detritus | Multidisciplinary Journal for Circular Economy and Sustainable Management of Residues

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WASTEWATER TREATMENT USING BLACK SOLDIER FLY LARVAE: THEORETICAL FRAMEWORK AND PRELIMINARY TESTING FOR CONTINUOUS REACTOR DESIGN

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Article Info:
Received:
12 February 2025
Revised:
11 April 2025
Accepted:
12 May 2025
Available online:
4 June 2025

Keywords:
Hermetia illucens
Wastewater
Biological treatment

ABSTRACT

The use of Black Soldier Fly (BSF) larvae has recently been introduced, through the so-called LarWaR process (LARvae for WAstewater treatment and Resource recovery), as a promising alternative biological treatment for high-organic-content wastewater. LarWaR has proven effective in managing high concentrations of organic substances while recovering high-value resources in the form of larval biomass. This study summarises the state of the art of key process aspects within a theoretical design framework, highlighting the persisting research gaps. Additionally, it investigates an appropriate treatability test for designing and reliably predicting the performance of a continuous reactor treating artificial wastewater. A series of preliminary batch tests were conducted to determine the expected substrate consumption rates (v_s) — fitted using a Michaelis-Menten-like relationship — and removal efficiencies at different loads (F/L, mgC/larva/day). A continuous test was then perfomed to validate the results from the treatability batch tests. The fitted Michaelis-Menten-like model for v_s values proved to be a reliable tool for reactor design, as the steady-state performance (v_s = 0.72 mgC/larva/day; η = 36%) closely matched predictions from the treatability test (v_s = 0.86 mgC/larva/day, η = 43%). Finally, the study explored the maximum effective reactor depth (h) for the LarWaR system through video monitoring of larval behavior in deep liquid substrates. Observations revealed physical limitations restricting larval movement to the first few centimeters (3-4 cm) of the liquid surface, providing crucial insights for optimizing reactor depth. These findings contribute to improving the design methodology and scalability of the LarWaR process.

1. INTRODUCTION

1.1 State of art

Black soldier fly (BSF) larvae are conventionally used as an alternative technology for the treatment of solid biowaste, with the benefit of effectively stabilizing and metabolizing putrescible waste, converting it into valuable larval biomass.

During the larval stage – the only feeding stage — which lasts between 2 to 4 weeks under optimal conditions, the larvae can efficiently consume and metabolize a wide variety of organic wastes such as food scraps, spoiled feed, animal manure, etc. (i.a. Isibika et al., 2021).

This process results in significant volume and mass reduction of the waste — up to 70% — while converting it into nutrient-rich larval biomass. The biomass, composed of up to 48% protein and 35% fat in the prepupal stage (Gold et al., 2018), is suitable for use in animal feed or as a feedstock for industrial applications (chitin, biolubricants, proteins, etc. Surendra et al., 2020). Overall, the use of BSF larvae presents an efficient, low-cost, and eco-friendly approach to managing putrescible waste, aligning with circular economy principles by recovering high valuable resources from waste streams (Cossu et al., 2020).

Recently, the LarWaR process (Larvae for Wastewater treatment and Resource recovery) has been introduced for treating high-organic-content wastewaters as well. This process offers a highly promising alternative to conventional biological processes, with removal kinetics up to three times higher than those of the conventional aerobic processes (Grossule et al., 2021; Grossule, 2024; Grossule and Lavagnolo, 2020), while recovering high-value resources (i.e. larval biomass).

While BSF larvae have been successfully used to treat solid biowaste (Grossule et al., 2024), their application to liquid substrates is impaired by the high larval mortality. As terrestrial organisms, BSF larvae are prone to drowning in pure liquid environments. LarWaR process overcomes

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https://doi.org/10.31025/2611-4135/2025.19493

this critical issue by means of a patented solution (Italian Patent n. 102021000016700; PCT/IB2022/055888; priority 25/06/2021), consisting in the use of a porous supporting material, to be installed in the reactor and to be saturated by the treatment targeted liquid. The aim is to provide a physical support for larvae mobility allowing them to dive for eating and reemerge for breathing (Grossule and Cossu, 2021).

Several key aspects of the LarWaR process have recently been investigated with a view to optimizing the process and advancing toward the definition of reactor design and sizing. Main findings can be summarised in the following theoretical design framework.

