UNIVERSITÀ DEGLI STUDI DI PADOVA
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MASTER THESIS

The Use of Black Soldier Fly Larvae in Wastewater Treatment: The Influence of Organic Concentration on Process Efficiency and Substrate Consumption Rate

Supervisor:

Prof.ssa VALENTINA GROSSULE

Student:

VANJA NOVAKOVIĆ
2085458

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I dedicate this thesis to my family, whose solid support has been my foundation throughout my education. Their love, encouragement, and sacrifices have made this accomplishment possible.

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Abstract

The application of Black Soldier Fly (BSF) larvae (Hermetia illucens) in wastewater treatment (WWT) presents an innovative and sustainable solution for the biological processing of high organic content wastewater. This study investigates the potential of Black Soldier Fly (BSF) larvae in treating wastewater with varying concentrations of organic matter, focusing on total organic carbon (TOC) removal. Experiments were conducted using two organic concentrations (1000 mg/L and 2500 mg/L) to examine the effects of organic loading rate on substrate consumption rate, treatment efficiency, and larval mortality. Specifically, the research aimed at confirming the applicability of a Michaelis-Menten like relationship between substrate consumption rate and organic load, with findings indicating that larvae exhibit increased substrate removal as organic load increases, though treatment efficiency declines at higher loads due to larval overload. Mortality was significantly higher at low organic loads, suggesting that insufficient nutrients contribute to higher death rates, while overloading at high concentrations reduced system efficiency without a dramatic rise in mortality. The study concludes that optimizing organic loading rates is essential to balance substrate removal and efficiency, highlighting the potential of BSF larvae as an effective tool for sustainable wastewater treatment.

1. INTRODUCTION

1.1. Role of BSF larvae in the WWT sector

In an era characterized by rapid scientific and technological advancements, it is challenging to perceive limitations to human capabilities. Nonetheless, the magnitude and immediacy of the challenges we face are equally significant. As global population rise and industries expand, the demand for clean water and effective wastewater treatment has become more urgent than ever. Traditional mechanical and chemical methods, while effective, are energy-intensive and often result in the production of harmful byproducts. Moreover, these methods are increasingly ill-suited to the complex and diverse wastewater compositions arising from modern industrial activities. In contrast, bio-based technologies, which leverage the natural processes of living organisms, are proving to be more sustainable, adaptable, and cost-effective. Among these technologies, the use of Black Soldier Fly Larvae has emerged as a promising alternative for treating high organic content wastewater (Grossule V. F., 2022).

Bio-based technologies, such as microbial bioreactors and enzymatic processes, are more environmentally friendly, utilizing the innate capabilities of organisms to degrade contaminants without the adverse effects of traditional chemical treatments. BSFL, in particular, offer an efficient, scalable solution by converting organic waste into valuable biomass. Unlike mechanical or chemical processes, bio-based treatments are energy-efficient, reduce reliance on harmful chemicals, and align with the growing regulatory emphasis on sustainable practices in wastewater management (Strategy, 2018).

The shift toward bio-based technologies is also driven by increasing regulatory pressures. In the EU, for example, the EU Bioeconomy Strategy aims to transition to a more sustainable, bio-based economy, encouraging the use of renewable biological resources in industrial processes, including wastewater treatment. This is further reinforced by the new draft of the Wastewater Directive, which places a strong emphasis on the recovery of valuable resources from wastewater, minimizing environmental pollution, and enhancing the circular economy (Directive, 1991).

One of the most significant advantages of using BSFL in WWT is the ability to recover valuable resources from wastewater. As the larvae consume organic material in the wastewater, they convert it into protein-rich biomass and frass (a nutrient-rich residue that can be used as organic fertilizer). The biomass produced by BSFL is particularly valuable, as it can be upcycled into animal feed or biofuels, offering a sustainable method for resource recovery. This not only reduces the organic load in wastewater but also transforms waste into usable products, contributing to the circular economy (Grossule V. F., 2022).

In the context of the EU's regulatory landscape, the new draft of the Wastewater Directive emphasizes the need for the recovery of nutrients like phosphorus and nitrogen from wastewater streams. The BSFL process aligns well with this objective, as the larvae can effectively capture and recycle these nutrients, thereby reducing the risk of eutrophication in water bodies. Phosphorus, for instance, is a non-renewable resource critical for agriculture, and its recovery from wastewater could help mitigate the environmental impact of mining for new sources. In this regard, BSFL could play a pivotal role in achieving the EU's sustainability goals by turning organic pollutants into valuable resources and closing the loop on waste management (Grossule V. F., 2022) (Directive, 1991) (Strategy, 2018).

The ability to upcycle wastewater contaminants into high-value products such as larval biomass and organic fertilizers not only provides economic incentives but also helps to reduce the environmental footprint of wastewater treatment. This is especially relevant as governments push for more stringent regulations on wastewater discharge, requiring innovative approaches that go beyond traditional treatment methods.

High organic content wastewater poses significant challenges for conventional WWT plants, primarily due to its high Biochemical Oxygen Demand (BOD) and elevated levels of suspended solids. These conditions can overwhelm traditional treatment systems, leading to inefficiencies and potential environmental impacts. For instance, food processing wastewater, which is rich in organic materials such as fats, proteins, and sugars, can exceed the capacity of typical biological or chemical treatments, leading to increased operational costs and reduced treatment efficiency.

BSFL offer a biological solution to the pretreatment of such highly organic wastewaters. These larvae are capable of consuming large amounts of organic material, significantly reducing the organic load before the wastewater enters the main treatment stages. Studies have demonstrated that BSFL can reduce Chemical Oxygen Demand (COD) and BOD levels by efficiently breaking down organic matter and converting it into larval biomass. This biomass can then be harvested and upcycled into high-value products, providing a cost-effective solution to managing high organic content wastewater (Grossule V. F., 2022).

Furthermore, conventional WWT processes often overlook the potential for resource recovery in these waste streams. While traditional systems focus on contaminant removal, they miss the opportunity to harvest valuable nutrients and materials embedded in the wastewater. BSFL-based systems address this gap by enabling the recovery of proteins, lipids, and other organic materials that can be repurposed for agricultural, industrial, or bioenergy applications. This capability aligns with the principles of a circular economy, turning waste into a resource and reducing the environmental impact of wastewater management (Directive, 1991).

As the demand for clean water grows and regulatory pressures mount, bio-based technologies like BSFL are becoming increasingly essential in the WWT sector. By offering a sustainable, adaptable, and cost-effective approach to treating high organic content wastewater, BSFL systems not only improve treatment efficiency but also recover valuable resources, promoting a circular economy. The ability to upcycle organic waste into usable products positions BSFL as a vital component of the future of wastewater management. As industries and municipalities seek to meet new regulatory standards and minimize their environmental impact, the role of BSFL in wastewater treatment will continue to grow, paving the way for more sustainable and efficient WWT technologies (Grossule V. F., 2022) (Directive, 1991) (Strategy, 2018).

1.2. LarWar process

While BSF larvae have been successfully exploited in the treatment of putrescible solid waste (e.g., food residues), the application to wastewater has been hindered by the fact that larvae are terrestrial organisms and fail to survive in a liquid environment.

To overcome this critical issue, Grossule and Cossu (2021) have proposed and patented a technical solution (Italian Patent n. 102021000016700; PCT/IB2022/055888; priority 25/06/2021) based on the adoption of a porous material to be installed in the reactor to support larval mobility. The porous material, saturated with the targeted wastewater, allows larvae to dive for eating and to re-emerge for breathing (Grossule et al., 2021).

The patented solution paved the way to the development of a wastewater treatment process based on use of BSF larvae (shortened in the acronym LarWaR stands for Larvae for Wastewater treatment and Resource recovery), which couples the wastewater treatment with the conversion of organic waste into biomass. This approach is being researched as a more sustainable option compared to traditional methods, as BSF larvae produce valuable biomass that can be harvested for use in animal feed or biofuel production. Studies show that BSF larvae perform well in treating wastewater from food industries and municipal solid waste (MSW) landfill leachate, effectively removing high levels of organic matter (Grossule V. F., 2023).

The design and optimization of BSF larvae reactors for wastewater treatment are critical for ensuring efficiency and scalability. Key aspects of this process include the selection of physical support materials, optimization of the parameters, and reactor configuration.

The organic load and the concentration of organic content in the substrate are two key factors influencing the performance of the LarWar process. Grossule et al., (2023) found that their effects on the larvae's growth, substrate consumption, and overall wastewater treatment efficiency can be summarized as follows:

The specific substrate consumption rate (υ_S) of BSF larvae follows a Michaelis-Menten curve, meaning that the consumption rate increases with organic load but approaches a maximum value (υ_{max}) beyond which further increases in load do not significantly enhance consumption.

Grossule et al., (2023) results show that at the maximum consumption rate for BSF larvae (υ_{max}) is 3.8 mg of TOC (Total Organic Carbon) per larva per day, with a saturation point (Km) of 34.3 mg TOC per larva.

Lower organic loads, below 10 mg TOC per larva, result in poor larval growth, lower maximum wet weights (35-45 mg/larva), low prepupation rates (0-2%), and higher mortality rates (2.5-7%). In contrast, higher organic loads lead to increased larval biomass and improved survival rates. For example, loads of 13-14.5 mg TOC per larva yield higher larval weights (38.3-52.6 mg/larva), and even greater loads (27-27.4 mg TOC per larva) further increase larval weights (53.9-67.9 mg/larva) (Grossule V. F., 2023).

A significant correlation between the specific substrate removal rate and organic load underscores the importance of optimizing this factor to achieve higher removal efficiencies in the reactor. Interestingly, studies show that the concentration of organic content within the substrate does not significantly affect the specific substrate consumption rate of BSF larvae. While the larvae consume organic matter more rapidly as the organic load (F/L) increases, the concentration itself does not play a large role in determining the consumption rate.

Bordin research aimed at achieving a 95% removal efficiency confirmed an average removal efficiency of 94% for both TOC (Total Organic Carbon) and COD, demonstrating the system's effectiveness. This was supported by a high correlation between the maximum specific substrate consumption rate and overall removal efficiency, validating the model's use in reactor design. The relationship between the specific substrate consumption rate (υ_S) and the organic load (F/L) was found to follow Michaelis-Menten kinetics, providing framework for optimizing reactor conditions (Bordin).

