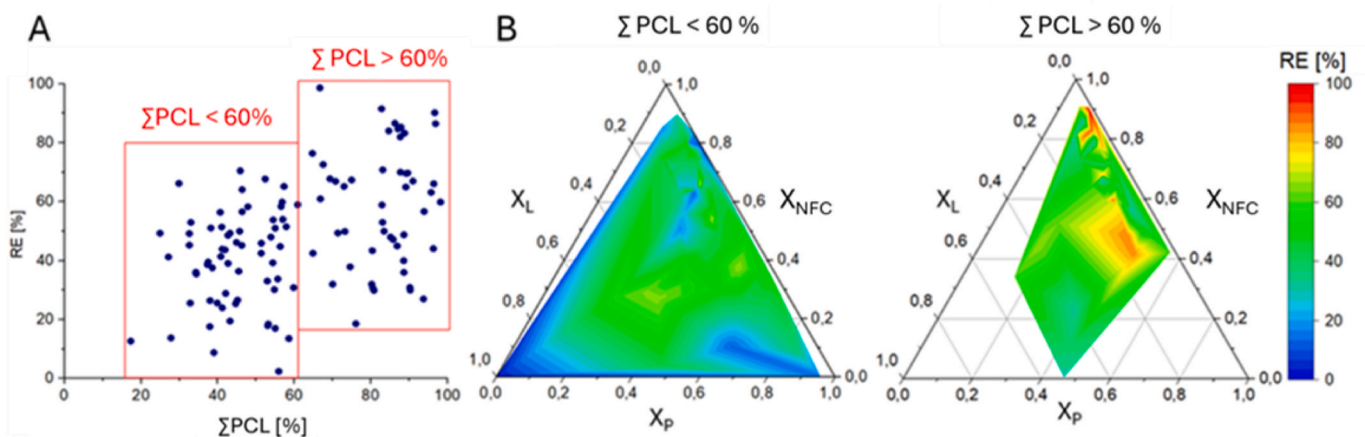
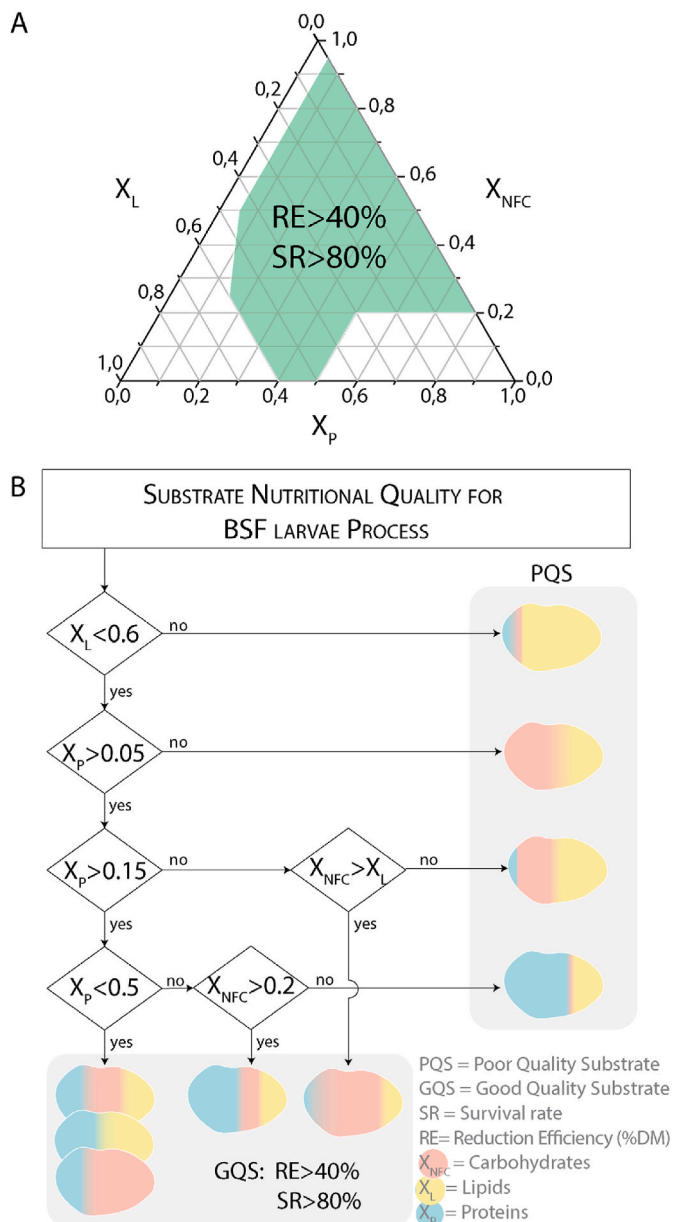


Fig. 6. Variation of the reduction efficiency (RE) in relation to (A) overall macronutrients concentration ( $\Sigma PCL$ ) and (B) relative abundance of macronutrients (i.e.  $X_p$ ,  $X_{NFC}$ , and  $X_L$ ), distinguishing when  $\Sigma PCL < 60\%$  and  $> 60\%$ . P = protein; NFC = non-fibre carbohydrates; L = lipids. Number of considered substrates: 51.

$X_{NFC} > X_L$ . Fig. 7B provides a flowchart to identify GQs considering the same thresholds for process performance.