1.2 Theoretical design framework

LarWaR reactor design and sizing involves the definition of the following key aspects (Figure 1): A) Suitability of wastewater for the LarWaR process; B) Larvae kinetics (i.e. specific substrate consumption rate; v_S, mgC/larva/day) and removal efficiencies (η, adimensional or %); C) Larval density (larvae/cm^2) and supporting material; D) Reactor volume and dimensions (surface-A; depth-h).

A) Suitability of wastewater for the LarWaR process

A wastewater can be considered suitable for LarWaR treatment process when it allows high larval development, low mortality and effective removal of organic matter, which occur under the following two conditions:

- Presence of high organic content, with concentrations exceeding the Treatment Threshold Limit (TTL), which may vary based on the quality of the liquid substrate. For example, Grossule et al. (2023) found that, in the case of their tested leachate, a TOC concentration above 2000 mgC/L was necessary to ensure optimal process performance.
- Appropriate nutritional quality of the organic substrate. Nutritional quality is related to the presence of dominant macronutrients such as proteins (P), non-fibre carbohydrates (NFC), and lipids (L), and their relative abundance (X_i, i = P, NCF or L, calculated as ratio between the single macronutrient concentration and sum of P, NCL and L). Substrates with good nutritional quality have been identified by Grossule et al. (2025), 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 0.05-0.15 a good quality substrate occurs if $X_{NFC} > X_L$.

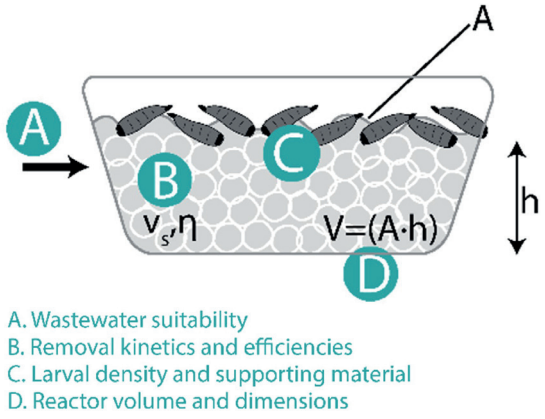

FIGURE 1: Scheme of key aspects to be defined in view of reactor design and sizing. v_s = specific substrate consumption rate (mgC/larva/day); η = removal efficiency (adim or %); V = volume (L); A = surface (cm^2); h = depth (cm).

If the wastewater meets these two conditions, it can be deemed suitable for the LarWaR process, and the design and sizing of the LarWaR reactor can proceed.

B) Process kinetics and removal efficiencies

Substrate removal kinetics, expressed by the specific substrate consumption rate (v_S, mgC/larva/day), and removal efficiency (η, adimensional or %) can be defined for a continuous reactor, using the following Equations (Equations 1 and 2):

$$v_S = \frac{Q(S_0 - S_e)}{X_0} \quad (1)$$

$$\eta = \frac{(S_0 - S_e)}{S_0} \quad (2)$$

where:
v_s = specific substrate consumption rate (mgC/larva/day)
η = removal efficiency (-)
Q = flowrate (L/d)
S_o, S_e = Influent and effluent substrate concentrations (mgC/L)
X_0 = initial number of larvae

According to Grossule et al. (2023), v_s is primary influenced by the organic load, i.e. by the Food/Larvae (F/L) ratio expressed as Total Organic Carbon (TOC) fed per larvae per day (mgC/larva/day). F/L can be expressed by combining the Equation 1 and Equation 2 as follow (Equation 3):

$$F/L = \frac{QS_o}{X_0} = \frac{QS_o}{X_0} \frac{(S_0 - S_e)}{(S_0 - S_e)} = \frac{v_s}{\eta} \quad (3)$$

According to the Equation 3, v_s is directly proportional to F/L, whereas η is inversely proportional.

v_s increases nonlinearly with F/L following a Michaelis-Menten like relationship (Grossule et al., 2023), which can be expressed according to Equation 4:

$$v_S = \frac{v_{S_{max}} \cdot F/L}{K_S + F/L} \quad (4)$$

where:
$v_{s,max}$ = max specific substrate consumption rate (mgC/larva/day)
K_s = half saturation constant (mgC/larva)

The relationships between F/L, v_s (fitting a Michaelis-Menten like relationship) and η, is graphically illustrated in Figure 2.

The Michaelis-Menten like relationship can be defined, for a specific wastewater, by means of a treatability test, which involves performing a series of batch tests to determine v_s and η values at different F/L ratios. This relationship can be then used to design the reactor volume (as described at the point D).