Optimizing Reactor Conditions for LarWar:

- Hydraulic Retention Time (HRT): BSF larvae reactors show a significantly lower HRT compared to conventional activated sludge systems, with HRTs being four to five times shorter. This highlights the system’s ability to process waste more quickly, reducing the necessary reactor size for the same throughput.

- Environmental Conditions**:** Maintaining optimal temperature (24-30°C) and moisture content (60-70%) is crucial for larval activity. These factors directly affect the larvae’s metabolic rate and growth, ensuring efficient substrate consumption.

- Support Material: The choice of support material is another important consideration. For instance, Kaldness media has shown superior performance in supporting larvae during the treatment process.

1.3. Research objective

Grossule et al., (2023) found a relationship similar to the Michaelis-Menten kinetics between the load and specific daily consumption rate of BSF, regardless of concentration. The goal of the current research was to confirm this relationship by testing two different diet concentrations. By systematically varying two different concentrations of the diet given to the BSF larvae, this study aims to verify the initial findings and better understand the mechanisms behind this relationship. This investigation could significantly improve the design parameters for BSF larvae reactors, optimizing their performance in wastewater treatment. Understanding if different diet concentrations affect consumption rates can provide crucial insights for scaling up BSF larvae systems and improving their overall effectiveness. This research seeks to confirm the previously observed kinetic relationship but also aims to contribute to sustainable wastewater treatment technology by providing empirical data and theoretical insights for future reactor design and operation strategies.

2. THEORETICAL DESIGN FRAMEWORK

2.1. Design parameters

The design process for the LarWar system aims at determining the reactor's Volume (V) and/or Hydraulic Retention Time. To achieve this, several key parameters need to be correlated. These include the desired treatment efficiency (η) of the LarWar process to be achieved, the larvae density, the Food/Larvae (F/L) ratio i.e. the organic load that influences the growth and substrate processing, and the specific substrate consumption rate (v_s), which denotes the rate at which larvae consume the substrate on a per-larvae basis.

These parameters are crucial for establishing the system's operational efficiency, as they directly influence the required reactor size and the HRT necessary to meet treatment goals. Ultimately, defining the reactor's volume or HRT depends on how well these parameters are balanced and optimized within the system.

The design process for the Black Soldier Fly Larvae treatment system starts with the formula for Hydraulic Retention Time (HRT):

$$HRT = \frac{V}{Q} \tag{2.1}$$

Where:

- HRT = Hydraulic Retention Time (day)
- V = Volume of the reactor (L)
- Q = Flow rate (L/day)

The following formulas describe the key parameters:

1. Treatment Efficiency:

The treatment efficiency is given by:

$$\eta = \frac{S0 - S}{S0} \cdot 100 \tag{2.2}$$

Where:

- η = Treatment efficiency (%)
- S_0 = Initial substrate concentration (mg/L)
- S = Final substrate concentration (mg/L)

2. Larval density:

Larval density can be defined in terms of both surface area and volume; however, both for liquid and solid substrates, surface larval density is more commonly utilized, as oxygen transfer primarily occurs at the surface. Larval metabolism is strongly dependent on atmospheric oxygen, as they surface to access air since they are unable to extract oxygen from the substrate and will suffocate in water.

$$Cls = \frac{Nlarvae}{A} \tag{2.3}$$

$$Clv = \frac{Nlarvae}{V} \tag{2.4}$$

Where:

- A= Surface Area (m^2)
- N_{larvae} = Number of larvae
- C_{ls} = Larval Superficial Density (larvae/cm^2)
- C_{lv} = Larval Volumetric Density (larvae/cm^2)

Grossule et al., (2024) found that the optimization of the treatment process depends on maintaining appropriate superficial larval density, with 16 larvae per cm² being optimal for balancing organic matter conversion and survival.

An important parameter is also the average depth at which the larval mass submerges in a container filled with material that is fully saturated by the liquid substrate. This measurement is used to set the liquid level within reaction tanks to maximize volume while ensuring the substrate is accessible and avoiding areas where wastewater treatment is inadequate. This parameter serves as a connecting factor between the two types of larval density, linking volumetric larval density and surface larval density through the following formula:

$$Cls = Clv \cdot H \tag{2.5}$$

Where:

- H= Submergence depth (m)

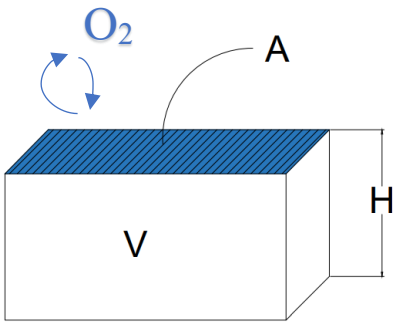

Figure 1. Illustration showing volumetric and surface measurements of larval density, emphasizing the relationship between larval distribution and oxygen transfer, which predominantly occurs at the surface. The figure highlights the significance of surface area over volume when calculating larval density in environments where oxygen availability is a key factor.

3. Specific Substrate Consumption Rate:

   This rate defines the amount of substrate consumed by one larva daily:

$$\vartheta s = \frac{Q \cdot (So - S)}{Nlarvae} \tag{2.6}$$

   Where:

   - ϑs = Substrate removal rate (mg/larvae/day)
   - Q = Volumetric Flow (L/day)

4. The organic loading rate or Food to Larvae ratio (F/L) is given by:

$$\frac{F}{L} = \frac{Q \cdot So}{Nlarvae} \tag{2.7}$$

Where:

- F/L = Daily specific organic load per larvae (mg/larvae/day)

To explore the relationship between the organic loading rate, treatment efficiency, and substrate removal rate, let's introduce the term (S_0−S) in both the numerator and denominator of the equation:

$$\frac{F}{L} = \frac{Q \cdot So}{Nlarvae} \cdot \frac{So - S}{So - S} \tag{2.8}$$

Thus, the final expression becomes:

$$\frac{F}{L} = \frac{\vartheta s \cdot 100}{\eta} \tag{2.9}$$

This formula clearly demonstrates that the organic loading rate is directly proportional to the substrate removal rate and inversely proportional to the treatment efficiency. In simpler terms, for a given substrate removal rate, as the efficiency increases, the required organic loading rate decreases.

Further, a relationship between HRT, treatment efficiency, volumetric larval density and substrate removal rate can be formulated by expressing the volume in terms of the volumetric larval density and the number of larvae, as well as expressing number of larvae in terms of the specific substrate consumption rate, we can incorporate the treatment efficiency to derive the following formula:

$$HRT = \frac{V}{Q} = \frac{\eta \cdot So}{Clv \cdot \vartheta s} \tag{2.10}$$

Which can be expressed also with superficial larval density and submergence depth:

$$HRT = \frac{So \cdot \eta}{Cls \cdot \vartheta s} \cdot H \tag{2.11}$$

This means that reactor volume can be expressed with submergence depth as:

$$V = \frac{Q \cdot So \cdot \eta}{Cls \cdot \vartheta s} \cdot H \tag{2.12}$$

To optimize a reactor design for wastewater treatment, the process begins by determining the required treatment efficiency based on regulatory standards or specific process requirements. This efficiency defines how much of the incoming substrate (pollutants) needs to be removed during treatment. Once the target efficiency is established, the next step involves calculating the optimal hydraulic retention time. This calculation is based on the desired substrate removal rate and the biological capacity of the treatment system, modeled using Michaelis-Menten-like relationship, which describes how the load affects the rate of substrate removal, which will be explained further on.

With the optimal HRT in hand, the flow rate (Q) can be set by solving for Q in the reactor volume equation (2.1). This equation links the flow rate to the volume of the reactor needed to achieve the required retention time for effective treatment.

Once the flow rate is set, the next step is to calculate the reactor volume that corresponds to the optimal flow rate and HRT. This ensures that the reactor is appropriately sized to handle the desired throughput while providing sufficient time for biological processes to degrade the organic matter.

Finally, the organic loading rate is adjusted to ensure the system is neither overloaded nor underloaded. If it is too high, the reactor might not remove enough substrate due to system overload, while too low can lead to inefficient use of the reactor's capacity. By balancing these parameters, the system can be optimized for both efficiency and cost-effectiveness.

2.2. The Michaelis-Menten-like relationship

The Michaelis-Menten equation is widely used to describe the relationship between substrate concentration and the reaction rate in enzymatic processes. It illustrates how the reaction rate increases with substrate concentration before reaching a maximum value, at which point the system becomes saturated. The equation is expressed as:

$$\vartheta = \frac{\vartheta max \cdot S}{Km + S} \tag{2.13}$$

Where:

- υ = Reaction rate or substrate removal rate (mg/L/day)
- υ_{max} = Maximum reaction rate (mg/L/day)
- S = Substrate concentration (mg/L)
- K_m= Michaelis constant (mg/L), which is the substrate concentration at half of the maximum reaction rate (υ_{max})

This model helps explain how, as the concentration of a substrate increases, the reaction rate also increases but eventually levels off as the system reaches its capacity for substrate processing. Initially, the system responds effectively to increases in substrate, but after a certain point, additional substrate does not significantly boost the reaction rate.

In research involving Black Soldier Fly larvae, the Michaelis-Menten equation can be adapted to describe the substrate removal process by larvae, where the focus shifts from substrate concentration to the organic loading rate per larvae. The specific substrate consumption rate can be described by the modified Michaelis-Menten equation:

$$vs = \frac{vmax \cdot \frac{F}{L}}{Km + \frac{F}{L}} \tag{2.14}$$

Where:

- υ_s = Specific substrate consumption rate (mg/larvae/day)
- F/L = Daily specific organic load per larvae (mg/larvae/day)
- υ_{max} = Maximum reaction rate per larvae (mg/larvae/day)
- K_m = Michaelis constant, which represents the value of F/L at which the reaction rate υ is half of υ_{max}

This equation highlights, as shown in the Figure 2, that as the organic loading rate increases, the substrate removal rate also increases but eventually reaches a maximum (υmax), beyond which further increases in load have minimal effect on the removal rate. The Km value is a critical point, indicating the F/L ratio at which the removal rate is half of its maximum capacity.