Generally, Fig. 7 can be used for a preliminary evaluation of the suitability of a specific substrate for larvae treatment based on its relative abundance of macronutrients ( $X_p$ ,  $X_{NFC}$ ,  $X_L$ ). Additionally, it can

help determine the optimal combination of different substrates to achieve a balanced substrate composition. Optimizing substrate macronutrient balance can be accomplished by mixing various types of biowaste, each offering an abundance of specific nutrients. For example, protein-rich waste (such as meat and fish), which has a high protein content



**Fig. 7.** Identification of good quality substrates (GQS) based on relative abundance of proteins ( $X_p$ ), non-fibre carbohydrates ( $X_{NFC}$ ) and lipids ( $X_L$ ). GQS are those achieving  $SR > 80\%$  and  $RE > 40\%$ . GQS are identified by means of triangular chart (A) and flowchart (B).  $X_p$ ,  $X_{NFC}$  and  $X_L$  were calculated as the ratio between the macronutrient concentration per substrate dry weight (i.e. P, NFC and L, %) and overall macronutrients concentration per substrate dry weight ( $\sum PCL$ , %).

but lacks carbohydrates, can be combined with carbohydrate-rich waste (like fruits and vegetables), which offers more carbohydrates but may be low in protein.

However, further laboratory tests should be performed to confirm the preliminary evaluation. In fact, other factors not considered in this general scheme, such as the origin and bioavailability of macronutrients, may also influence process performance (Barragán et al., 2018; Ribeiro et al., 2022; Spranghers et al., 2017). For example, the low performance of BSFL was observed when they were fed with apples, whose carbohydrates are primarily composed of simple sugars (Ribeiro et al., 2022; Broeckx et al., 2021b). Further, other nutrients such as vitamins and minerals as well as the amino acid profile might affect the performance (Edelman and Colt, 2016). Another important aspect to consider is the

presence of toxic substances. For instance, heavy metals in fish and polyphenols in winery by-products are a possible inhibitor of the larval process (Meneguz et al., 2018b; Lopes et al., 2020; Isibika et al., 2021). Finally, the analysed amounts of macronutrients may not fully represent the actual bioavailable content for larvae. For instance, Meneguz et al. (2018b) noted that the nutrients within grape seeds, quantified during the analytical characterization of the substrate, were not entirely accessible because the seeds were not thoroughly milled during pre-treatment prior to feeding the larvae.

#### 4. Conclusions

The treatment of biowaste using the BSF larvae process is influenced by the nutritional quality of substrate, which can be related to the overall concentration and relative abundance of dominant macronutrients, such as proteins (P), non-fibre carbohydrates (NFC), and lipids (L). Knowing how these substrate quality parameters influence the process performance is fundamental to determine the suitability of a substrate to BSFL process and to optimise it. However, a comprehensive understanding of how all possible scenarios, in terms of substrate quality, impact the process performance is still missing. This study performed a systematic literature review to gather and synergize all literature findings regarding the impact of the substrate quality (in terms of  $\sum PCL$  and macronutrients relative abundance –  $X_p$ ,  $X_{NFC}$ ,  $X_L$ ) on process performance, in terms of reduction efficiency (RE) and survival rate (SR).

From the results the following conclusions can be drawn:

- Most of literature studies investigated the treatment food waste, vegetable and fruit waste, and artificial substrates which are characterized by the best nutritional quality, with the highest  $P + C + L$  concentrations and well-balanced macronutrient profiles ( $NFC > P > L$ ). This results into limited knowledge regarding poor nutritional quality substrates.
- SR (%) is independent of  $\sum PCL$  while it varies with relative abundance of macronutrients.
- RE (%) increases with the increase of  $\sum PCL$ .
- Good nutritional quality substrates have been assumed to be suitable for larvae treatment when  $SR > 80\%$  and  $RE > 40\%$ . Accordingly, good nutritional quality substrates have been identified based on the relative abundance of macronutrients for any  $\sum PCL$  when  $X_L < 0.6$ ,  $X_p > 0.05$ , and when  $X_p > 0.15$  if  $X_{NFC} > 0.2$ . Additionally, when  $X_p$  ranges between 0.05 and 0.15 a good quality substrate occurs if  $X_{NFC} > X_L$ .
- These findings can be used for a preliminary evaluation of the suitability of a specific substrate for larvae treatment based on its relative abundance of macronutrients ( $X_p$ ,  $X_{NFC}$ ,  $X_L$ ) or to determine the optimal mix of different substrates to achieve a suitable substrate.
- However, further laboratory tests should be performed to confirm the preliminary evaluation, checking eventual influence of other factors not considered in this general scheme, such as the origin and bioavailability of macronutrients, presence of toxicants or availability of vitamins minerals.
- Further studies should be performed, preferably by means of artificial diets and constant environmental conditions, to better investigate atypical substrate quality scenarios and to include other process performance parameters (such as bioconversion factor).

#### CRedit authorship contribution statement

**Valentina Grossule:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Mia Henjak:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Giovanni Beggio:** Writing – review & editing, Methodology. **Jeffery K. Tomberlin:** Writing – review & editing, Formal analysis.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Valentina Grossule reports financial support was provided by European Union (Next generation EU programme) and Padova University (STARS@UNIPD programme). If there are other authors, they declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2024.123605>.



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## Data availability

Data will be made available on request.

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