C) Larval density and supporting material

Definition of optimal larval density and supporting material is crucial for the design of the LarWaR process.

According to Grossule (2024), the optimal larval density should maximize removal efficiencies without compromising survival rate (i.e. avoiding larval starvation and competition for breathing), while the optimal supporting material should maximise porosity (n=liquid volume/total reactor volume filled with supporting material=V_l/V_{tot}) without compromising the ability of larvae to move and survive.

Larval density is typically defined as surface area density (Cl_v, larvae/cm^2), rather than volumetric density (Cl_v, larvae/cm^3), for both liquid and solid substrates. This is because larval metabolism depends strongly on atmospheric oxygen, which is primarily available at the substrate-air interface on the substrate surface. Previous studies have investigated the optimum larval density for solid substrates, suggesting values between 1 and 5 larvae/cm^2 to avoid overcrowded conditions, which can slow larval development, reduce survival rates, and hinder process optimization due to feed competition (Abduh et al., 2018; Cattaneo et al., 2025; Parra Paz et al., 2015).

In contrast, under liquid substrate feeding, higher larval densities can be used without negatively affecting the process. Grossule (2024) investigated LarWaR performance under different supporting materials (plastic granules, Pall rings and geomat) featuring different porosities (35%, 75%, 92% respectively), and different superficial larval densities (4, 8, 16 and 32 larvae/cm^2). The study concluded that materials with higher porosity support greater larval densities and, consequently, higher organic loads with reduced reactor volumes. However, porosity exceeding 90% might not provide sufficient solid support to larvae. Optimum conditions were observed at a larval density of 16 larvae/cm^2 with pall rings (featuring a porosity n=75%) as supporting material.

D) Reactor volume and dimensions

In order to define the reactor volume, the hydraulic retention time (HRT, days) can be primarily calculated as follow:

$$HRT = \frac{V_l}{Q} = \frac{S_0 \cdot \eta}{Cl_A \cdot v_s} \cdot h \quad (5)$$

where:
$V_l = n \cdot V_{tot}$ = liquid volume
$Cl_A = X_0/A$ = larval surface area density (larvae/cm^2)
A = surface area (cm^2)
h = reactor depth (cm)

From equation of HRT, liquid and total reactor volumes can be calculated as follows:

$$V_l = HRT \cdot Q = \frac{Q \cdot S_0 \cdot \eta}{Cl_A \cdot v_s} \cdot h = \frac{Q \cdot S_0}{Cl_A \cdot F\ L} \cdot h \quad (6)$$

$$V_{tot} = \frac{V_l}{n}$$

In Equation 6, once the Michalis-Menten-like relationship has been determined, the design F/L can be chosen as a trade-off between maximizing loads (and minimizing volumes, as shown in Equation 5, Figure 2) and achieving acceptable removal efficiencies. Once the F/L is fixed, the only remaining parameter in Equation 6 needed to calculate the reactor volume is the reactor depth (h).

1.3 Research gaps and aims of the study

Considering the above-mentioned key aspects investigated in previous studies (Figure 1), several research gaps still persist:

A) *Suitability of wastewater for the LarWaR process*. Grossule et al. (2023) suggested a TTL of 2000 mgC/L, based on the treatment of an artificial leachate. However, this concentration limit may vary depending on the quality of the liquid substrate. Further studies are required to generalise the TTL under optimal nutritional quality of the liquid substrate.

B) *Process kinetics and removal efficiencies*. v_s values were determined for different artificial wastewaters and organic loads through batch tests, yielding variable values between 0.25 and 2.25 mgC/larva/day under substrate loads ranging from 0.1 to 2.6 mgC/larva/day (Grossule et al., 2023). However, v_s values obtained under batch conditions may be overestimated, particularly under long HRT conditions, which can facilitate parallel substrate attenuation mechanisms beyond larval metabolism, such as bacterial oxidation, adsorption, precipitation, (Grossule et al, 2023).
Appropriate treatability batch tests with suitable HRT should be established to determine the Michaelis-Menten-like relationship and validated through continuous experiments.

C) *Larval density and supporting material*. Grossule (2024) identified optimum conditions at Cl_A = 16 larvae/cm^2 with pall rings. However, Cl_A can be further optimized by investigating densities between 16 and 32 larvae/cm^2, while alternative supporting materials with porosities ranging between 75% and 90% should also be tested. Moreover, a crucial aspect regarding the supporting material remains unaddressed: it should not only provide adequate support for the larval mobility while maximizing porosity but also facilitate the effective separation of larval biomass at the end of the larval stage.