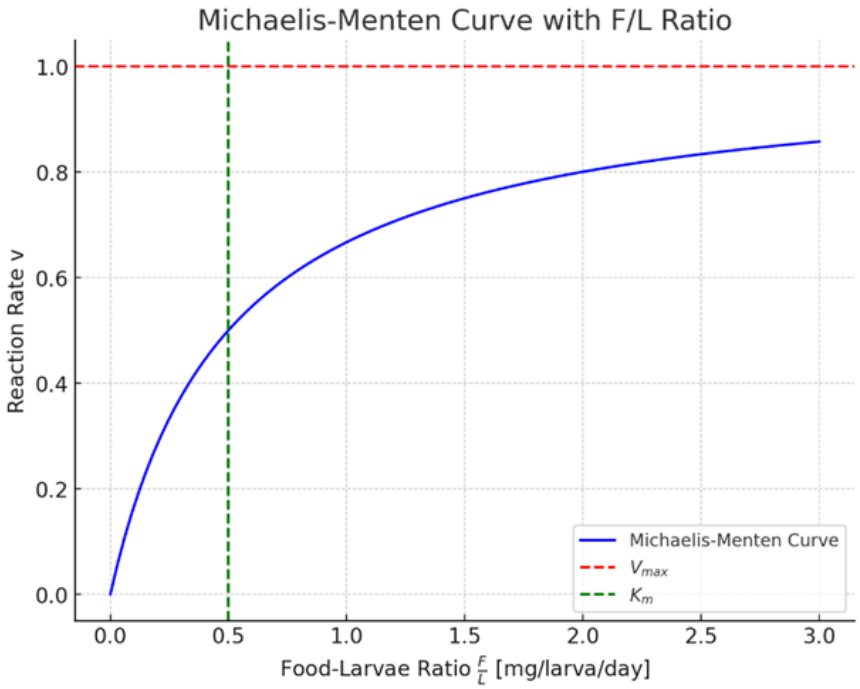

Figure 2. Figure showing the Michaelis-Menten curve illustrating the relationship between the Food-Larvae Ratio and the Specific Substrate consumption rate. The blue curve represents the reaction rate as a function of the F/L ratio, which increases rapidly at lower F/L values but begins to plateau as it approaches the maximum reaction rate shown by the red dashed line. The green dashed line indicates the Michaelis constant, which represents the F/L ratio at which the reaction rate reaches half of Vmax. This figure highlights the diminishing returns in reaction rate as F/L increases, following a classic saturation curve typical of Michaelis-Menten kinetics.

This inverse relationship from formula 2.9 is crucial in the design and operation of biological reactors, as it helps engineers optimize the system for maximum efficiency under varying conditions. By understanding and controlling these parameters, the reactor can be adjusted to achieve the desired treatment outcomes while minimizing resource use. After demonstrating the relationship between the specific substrate consumption rate and the organic load, we can graphically represent also the relationship between the efficiency and specific substrate consumption rate as functions of organic load:

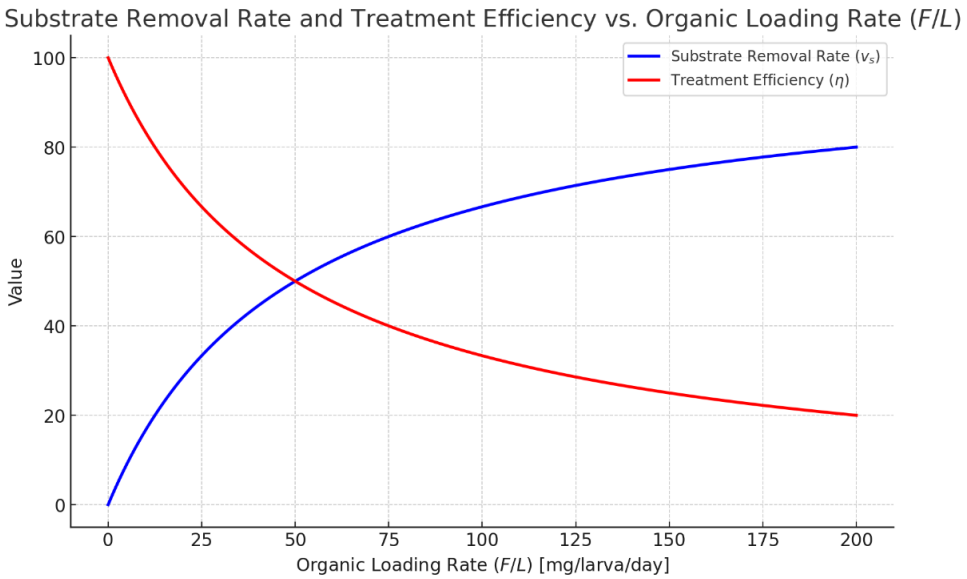

Figure 3. Figure shows the relationship between Substrate Removal Rate and Treatment Efficiency as functions of Organic Loading Rate (F/L) in mg/larva/day. The blue curve represents the Substrate Removal Rate, which increases exponentially with rising organic loading, indicating that larvae consume more substrate as the load increases. Conversely, the red curve represents Treatment Efficiency, which decreases exponentially as the Organic Loading Rate increases, reflecting a decline in system efficiency due to larval overload. The intersection of these curves highlights the trade-off between maximizing substrate removal and maintaining high treatment efficiency, suggesting an optimal loading range where both parameters are balanced.

3. MATERIALS AND METHODS

3.1. Research program

In order to reach the objective of the research, two set of tests were conducted. The first test, LC, consisted of 18 boxes, in which the concentration was fixed as 1000 mg/L, and 6 different loads, were tested. The second test, HC, had the same configuration, but with a concentration of 2500 mg/L. The tests were conducted to confirm once again the relationship between the organic load rate, substrate removal rate and efficiency of the process.

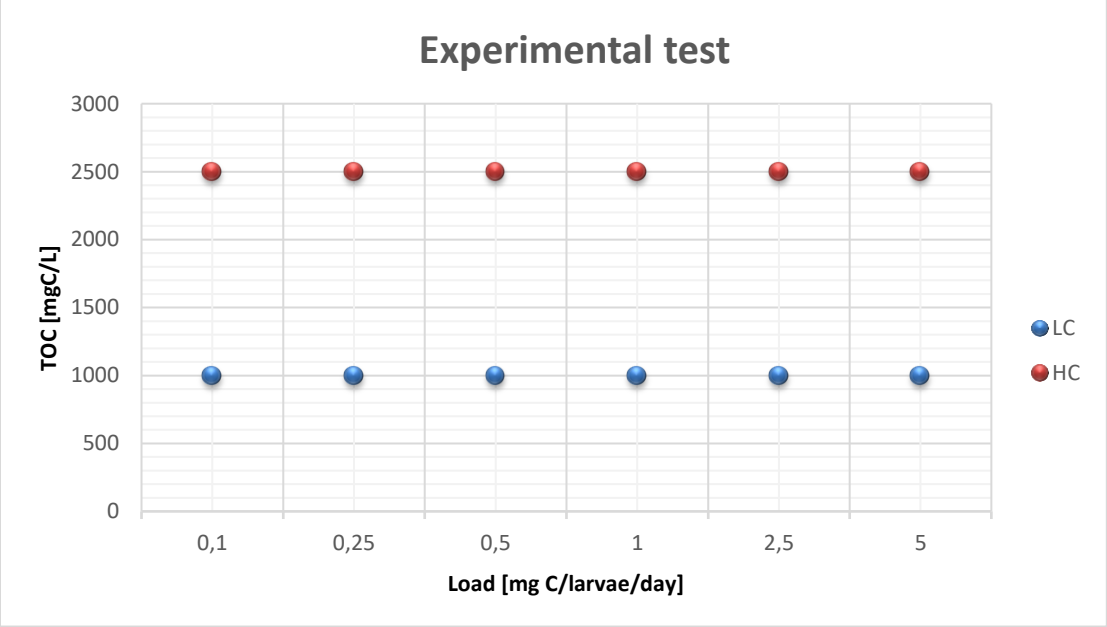

Figure 4. The figure presents a graph plotting Total Organic Carbon (TOC) concentration in mg/L on the y-axis against various load levels (mg C/larvae/day) on the x-axis. Two datasets are represented: one for LC (blue circles) and the other for HC (red circles).

The experiment was performed by placing in plastic boxes young BSF larvae separated manually. Larvae were physically supported by a plastic granular bed (Kaldness), which was fully saturated by the feeding solution. Each of the tests had 3 replicates, marked as A, B, C. That gives 36 boxes of tests in total. In the Table 1, the number of larvae and the feeding substrate's volume for each of the tests is shown. The samples were named based on their TOC concentration and load, as

shown in the table provided, with corresponding volumes and larvae numbers adjusted accordingly.

Test name	TOC	Load (mg C/larvae*day)	Volume (mL/day)	No. Larvae
LC_0.1	1000	0,1	50	357
LC_0.25	1000	0,25	50	143
LC_0.5	1000	0,5	50	71
LC_1	1000	1	200	143
LC_2.5	1000	2,5	200	57
LC_5	1000	5	300	43
HC_0.1	2500	0,1	50	893
HC_0.25	2500	0,25	50	357
HC_0.5	2500	0,5	50	179
HC_1	2500	1	200	357
HC_2.5	2500	2,5	200	143
HC_5	2500	5	300	107

Table 1. This table outlines the experimental setup for different sample batches (LC and HC) under varying conditions of Total Organic Carbon concentrations and load rates. The batches are categorized into two groups: LC (highlighted in blue) with a TOC concentration of 1000 mg/L, and HC (highlighted in red) with a TOC concentration of 2500 mg/L. Each batch was tested with six different load rates, ranging from 0.1 to 5 mg C/larvae/day. The volume used in the experiments varied between 50 mL and 300 mL, while the number of larvae per setup ranged from 43 to 893. All the tests were conducted in batch-type reactors.

3.2. Equipment and growth conditions

The biological material used in this study consisted of BSF larvae, which were sourced from a local supplier. The larvae used in the experiments were 10 days old and had an average weight of 0,011 g. Prior to the experiments, the larvae were in their natural habitat conditioned by room temperature.

The substrate provided to the larvae consisted of glucose powder (D (+) Glucose anhydrous - RPE - For analysis - Plastic bottle 500 g - CAS = 50-99-7**)** and whey protein powder (Yamamoto® Nutrition Iso-FUJI 100% NATURAL Volactive® 700 grams), which was prepared by dissolving powders in distilled water. This substrate served as the

primary food source for the larvae throughout the study.