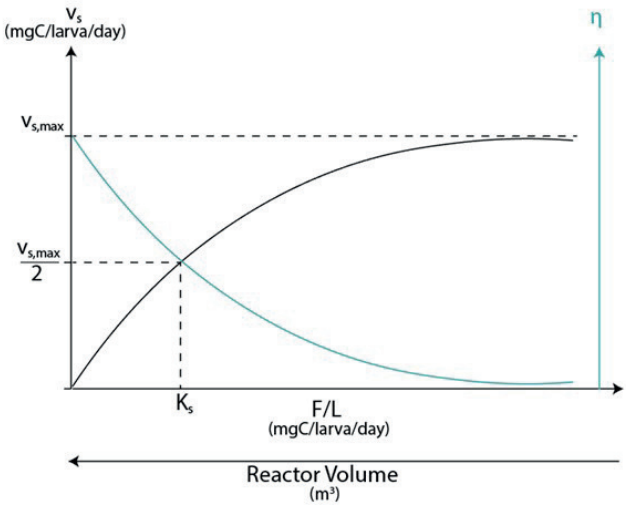

FIGURE 2: raphical illustration of the relationships between F/L, v_s (fitting a Michaelis-Menten like relationship) and η. Reactor Volume is then inversely proportional to F/L according to Equation 5.

D) *Reactor volume and dimensions*. In Equation 6, the only parameter that has been completely overlooked by research so far is the reactor depth (h), which corresponds to maximum substrate depth within which larvae can operate effectively. Some studies have investigated the effect of solid substrate depth on BSF larvae performance, concluding that larval survival and waste reduction efficiency are significantly impaired when depths exceed 5 cm (Abduh et al., 2018; Brits 2017; Dortmans et al., 2017). Greater depths may limit natural air circulation within substrate, leading to anaerobic conditions once interstitial air is depleted, which hinders larval respiration. When dealing with liquid substrates, larval respiration can only occur at the liquid-air interface, posing an even greater challenge since interstitial air within the waste mass is unavailable. However, no data are currently available regarding the maximum depth accessible to larvae under complete absence of free oxygen within liquid substrate.

Considering the abovementioned research gaps, present study conducted three tests to investigate the following issues:

- Treatability test: to determine the Michaelis-Menten-like relationship through a series of batch tests conducted under appropriately short HRT to minimize parallel substrate attenuation mechanisms.
- Continuous test: To validate the v_s and η values obtained through the preliminary treatability batch test.
- "Big Brother" test: To determine the maximum liquid substrate depth, representing the effective liquid height of the LarWaR reactor (h).

2. MATERIALS AND METHODS

2.1 Treatability test

A preliminary treatability test was performed on artificial wastewater through a series of batch tests to determine the v_s and η values at different F/L ratios, with the goal of identifying the Michaelis-Menten-like relationship (Equation 4, Figure 2).

Batch reactors consisted of plastic boxes (13.5 cm x 13.5 cm x 5.5 cm) containing young BSF larvae (6-days-old, 13 mg as average wet weight per larva) and granular plastic material (VALOX®, 2-3 mm diameter), completely saturated with artificial wastewater, which was replaced daily (HRT = 1 day) up to 10 days. The HRT was selected as a first attempt (to be validated through continuous testing), to be shorter than that of Grossule et al. (2023; ~7 days) in order to limit the possible effect of parallel substrate attenuation mechanisms and the overestimation of v_s values.

Each reactor was covered with a permeable non-woven fabric and sealed with a perforated plastic lid. The test was conducted in thermally insulated room maintained at 25-30°C with a photoperiod of 18/6 h Light/Dark.

Five F/L ratios were tested in triplicate (0.11, 0.53, 1.06, 2.65, 5.29 mgC/larva/day), adjusting the daily volume of feeding artificial wastewater (30-250mL) and larvae density (1-6 larvae/cm^2) accordingly.

Artificial wastewater was prepared according to the recipe described by Grossule et al. (2023) with the following concentrations: TOC = 3980 mgC/L, COD = 13100 mgO_2/L, BOD_5 =4489 mgO2/L, TKN =3598 mgN/L, NH_4 = 3197 mgN/L, Organic nitrogen = 401 mgN/L.

Artificial wastewater from Grossule et al. (2023) was selected, being previously tested and proven suitable for the LarWaR treatment process, characterized by a high organic content and an appropriate nutritional quality of the organic substances.

At the time of wastewater replacement, the extracted volume was measured, sampled and analysed for TOC concentration. Larvae were sampled every two days, individually weighted and returned to the box. Larval development was monitored by measuring the average larval wet weight. Wastewater treatment performance was assessed by measuring average v_s and η values. v_s values were fitted using a Michaelis-Menten like relationship (Equation 4) and compared with those obtained from longer HRT batch tests (~7 days) by Grossule et al. (2023).

2.2 Continuous test

A continuous test was performed in order to validate the v_s and η values obtained through the preliminary treatability test. The experimental setup consisted of five reactors connected in series, each composed of a plastic box (13.5 cm x 13.5 cm x 5.5 cm) containing granular plastic material (VALOX®, 2-3 mm diameter), completely saturated with 150 mL artificial wastewater. Each reactor was covered with a permeable non-woven fabric and sealed with a perforated plastic lid. A peristaltic pump continuously fed the reactors with wastewater from a starting storage tank, while effluent was collected in a final storage tank (Figure 3). 160 BSF larvae (6-days-old, 15 mg as average wet weight per larva) were placed in each reactor. The en-

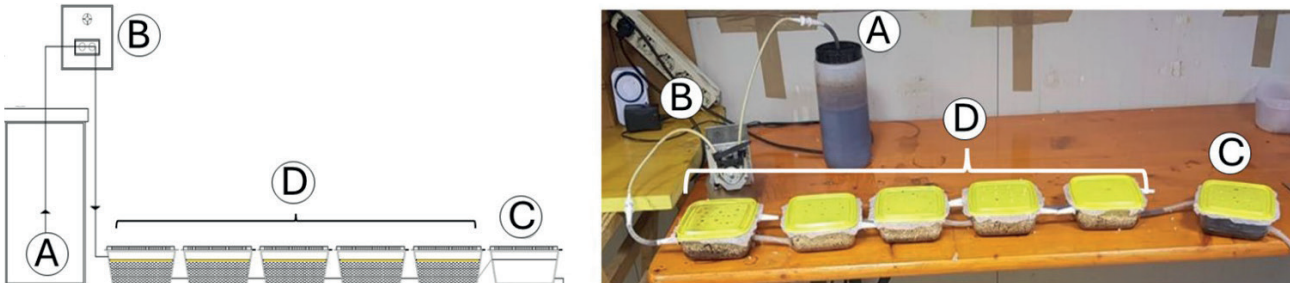

FIGURE 3: Scheme (left) and picture (right) of the test set up. A) Input wastewater storage tank; B) peristaltic pump; C) effluent wastewater collection tank; D) LarWaR reactors connected in series.

vironmental conditions and the recipe for preparing the artificial wastewater were the same as those used in the treatability test. The reactors were operated at a flow rate of Q = 0.426 L/d (HRT = 1.76 days), under a F/L = 2 mgC/larva/day, calculated using Equation 3 (S_0 = 3760 mgC/L, X_0 = 800 larvae).

Daily, fresh influent wastewater was provided and output storage tank was emptied. Approx. every 2 days, effluent wastewater was sampled and analysed for TOC concentrations and larvae were sampled and weighted to monitor the variation of the average larval wet weight.

The reactor was operated for 12 days until reaching the steady state, when TOC effluent concentrations were virtually constant.

At steady state conditions, v_s and η values were calculated using to Equations 1 and 2, respectively, and compared to expected results both from treatability test (HRT = 1 day) and Grossule et al., (2023), which were obtained from longer HRT batch tests (~7 days).

2.3 "Big brother" test

The experiment was conducted by placing 15-days old BSF larvae into glass batch reactors (10 cm in diameter and 14 cm in height) containing supporting material fully saturated with artificial wastewater at a water depth of 8 cm. Each reactor was covered by a permeable non-woven fabric to avoid oviposition by other flies but allowing air circulation.

Four larval densities were tested (2, 4, 8, and 16 larvae/cm²), using pall rings as supporting material (Grossule 2024). Larval densities were selected to investigate the potential impact of larval density on larval behaviour, remaining below the threshold density reported by Grossule (2024). Larvae behaviour was monitored for 48 hours by means of a video camera recording continuously at a frame rate of 29 fps.

The artificial wastewater was prepared by diluting low-fat milk with tap water (1:10) and served as high-quality diet to prevent any influence of poor quality diet on the results (Grossule et al., 2024).

All tests were conducted under room temperature (approx. 20°C) under continuous lighting.