The 36 batch reactors used in this study were plastic containers with a capacity of 500 mL. The containers were filled with Kaldness saturated with different volumes of feeding substrate. The reactors were designed to allow for easy access to the larvae and substrate, and they were equipped with a permeable non-woven fabric covers to prevent larvae escape and ensure adequate ventilation. All tests were carried out in a thermal insulated room under the same environmental conditions suggested by Grossule and Lavagnolo (Grossule V. a., 2019): temperature range 25-30 °C; photoperiod Light/Dark of 18/6h.

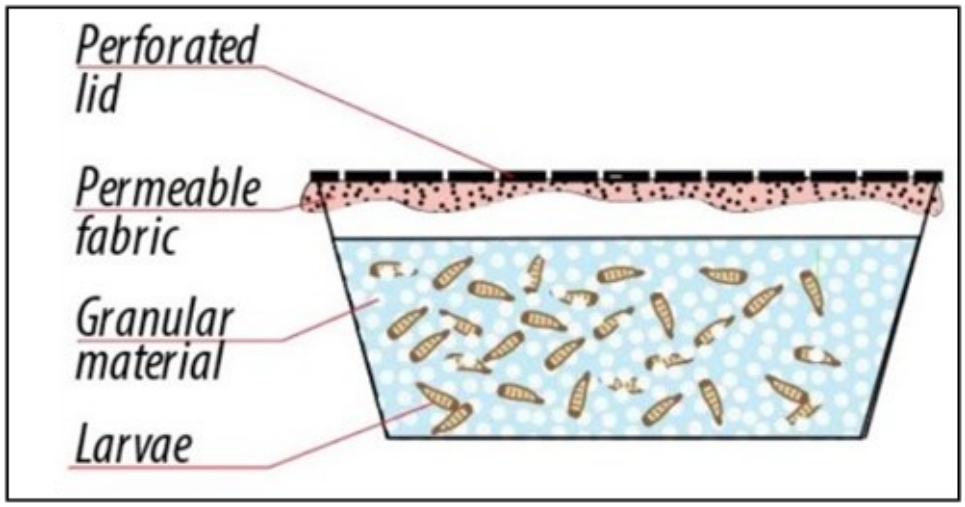

Figure 5. Scheme of the testing reactor for research purposes consisting of a perforated lid, permeable fabric, granular material and larvae that is designed for them to easily climb up for air (Grossule V. a., 2019)

The reactors were then sealed and placed in an incubator to maintain the specified environmental conditions. Samples were collected from the reactors daily to be analyzed weekly for total organic carbon. The TOC analysis was conducted using a SHIMADZU Total Organic Carbon Analyzer (TOC-VCSN) as described further in the text.

3.3. Feeding, operation and monitoring

The preparation of wastewater samples involved creating solutions with the desired TOC concentrations for experimental use. For each TOC level, appropriate amounts of glucose and whey were dissolved in distilled water to achieve the desired concentration:

- For a TOC concentration of 2500 mg/L, a solution was prepared by dissolving 5.8 g

of glucose and 6.4 g of whey in 2 L of distilled water.

- For a TOC concentration of 1000 mg/L, a solution was prepared by dissolving 2.32 g of glucose and 2.56 g of whey in 2 L of distilled water.

These prepared wastewater solutions were used directly in the experimental conditions, providing the necessary TOC levels without further dilution.

The diluted wastewater samples were then distributed according to the experimental load requirements:

- For loads of 0.1, 0.25 and 0.5 the amount of diluted wastewater was 50 mL,
- For loads of 1 and 2.5 the amount of diluted wastewater was 200 mL,
- For load of 5 the amount of diluted wastewater was 300 mL.

Feeding was conducted daily, excluding weekends, over a six-week period to ensure consistent experimental conditions. The liquid feed was carefully extracted using a net placed over the boxes, and syringes were employed for precise removal.

Each week, ten larvae were randomly selected from each box and individually weighed using an analytical balance. After weighing, the larvae were returned to their respective boxes. During each feeding replacement, the remaining liquid was measured, sampled, and stored for subsequent weekly total organic carbon analysis.

Prepupae, distinguished by their darkened coloration, as seen in figure 6, were collected, cleaned, weighed, and then frozen for further analysis. The study monitored larval development and growth by tracking changes in larval wet weight, mortality rates, the number of prepupa, fluctuations in TOC concentration, and variations in wastewater volumes.

Figure 6. The image depicts a prepupa of the studied species, showing the characteristic transitional phase between the larval and pupal stages. The prepupa has a distinct elongated, cylindrical body with a darker coloration compared

to the larval stage. The segmentation of the body is visible, though less pronounced due to the shrinkage typical in this phase. The overall size of the prepupa is smaller compared to a fully developed larva, and it appears motionless, as is typical during this phase.

3.4. Analytical procedure

3.4.1. SHIMADZU Total Organic Carbon Analyzer (TOC-VCSN)

The Total Organic Carbon content of the samples was measured using a SHIMADZU Total Organic Carbon Analyzer. Prior to analysis, the samples were acidified with phosphoric acid to remove inorganic carbon, and the resulting solution was sparged with high-purity air to purge the CO_2. The remaining organic carbon was oxidized to CO_2 using high-temperature catalytic combustion in the presence of a platinum catalyst. The CO_2 generated was then quantified using a non-dispersive infrared (NDIR) detector, and the TOC concentration was calculated based on the detected CO_2 levels.

Calibration of the TOC-VCSN was performed using certified TOC standards with known concentrations, and quality control was ensured by analyzing blank samples and replicates alongside the experimental samples. Data obtained from the TOC analysis were used to assess the efficiency of organic matter degradation by the BSF larvae in the batch reactors.

The TOC analyzer subtracts the inorganic carbon (IC) content, if present, from the total carbon (TC) measured to determine the TOC. The equation used is:

$$TOC = TC - IC \tag{3.1}$$

The TOC concentration is then displayed on the instrument's interface, typically in parts per million (ppm) or milligrams per liter (mg/L) (Corporation, 2024).

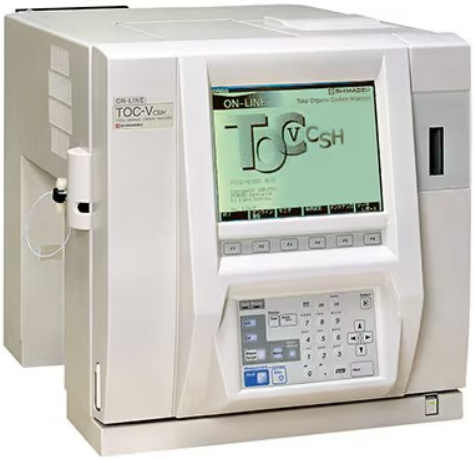

Figure 7. SHIMADZU Total Organic Carbon Analyzer (TOC-VCSN) (Corporation, 2024)

3.4.2. Analytical balance

An analytical balance is a precision instrument designed to measure mass with high accuracy, typically down to 0.1 milligrams (mg) or finer. The object or material to be weighed is gently placed on the weighing pan. The balance is extremely sensitive, so any slight movement, air current, or vibration can affect the reading, which is why the weighing chamber is used. When the sample is placed on the pan, it exerts a downward force due to gravity. This force causes a small deflection in the balance's internal mechanism, which is often a lever or beam system. The key to an analytical balance's accuracy lies in its electromagnetic force compensation system. This system generates an electromagnetic force that counteracts the deflection caused by the sample's weight. The balance automatically adjusts the current in the electromagnetic coil until the beam or lever returns to its original position, effectively balancing the force exerted by the sample. The amount of current required to balance the beam is directly proportional to the mass of the sample. The balance's internal electronics convert this current into a mass reading, which is displayed on the screen. To ensure accurate measurements, the balance is equipped with a draft shield or enclosure to minimize the effects of air currents, and some models may also include temperature control or calibration features to account for environmental variations.

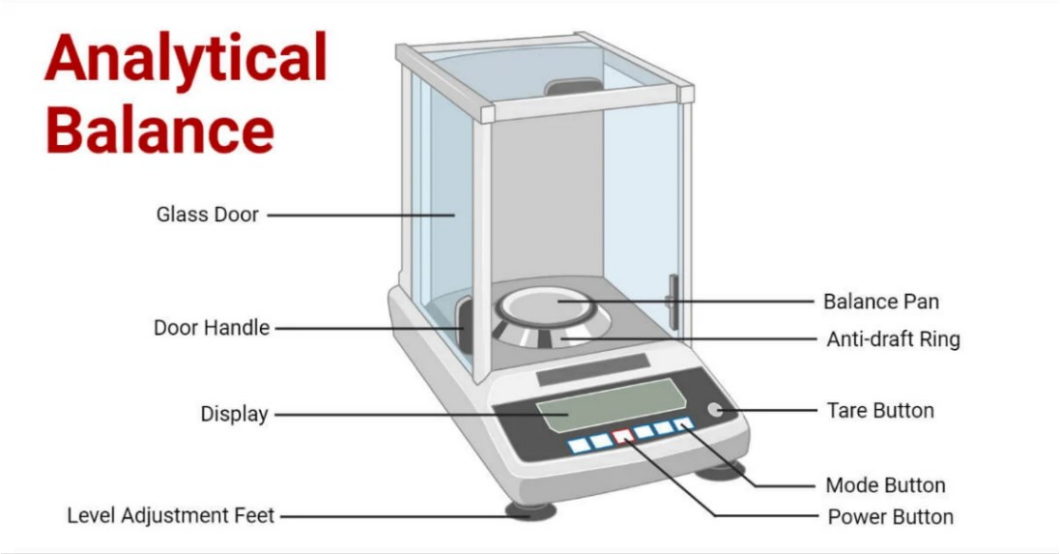

Figure 8. The image shows key components of an analytical balance, a precision instrument used for measuring small mass values. Visible parts include the balance pan, located at the center of the balance, where samples are placed for measurement. Surrounding the pan is an anti-draft ring and a glass door, designed to prevent air currents from affecting the measurement accuracy. Below the weighing pan, the digital display shows the measurement reading with high precision, typically to several decimal places. The control panel is located near the display, featuring buttons to operate functions like taring, calibration, and unit selection. The balance is housed with a level adjustment feet, ensuring stability during operation. (Prakriti, 2022)

3.4.3. Mortality and Yield rate

Two important parameters were calculated in order to better understand the results.