Scheme of the tests and reactors set up is illustrated in Figure 4.

2.4 Analytical methods

TOC was determined using a TOC–VCSN Shimadzu Analyzer, COD and BOD5 were determined according to the standard Italian method IRSA-CNR (29/2003 vol. 2 n. 5130; 29/2003 vol. 2 n. 5120 B2). Ammonia nitrogen was measured with a distillation-titration procedure and TKN was measured through a distillation-titration procedure after an acid digestion phase. Organic nitrogen was then calculated by subtracting N-NH_4 from TKN.

3. RESULTS AND DISCUSSION

3.1 Treatability test

Figure 5 illustrates the variation of specific substrate consumption rates (v_s, mgC/larva/day) with F/L (mgC/larva/day) and the fitted Michaelis Menten-like relationship (Equation 4), derived from both the treatability test (under an HRT = 1 day) and Grossule et al., (2023) (under longer HRT batch tests ~7 days). The v_s values obtained by Grossule et al. (2023) were significantly higher than those from the treatability test, particularly at high F/L, demonstrating the possible impact of long HRT on the overestimation of substrate consumption rates. v_s values from both treatability test and Grossule et al. (2023) followed a Michae-

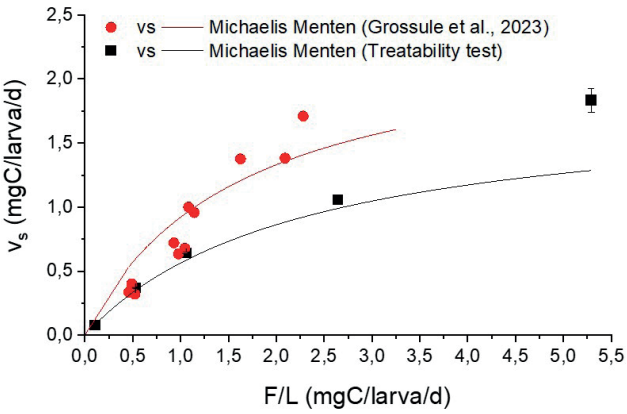

FIGURE 5: Variation of specific substrate consumption rates (v_s, mgC/larva/day) with F/L (mgC/larva/d) and the fitted Michaelis Menten-like relationship (Equation 4) obtained from both the treatability tests (under an HRT = 1 day; R^2=0.83) and from Grossule et al., (2023) (under longer HRT batch tests ~7 days, R^2=0.74).

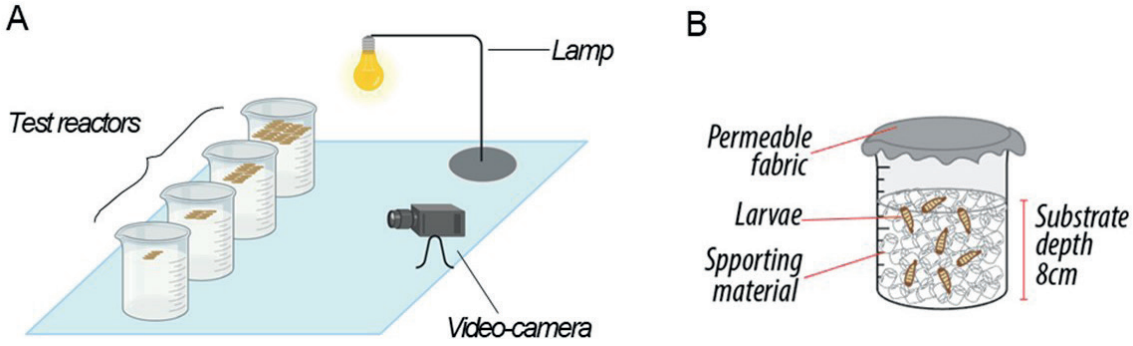

FIGURE 4: A) Scheme of tests set up for the testing of four larval densities (2, 4, 8, 16 n. larvae/cm²); B) Reactors set up (Pall rings as supporting materials; Grossule, 2024).

lis-Menten-like relationship (Equation 4), with the following parameters:

- Treatability test: $v_{s,max}$ = 1.8336, K_s = 2.2552
- Grossule et al. (2023): $v_{s,max}$ = 2.395 mgC/larva/day, K_s = 1.6mgC/larva/day

Based on these parameters, the expected v_s and η values for the continuous test (calculated with Equations 4 and 3, respectively) were:

- Treatability test: v_s = 0.86 mgC/larva/day, η = 43%
- Grossule et al. (2023): v_s = 1.33 mgC/larva/day, η = 67%

3.2 Continuous test

Figure 6 illustrates the variation over time in effluent TOC concentrations and removal efficiencies in the continuous reactor (Figure 6A), as well as the variation over time in the average larval wet weight in the five-box series of the continuous reactor (Figure 6B).

The TOC concentration dropped from 3,760 mgC/L to 2,690 mgC/L by day 6, achieving a removal efficiency of 30%. It further decreased to 2,400 mgC/L by day 12, reaching a removal efficiency of 37% at which point virtually steady-state conditions were achieved. Larval development was greater in the last box compared to the first in the series, with the maximum average larval wet weight at the end of the test reaching 96.6 mg/larva (SE 10.1) in box D1 and 51.3 mg/larva (SE 5.7) in box D5.

Figure 7 illustrate the variation of v_s (mgC/larva/day) with F/L (mgC/larva/day), including the fitted Michaelis-Menten-like relationship and the η (%) obtained from the treatability test. In the same figure, the results from the continuous test are highlighted in red. At steady-state conditions, v_s and η in continuous test were 0.72 mgC/larva/day and 36%, respectively, closely matching the values predicted by the treatability test (v_s =0.78 mgC/larva/day, η = 39%). This agreement validates the expected results and confirms the appropriateness and reliability of the treatability test, performed at shorter HRT compared to Grossule et al. (2023). In contrast, batch tests at high HRT performed by Grossule et al. (2023), being possibly affected by parallel substrate attenuation mechanisms, overestimated both v_s and η values.

3.3 "Big brother" test

From the video monitoring, an initial acclimation period of the larvae was observed, during which they were primarily distributed along the dry walls of the reactors, attempting to escape. The acclimation phase ended after a few hours, when larvae began to move within the liquid-solid matrix, searching for food and a dark environment, being larvae photophobic (Salam et al., 2022). From this point forward, regardless of larval density, larvae mostly remained within the top 3-4 cm of the reactor (Figure 8), although occasionally some larvae moved toward the bottom.

4. CONCLUSIONS

The definition of an appropriate treatability test for designing and reliably predicting the performance of a continuous reactor was investigated through a series of preliminary batch tests using artificial wastewater. These tests aimed to determine the expected v_s values (fitted by a Michaelis-Menten curve) and η values at different F/L ratios, which were then validated using a continuous test operated at a fixed F/L.

Treatability tests, performed through short HRT batch tests (HRT =1 day), minimized the influence of parallel sub-

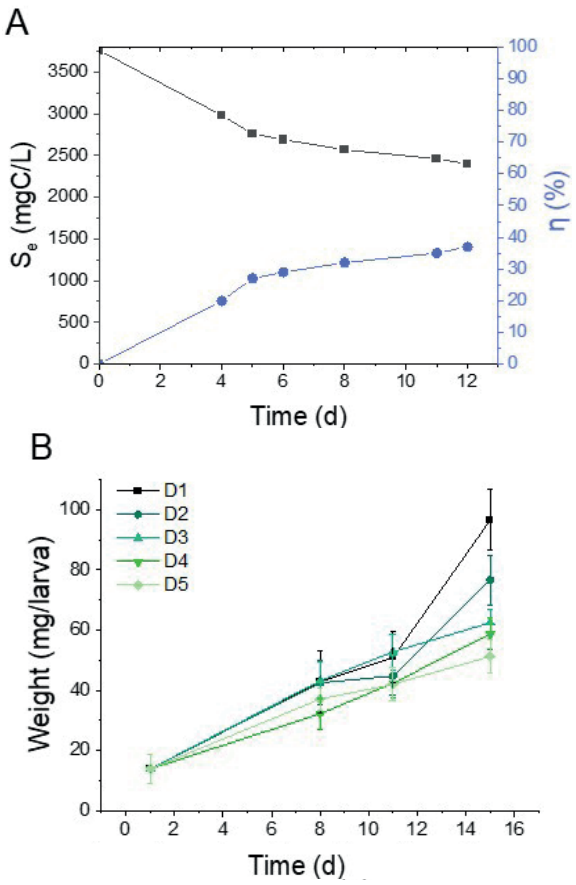

FIGURE 6: (A) Variation over time in effluent TOC concentrations and removal efficiencies in the continuous reactor. (B) Variation over time in the average larval wet weight in the five-box series of the continuous reactor.