The mortality rate is calculated by comparing the number of dead larvae to the initial number of larvae. This can be done using the formula:

$$Mortality\ Rate = \left(\frac{Number\ of\ Dead\ Larvae}{Initial\ Number\ of\ Larvae}\right) \times 100 \qquad (3.2)$$

At the conclusion of the test period, the larvae are manually counted to determine the number of survivors. This involves carefully inspecting the container and counting all the live larvae present. Any larvae that are not moving, are discolored, or show signs of decomposition are classified as dead.

The yield is calculated by comparing the change in the biomass of the larvae to the change in the substrate concentration (i.e., the organic matter consumed). This can be done using the formula:

$$Y = \frac{\Delta X}{\Delta S} \cdot 100 \quad (3.3)$$

Where:

- ΔX is the change in biomass of the larvae (final mass minus initial mass),
- ΔS is the change in substrate concentration (initial substrate concentration minus final substrate concentration in mass unit).

At the conclusion of the feeding period, the larvae are carefully collected from the wastewater solution and weighed to determine the final biomass. The initial biomass is recorded at the start of the experiment. Simultaneously, the substrate concentrations are measured at the beginning and the end to calculate the total amount of organic matter consumed by the larvae during the experiment.

4. RESULTS AND DISCUSSION

4.1. Growth of larvae

In the LC test, we observe the growth of black soldier fly larvae across six different substrate concentrations labeled LC_0.1, LC_0.25, LC_0.5, LC_1, LC_2.5, and LC_5. Larval growth in all concentrations shows an upward trend, peaking around week 5 before a slight decline by week 6.

- LC_5 (blue circle) shows the highest growth rate, with a steep increase in biomass peaking at week 5 (47 mg) and a slight decline afterward.

- LC_2.5 (flake) and LC_1 (blue cross) also exhibit strong growth, though they peak slightly lower than LC_5 where LC_2.5 reaches 35 mg, and LC_1 peaks at 38 mg.

- The lower concentrations (LC_0.1, LC_0.25, and LC_0.5) result in slower growth, with LC_0.1 exhibiting the lowest peak growth of 26 mg by week 5. This suggests that lower concentrations provide insufficient nutrients to support rapid larval biomass increase.

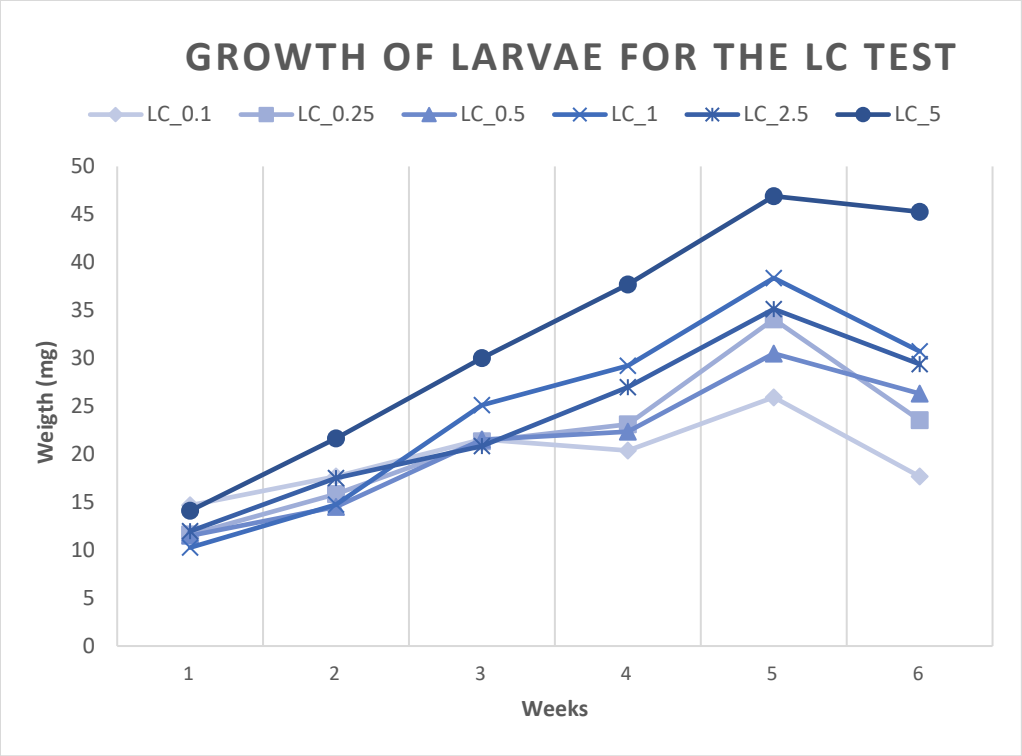

Figure 9. This graph shows the growth of black soldier fly larvae over six weeks, for six different substrate concentrations in the LC test: LC_0.1 (trapezoid), LC_0.25 (square), LC_0.5 (triangle), LC_1 (cross), LC_2.5 (flake), and LC_5 (circle). The larvae at the highest concentration, LC_5, exhibit the most significant growth while the lower concentrations (LC_0.1, LC_0.25, and LC_0.5) show slower growth. After reaching peak growth, most concentrations show a slight decline in biomass by week 6.

In the HC test, we observe the growth of black soldier fly larvae across six different substrate concentrations, denoted as HC_0.1, HC_0.25, HC_0.5, HC_1, HC_2.5, and HC_5.

- All concentrations exhibit an upward trend in larval growth until around week 5, with most concentrations peaking at that point, after which a slight decline is noticeable by week 6.

- HC_5 (red circle) shows the highest growth rate throughout the entire period, reaching a maximum around week 5 (75 mg) and slightly decreasing thereafter.

- HC_2.5 (red flake) and HC_1 (red cross) also exhibit significant growth but slightly decline after week 5, with HC_2.5 reaching a maximum of approximately 70 mg and HC_1 a maximum of around 65 mg.

- Lower concentrations (HC_0.1, HC_0.25, and HC_0.5) show relatively slower growth. The larvae fed on these lower concentrations achieve a peak biomass of around 51mg or less, suggesting that lower substrate concentrations limit larval growth potential.

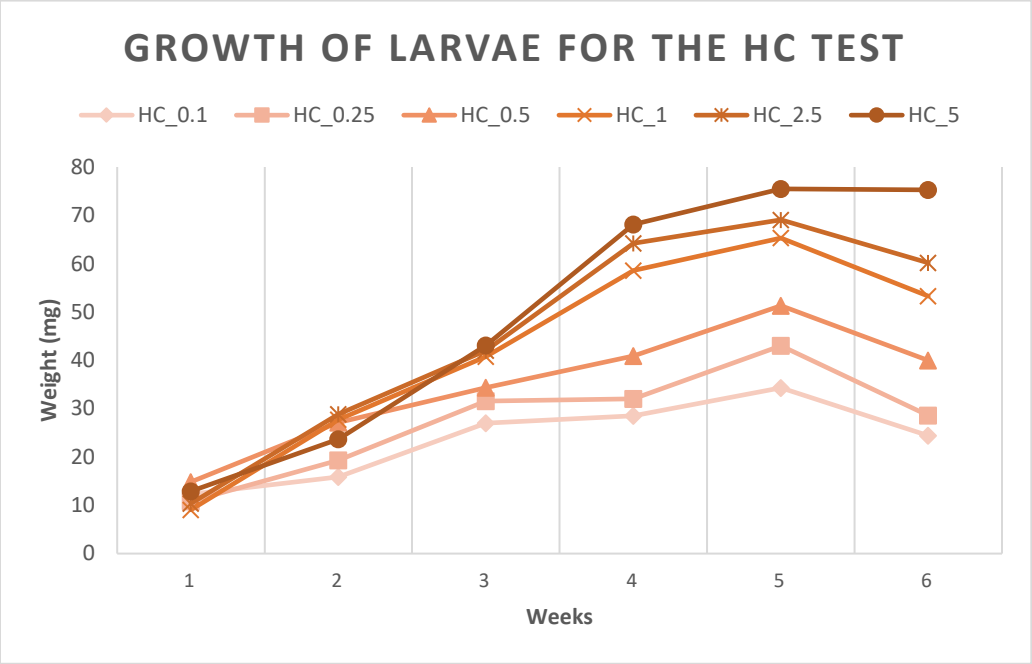

Figure 10. This graph displays the growth of black soldier fly larvae over six weeks for six substrate concentrations in the HC test: HC_0.1 (trapezoid), HC_0.25 (square), HC_0.5 (triangle), HC_1 (cross), HC_2.5 (flake), and HC_5 (circle). The larvae fed the highest concentration, HC_5, achieved the greatest growth, while larvae in the medium concentrations, HC_1 and HC_2.5, exhibited moderate growth. The lower concentrations (HC_0.1, HC_0.25, and HC_0.5) showed slower growth, followed by a slight decline in week 6 for all the tests.

In LC, higher concentrations (LC_5 and LC_2.5) support better larval growth, aligning with the HC test results. The consistent pattern of peaking around week 5 across all concentrations in both tests suggests that larval growth might be limited by nutrient availability or other environmental factors beyond this point, as the decline is noticeable after the peak.

In HC, the higher the concentration (particularly HC_5 and HC_2.5), the greater the larvae's ability to increase their biomass, likely due to the higher availability of organic material (glucose and protein) for consumption. However, the decline after week 5 in most cases could suggest a depletion of resources or an adaptation limit to the substrat. The reason could also be reaching the prepupal state.

4.2. Mortality

The mortality rates indicate a clear difference between the LC and HC tests. In the LC test, higher mortality is observed across all concentrations, suggesting that larvae may not thrive well under lower-carbon conditions, possibly due to limited nutrient availability. The sharp rise in mortality at lower feed levels (0.25 mg/larva/day) supports this, as larvae may not have received enough nourishment.

In contrast, the HC test shows lower mortality overall, particularly at 0.5 mg/larva/day, which seems to be the optimal concentration for minimizing death. This indicates that higher-carbon conditions (as in the HC test) provide a more suitable environment for larval survival, likely due to better nutrient availability.

- In the LC test, the mortality rates are generally higher compared to the HC test. Mortality ranges from 41% to 61%, with the highest mortality observed at the 0.25 mg/larva/day concentration (61.5%) and the lowest at 0.5 mg/larva/day (41.3%). The mortality remains high across all concentrations, suggesting that the larvae in the LC test faced harsher conditions or nutrient limitations.
- In the HC test the mortality rates are notably lower, ranging from 21% to 51%. The lowest mortality is observed at 0.5 mg/larva/day (21.6%), while the highest occurs at 1 mg/larva/day (45.1%). Mortality increases as the substrate concentration increases from 0.5 to 5 mg/larva/day with an exception of the HC_2.5 but remains consistently lower than the LC test at equivalent concentrations.