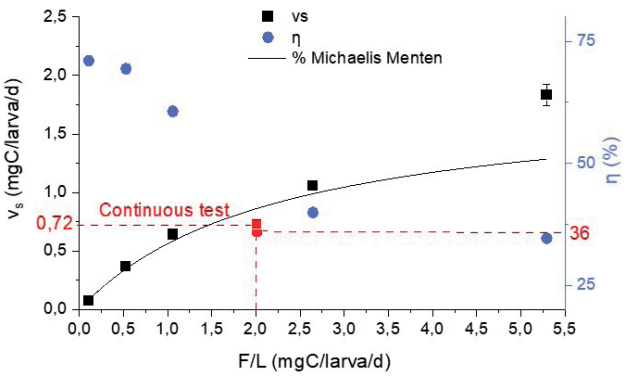

FIGURE 7: Variation of v_s (mgC/larva/day) with F/L (mgC/larva/d) (with the fitted Michaelis Menten-like relationship, R^2=0.83) and the η (%) obtained from the treatability test. In red the results from continuous test: v_s=0.72 mgC/larva/d, η= 36%.

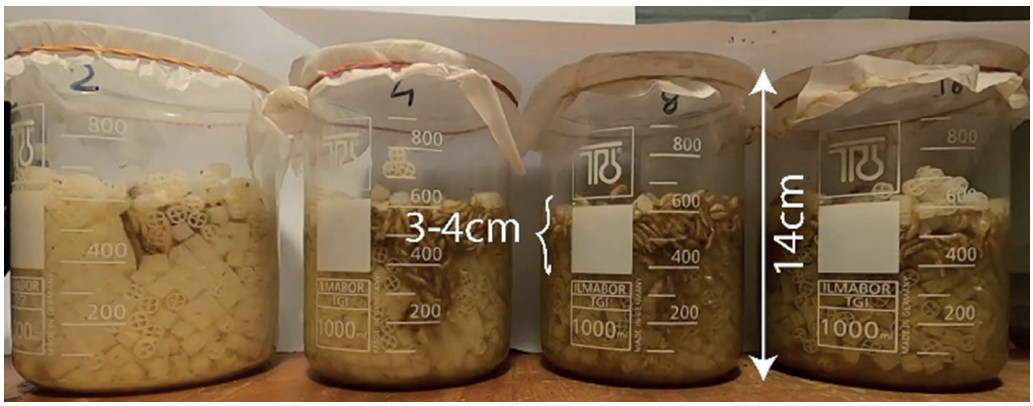

FIGURE 8: Video frame from the video monitoring of the 'Big Brother' test.

strate attenuation mechanisms and prevented the overestimation of v_s values, providing a more reliable tool to predict the performance of a continuous reactor. Consequently, the fitted Michaelis-Menten-like relationship was proven to be a reliable tool for designing and predicting the performance of the continuous reactor. At steady state conditions the reactor achieved v_s and η values closely matching those predicted by the treatability test. Additionally, the continuous reactor exhibited a startup period of just 12 days — significantly shorter than the typical startup periods required by conventional biological processes (ranging from weeks to months) (Ferraris et al., 2009; Khalili et al., 2013). The startup period may also be further reduced by using older larvae as incolum.

Video monitoring of larval behavior in deep liquid environments was conducted to investigate the maximum effective depth (h) of the LarWaR reactor. The analysis identified physical limitations to larval movement, restricting their effective activity to the first few centimeters of the liquid substrate.

By defining and validating reliable treatability tests and identifying the effective depth (h) of the LarWaR reactor, this study has contributed to improving the scalability of the LarWaR process. However, technical and operational challenges persist in scaling up the process. These challenges include:

i. Identifying the optimal support material and the most effective method for separating larval biomass at the end of the larval stage;
ii. Developing an efficient full-chain management system for larval biomass, from the provision of young larvae for inoculation to the marketability of larval biomass at the end of the process, including legal considerations;
iii. Determining the best trade-off between maximizing treatment efficiency (low loads, larger reactor volumes, and lower biomass generation) and maximizing larval biomass production (high loads, smaller reactor volumes, and lower treatment efficiency).

Future research will focus on designing and implementing a pilot-scale test on real wastewater streams, guided by the proposed design framework.

ACKNOWLEDGEMENTS

Funded by the European Union
NextGenerationEU

And by STARS@UNIPD programme – LarWaR-basics Project: Use of Black Soldier Fly Larvae for innovative Wastewater treatment and Resource recovery

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