Overall, larvae in the HC test were more resilient, with lower mortality across all concentrations compared to the LC test. This suggests that both organic load and concentration play a key role in determining larval mortality.

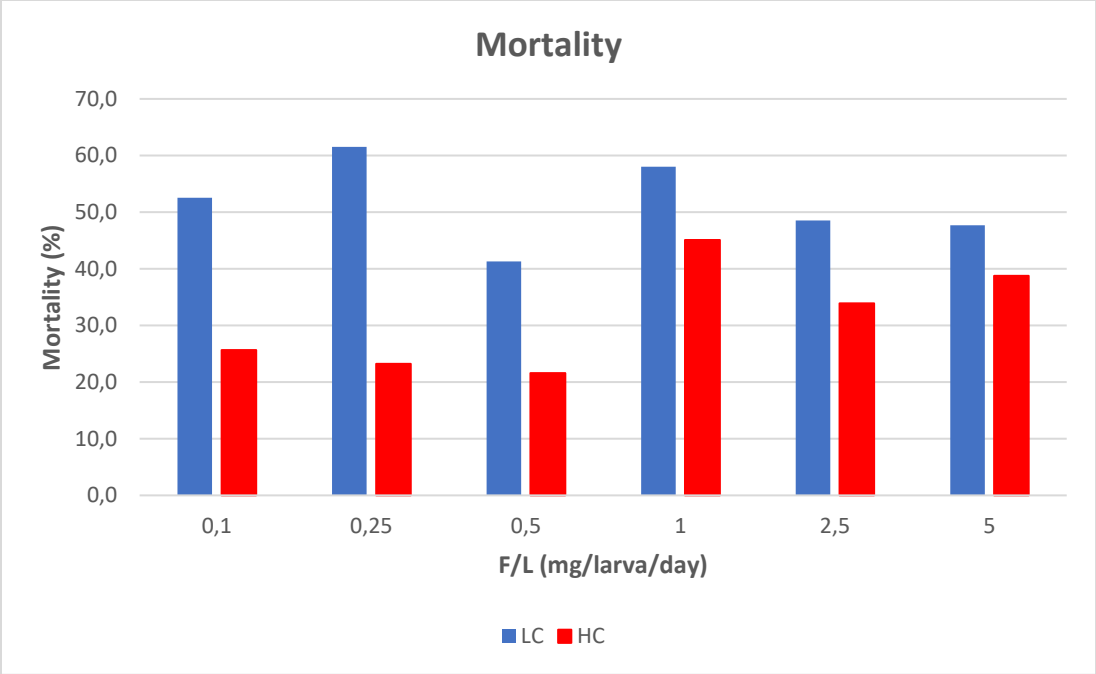

Figure 11. This bar graph shows the mortality rates (%) of BSF larvae across different feed concentrations (F/L, mg/larva/day) for the LC and HC tests. The blue bars represent the LC test, and the red bars represent the HC test. The substrate concentrations range from 0.1 to 5 mg/larva/day. In the LC test (blue bars), the mortality rates are generally higher compared to the HC test (red bars).

The number of prepupae is an important parameter for evaluating the developmental success of BSF larvae under different feeding conditions. Across all substrate concentrations from LC_0.1 to LC_2.5, no prepupae were observed. The only occurrence of prepupal formation in the LC test is at the highest concentration, LC_5, where 1 prepupa was recorded. This suggests that the larvae in the LC test were unable to develop into prepupae at lower concentrations, possibly due to insufficient nutrients. In contrast, the HC test shows significantly higher prepupal formation. The number of prepupae increases progressively with the concentration, peaking in HC_1.

This pattern suggests that larvae in the HC test are better able to develop into the prepupal stage, particularly at higher substrate concentrations (HC_0.5 and above). The peak of 6 prepupae at HC_1 suggests an optimal concentration for prepupal formation.

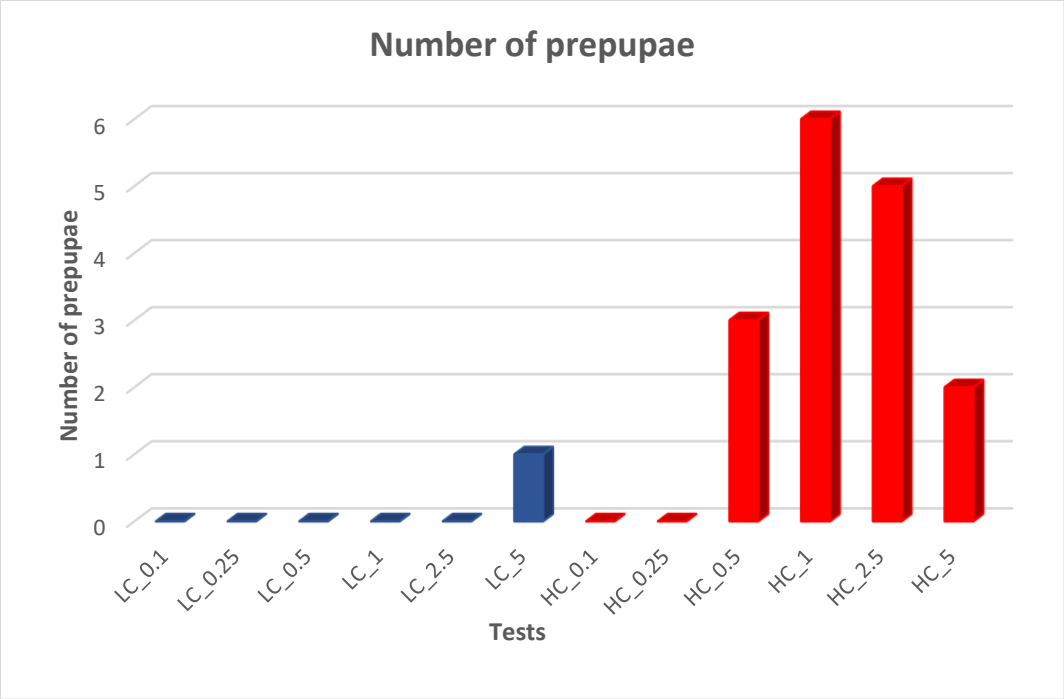

Figure 12. This bar graph displays the number of prepupae formed in both LC and HC tests across different substrate concentrations. The blue bars represent the LC test, and the red bars represent the HC test. The concentrations range from LC_0.1 to HC_5 mg/larva/day.

The results indicate that prepupal development is heavily dependent on substrate concentration and the organic carbon content of the diet. In the LC test, larvae only reached the prepupal stage at the highest concentration (LC_5), with just one prepupa observed, indicating that lower-carbon conditions hinder the development process.

In contrast, larvae in the HC test developed into prepupae across all concentrations from HC_0.5 to HC_5, with the number of prepupae increasing at HC_1 and HC_2.5. The presence of prepupae at multiple concentrations in the HC test suggests that higher carbon availability supports the larvae's full development cycle.

The optimal prepupal formation was observed at HC_1, indicating that this concentration provides the most suitable environment for larvae to mature into prepupae, while extremely high concentrations (HC_5) led to a slight reduction in the number of prepupae formed, possibly due to resource overabundance or environmental stress.

4.3. Yield

Yield is an important parameter to assess the efficiency of organic matter conversion by BSF larvae.

- In the LC test, the yield remains relatively low across all concentrations, with a slight increase at 0.5 mg/larva/day reaching around 0.61, followed by a return to lower yield levels at higher concentrations. This suggests that larvae in the LC conditions were not efficient at converting organic material into biomass across the entire range of tested concentrations.
- In the HC test, the yield is significantly higher across all concentrations, with a maximum yield of approximately 3 at the lowest concentration of 0.1 mg/larva/day. As the organic load increases, the yield decreases steadily, reaching approximately 0.4 at the highest concentration of 5 mg/larva/day. This suggests that larvae in HC conditions were most efficient at converting organic material into biomass at lower concentrations, with decreasing yield as the substrate concentration increased.

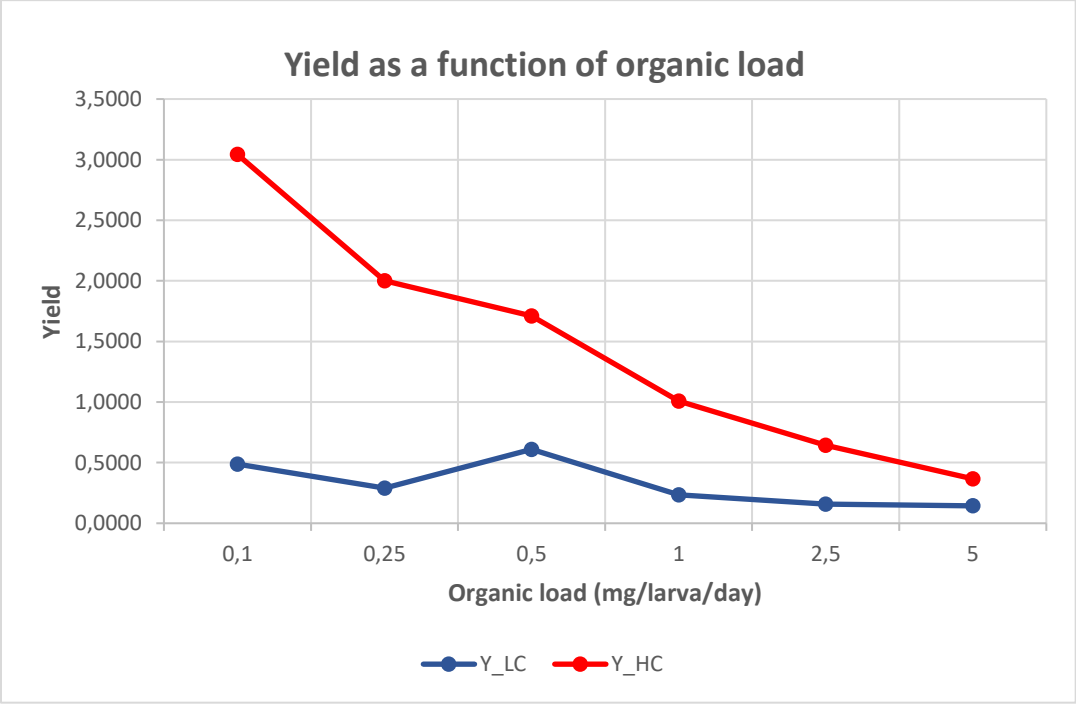

Figure 13. This graph shows the yield (%) as a function of organic load (mg/larva/day) for both LC and HC tests. The blue line represents the LC test (Y_LC), and the red line represents the HC test (Y_HC).

The yield data highlights a clear difference in the organic matter conversion efficiency between the LC and HC tests. The LC test shows consistently low yield percentages across all substrate concentrations, indicating that the larvae were unable to efficiently convert the provided organic load into biomass. The slight peak at 0.5 mg/larva/day may indicate an optimal, but limited, concentration for larvae in low-carbon conditions.

In contrast, the HC test shows much higher yields, particularly at lower organic load concentrations (0.1 mg/larva/day), where larvae reached the maximum conversion efficiency. As the organic load increased, the yield dropped significantly, suggesting that larvae in the HC test were less efficient at higher concentrations, possibly to the limiting factors.

Overall, the results suggest that HC conditions are more favorable for maximizing larval yield, particularly at low to moderate organic loads. However, as the substrate concentration increases, the larvae's efficiency in converting organic matter into biomass decreases. The LC conditions, on the other hand, result in consistently lower yields, indicating poorer conversion efficiency across all tested concentrations.

4.4. Substrate consumption rate

To assess whether the concentration of the substrate had any significant impact on the consumption rate, Michaelis-Menten-like curves were generated for each of the two substrate concentrations (Figure 14). The data for both concentrations show a distinct rise in the substrate consumption rate as the organic load increases. The curves for both concentrations showed similar shapes, with nearly identical plateaus at higher loads, indicating that the consumption rate is primarily governed by the load rather than the concentration of the substrate.

The curve was plotted in Origin between the specific daily consumption rate of Black Soldier Fly (BSF) larvae and the diet concentration, labeled as Vs low c and Vs high c, corresponding to two different diet concentrations, mentioned previously as LC and HC. The data was fitted using a Michaelis-Menten kinetic model, as suggested by previous research (Grossule V. F., 2023).

The substrate consumption rates for both concentrations were recorded, and the results are presented in Table 2 and Figure 14. In each case, the substrate consumption rate increased with increasing load, following a saturation curve similar to the classical Michaelis-Menten kinetics.

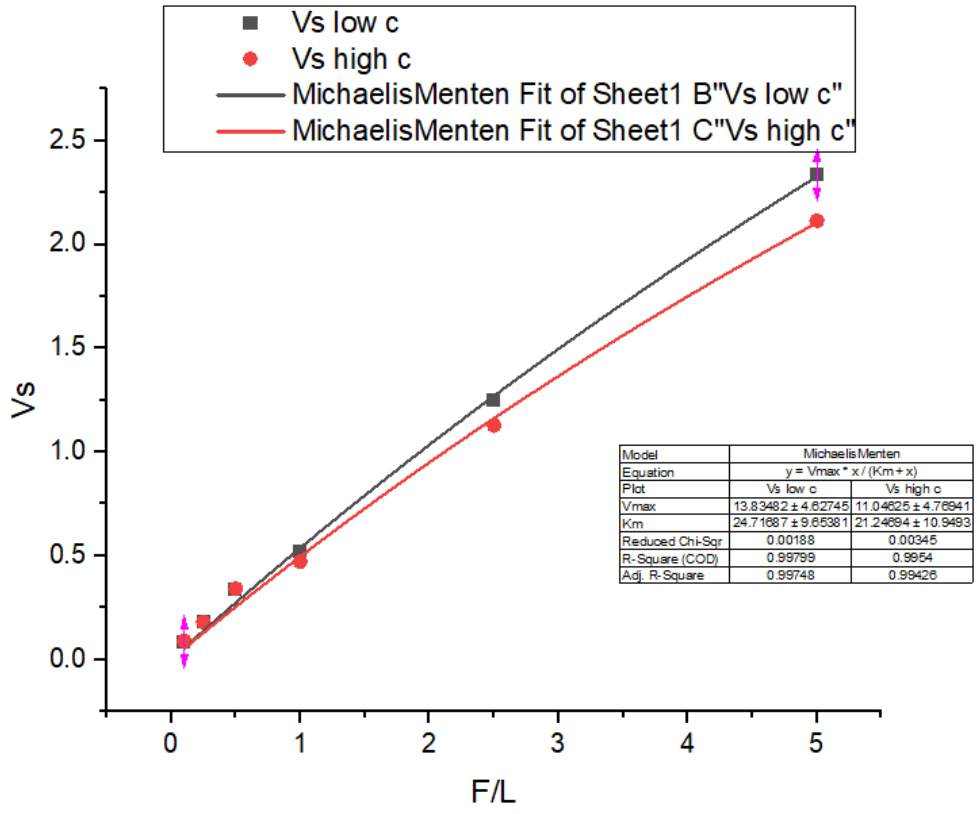

Figure 14. The Origin graph representing the Michaelis-Menten curve for both low and high concentrations show two curves, each plotting the organic load against the specific substrate consumption rate. The curve for the LC (Vs low c, represented by the gray line) curve is seems to be higher at lower substrate concentrations due to the higher Vmax value, indicating greater potential for substrate conversion in this condition. In contrast, the HC (Vs high c, represented by the red line) curve would reach a slightly lower maximum velocity (Vmax), and the substrate consumption rates would increase more rapidly at lower substrate concentrations due to the lower Km value, showing a higher affinity for the substrate.

Despite the difference in concentration, the curves followed a nearly identical trajectory, with both reaching similar maximum consumption rates (υ_{max}) and half-saturation constants (Km). This similarity suggests that the organic load, rather than substrate concentration, is the primary factor influencing the kinetics of substrate consumption in BSFL-based WWT.

For LC, the model fit yielded a maximum consumption rate (υ_{max}) of 13.84 mg/day with a Michaelis constant (Km) of 24.72 mg. The R^2 value of 0.99799 and adjusted R^2 of 0.99748 indicate an excellent fit to the Michaelis-Menten model, suggesting that the consumption rate of BSF larvae follows a saturation-type behavior in response to increasing food concentration.

For HC, which represents the second diet concentration, the results were similarly robust, with a maximum consumption rate (υ_{max}) of 11.05 mg/day and a Km of 21.25 mg. The R^2 and adjusted R^2 values of 0.9954 and 0.9943 show a strong correlation with the Michaelis-Menten model.

Model	**Michaelis-Menten**	
Equation	y = Vmax * x / (Km + x)	
Plot	Vs low c	Vs high c
Vmax	13.83482 ± 4.62745	11.04625 ± 4.76941
Km	24.71687 ± 9.65381	21.24694 ± 10.9493
Reduced Chi-Sqr	0.00188	0.00345
R-Square (COD)	0.99799	0.9954
Adj. R-Square	0.99748	0.99426

Table 2. This table presents the results of fitting a Michaelis-Menten model to substrate conversion data under two conditions: Vs low concentration (LC) and Vs high concentration (HC). The table provides the fitted values for the maximum substrate consumption rate (Vmax), the Michaelis constant (Km), and several statistical measures of model performance, including the reduced chi-square, R-squared (COD), and adjusted R-squared values.

These results support the hypothesis that BSF larvae exhibit a saturation-type response to diet concentration, and that both diet concentrations tested conform to the Michaelis-Menten kinetics.

The key finding of this study is that the substrate consumption rate in BSFL-mediated WWT is primarily dependent on organic load and not on the substrate concentration. The Michaelis-Menten-like curves generated for different concentrations were nearly identical, both in terms of maximum consumption rate and the substrate concentration at which half the maximum rate was achieved.

When comparing these findings to the results of Grossule et al. (2023), the maximum consumption rate (Vmax) and saturation point (Km) differ slightly, but the general trends align. Both studies highlight the ability of BSFL to consume substantial amounts of organic matter at high loads,

though differences in experimental conditions, such as the organic concentrations used, likely explain the observed variations in specific values. Grossule et al. observed a Vmax of 3.8 mg TOC/larva/day and a Km of 34.3 mg TOC/larva, while this study shows higher Vmax and Km values, reflecting differences in wastewater composition and larval handling.

The two diet concentrations, represented by LC and HC, provided distinct insights into the BSF larvae's consumption behavior. The slightly higher Vmax observed for LC suggests that at lower diet concentrations, larvae exhibit a higher potential maximum consumption rate compared to HC, where the Vmax was slightly lower. This could be due to differences in nutrient density or digestibility of the food at different concentrations. Additionally, the lower Km value for HC indicates that the larvae reach half-maximal consumption at a lower food concentration than LC, suggesting a more efficient response to the higher diet concentration in the HC group.

4.4.1. Efficiency

The efficiency in the LC test starts at a relatively high value of 85.9% at 0.1 mg/larva/day, but decreases as the organic load increases. By the highest organic load (5 mg/larva/day), the efficiency drops to 46.7%. This decline suggests that larvae in LC conditions become less efficient at higher organic concentrations, possibly due to nutrient limitations or saturation effects.

The efficiency in the HC test follows a similar trend, with the highest efficiency (89.9%) observed at the lowest organic load of 0.1 mg/larva/day. However, like in the LC test, the efficiency decreases steadily as the organic load increases, reaching 42.3% at the highest organic load (5 mg/larva/day). This indicates that, while the larvae in HC conditions are initially more efficient, their efficiency also diminishes with increasing substrate concentration.

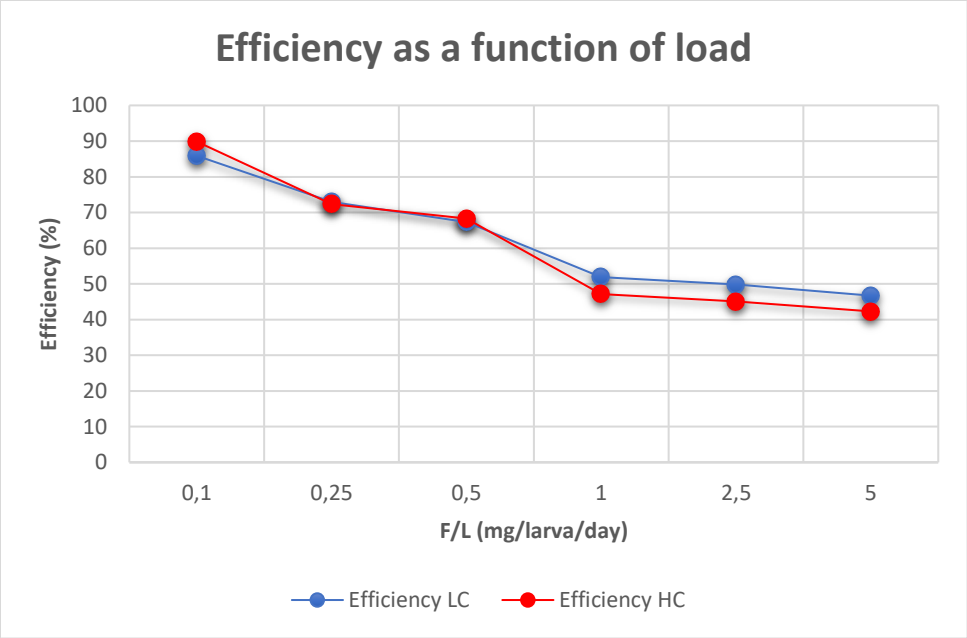

Figure 15. The graph provides the efficiency data for the conversion of organic load into biomass by BSF larvae in LC and HC tests. Efficiency (%) is shown for both LC (blue line) and HC (red line) tests at different organic loads (mg/larva/day)

4.5. Discussion

The mortality results from this study demonstrate the critical role of organic loading rates in determining the survival of Black Soldier Fly larvae during wastewater treatment. Under low concentration conditions, the mortality rate was significantly higher, especially at lower organic loads. For example, at a loading rate of 0.1 mg/larva/day, mortality exceeded 50%, indicating that larvae in nutrient-poor environments struggle to survive due to a lack of essential organic matter for metabolic processes. This suggests that under conditions where organic carbon is limited, larvae may experience nutritional deficiencies, which ultimately lead to higher death rates.

In contrast, the high concentration conditions showed lower mortality across most organic loads, which indicates that nutrient-rich environments provide a more favorable setting for larval survival. However, at higher organic loads (e.g., 5 mg/larva/day), while mortality did not significantly increase, the treatment efficiency declined, suggesting that larvae may become overwhelmed by excess nutrients. This reflects a saturation point beyond which larvae can no longer efficiently process additional organic material, despite maintaining relatively high survival

rates. These findings suggest that there is an optimal organic loading rate for wastewater treatment using BSFL, where both mortality and treatment efficiency are balanced. Identifying this balance is essential for maximizing both larval health and the overall effectiveness of the treatment system.

The following results provide a graphical representation of the previously discussed relationship between substrate removal rate and treatment efficiency as functions of organic loading rate in BSF larvae systems. The graph visually supports the hypothesis that increasing substrate availability leads to higher removal rates but diminished treatment efficiency. Specifically, it highlights the expected exponential increase in substrate consumption (blue curve) alongside the declining trend in system efficiency (red curve), confirming the trade-off observed at varying organic loads. This dataset now provides concrete evidence for identifying an optimal loading range where both substrate removal and treatment efficiency are balanced.

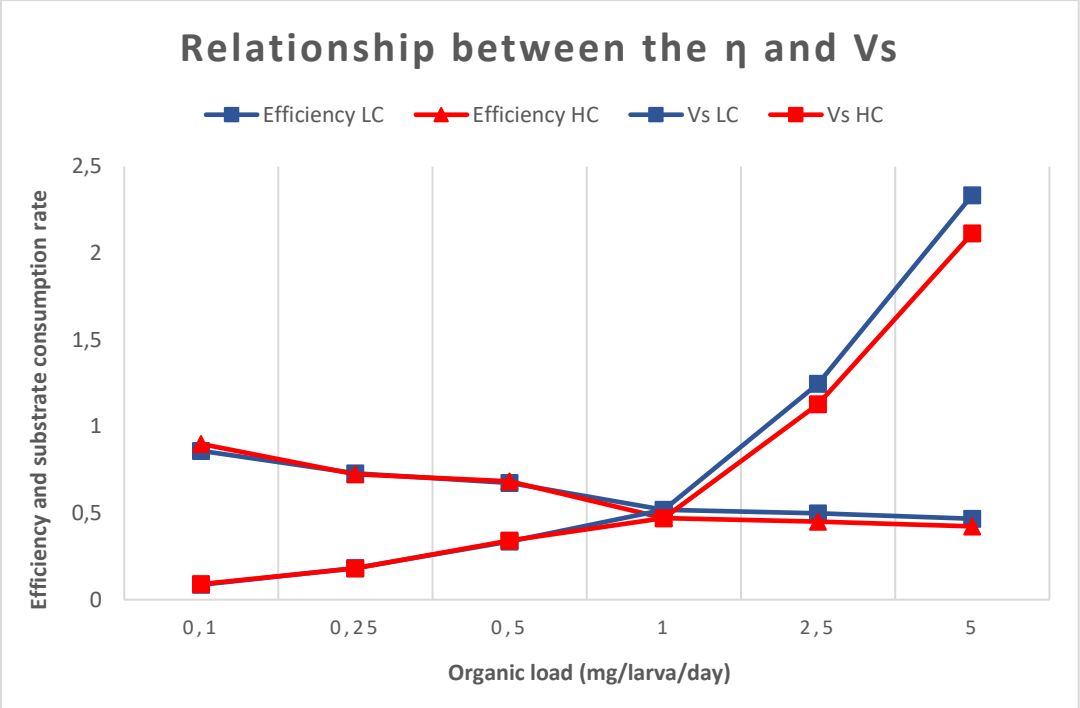

Figure 16. This graph presents the relationship between efficiency and substrate consumption rate (Vs) as functions of organic load for BSF larvae, under LC (blue) and HC (red) conditions. The efficiency curves, depicted by the triangular markers, show a decreasing trend with increasing organic load, suggesting a reduction in larvae's conversion efficiency as substrate availability rises.

The efficiency and specific substrate consumption rate show opposing trends, with efficiency dropping as specific substrate consumption rate rises. This demonstrates a trade-off between how much substrate the larvae can process and how effectively they convert it into biomass.

The optimal loading point on the graph, representing the relationship between substrate removal rate and treatment efficiency as functions of organic loading rate, is crucial for balancing both parameters effectively. As shown in the graph, this optimal load occurs at approximately 1 mg/larva/day, where the substrate removal rate (blue curve) and treatment efficiency (red curve) intersect.

At this loading rate, the larvae exhibit their highest substrate consumption without a dramatic drop in efficiency. This is the point at which the system achieves a balance between maximizing the amount of substrate being processed and maintaining relatively high treatment efficiency. Beyond this point, the blue curve continues to rise, indicating that larvae consume more substrate, but the red curve's sharp decline shows that the system's efficiency drops significantly.

Thus, 1 mg/larva/day can be identified as the optimal organic loading rate for the system. At this load, the substrate is removed at a high rate without overwhelming the larvae's capacity to convert the substrate into biomass efficiently. Higher loads would further increase substrate removal but at the cost of a substantial decrease in efficiency, which could compromise the overall performance of the system. This suggests that beyond this load, the system enters a state of overload, reducing the efficiency of the waste treatment process.

These results have important implications for the design and optimization of BSF larvae reactors, particularly in wastewater treatment applications. By demonstrating that larvae consumption follows Michaelis-Menten kinetics, this study provides a predictive framework for determining optimal feeding rates in BSF-based reactors. The lower Km values for the HC implies that feeding larvae with more concentrated substrates could result in more efficient processing, potentially reducing the total substrate required while maintaining high consumption rates.

Furthermore, the application of Michaelis-Menten kinetics to BSF larvae consumption offers a valuable tool for scaling up these systems. Reactor design can be optimized to provide food

concentrations that align with the Km values observed, maximizing the efficiency of the larvae's consumption behavior. This will enhance the performance of BSF larvae reactors, particularly in the treatment of high-organic-load wastewater, where substrate concentration and consumption efficiency are key design parameters.

This study not only confirms the relationship between diet concentration and substrate consumption rate as described by the Michaelis-Menten like relationship but also provides new insights that can aid in the development of more efficient BSF larvae systems for sustainable wastewater treatment.

5. CONCLUSION

This study aimed to validate the Michaelis-Menten-like kinetic relationship between substrate concentration and the consumption rate of Black Soldier Fly larvae, with a focus on their application in wastewater treatment. By examining the larvae's response to two different substrate concentrations (1000 mg/L and 2500 mg/L), several important conclusions were drawn that both confirm the kinetic relationship and highlight key considerations for reactor optimization.

An expected finding was the higher mortality rate in LC compared to HC, indicating that lower substrate concentrations may impose stress on the larvae. This suggests that while lower concentrations may be more efficient in terms of consumption, they could negatively impact larval development. The higher survival rates in HC suggest that slightly higher substrate concentrations may be more favorable for maintaining larval health over extended periods, despite their marginally lower consumption efficiency.

The relationship between efficiency and substrate consumption rate in this study reveals an important trade-off in BSF larvae-based wastewater treatment. As organic loading rates increase, the substrate consumption rate also increases, indicating that larvae can metabolize higher amounts of organic matter when more substrate is available. However, this comes at the cost of treatment efficiency, which declines significantly at higher loading rates due to larvae being overwhelmed by excess nutrients. The optimal balance is found at moderate loading rates, where larvae are able to consume substrate efficiently without experiencing metabolic overload, maintaining both high consumption rates and effective system performance.

The specific substrate consumption rate increased nonlinearly with organic loads, regardless of the substrate concentration fitting a Michaelis-Menten like relationship. Substrate concentration influenced larval growth and thus the potential amount of recoverable larval biomass, but produced no effect on specific substrate consumption rate. The substrate consumption rate values can be used in treatment unit design, based on organic loads, regardless of organic concentration.

While the study successfully validates the Michaelis-Menten kinetic relationship for BSF larvae, it also highlights the need to balance substrate concentration with larval health. Lower substrate

concentrations, although more efficient in terms of organic matter reduction, can lead to higher mortality. Conversely, higher concentrations may reduce efficiency but improve larval survival. These findings provide valuable insights for optimizing BSF-based reactors for wastewater treatment, particularly regarding the balance between substrate concentration, larval health, and system efficiency.

Acknowledgment

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