Journal of Environmental Management 338 (2023) 117775

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Treatment of wastewater using Black Soldier Fly larvae: Effect of organic concentration and load

Valentina Grossule [a,*], Ding Fang [b], Maria Cristina Lavagnolo [a]

[a] DICEA, Department of Civil, Architectural and Environmental Engineering, University of Padova. Via Marzolo 9, 35131 Padova, Italy
[b] School of Environment, Tsinghua University, Beijing, 100084, PR China

ARTICLE INFO

Keywords:
Hermetia illucens
Wastewater
Resource recovery
Organic load
Biological treatment

ABSTRACT

Recent studies have investigated the use of Black Soldier Fly (BSF) larvae as a promising biological treatment process for high organic content wastewater (i.a. Leachate from municipal solid waste landfill, food processing effluents), achieving both high treatment efficiency and production of secondary resources from larval biomass (i.a. Proteins and lipids). The present study was aimed at achieving a better understanding of how organic concentration and load might influence treatment performance. Larvae were fed with three artificial wastewaters characterised by same organic substances quality (degree of biodegradability and oxidation of the organic content measured respectively as BOD/COD and TOC/COD ratios) but different organic concentrations. Each type of wastewater was tested at four different loads. Treatment performance was assessed by monitoring both larval growth (in terms of weight variation, mortality and prepupation), and variation of wastewater quality and quantity to determine organic substrate consumption (measured in terms of Total Organic Carbon, TOC). Larval starvation was observed in all tests when TOC concentrations dropped below approx. 1000 mg C/L, which, for the tested wastewater, could be assumed as the limit value for adopting BSF larvae process. Substrate concentration in the feed (mgC/L) influenced larval growth (in terms of maximum wet weight, prepupation and mortality) only when organic load was above 10 mgC/larva: the higher the load, the higher the positive impact of the substrate concentration. On the contrary, the specific substrate consumption rate (v_S, mgC/larva/day) appeared not to be influenced by substrate concentration but only by the organic load, with a Michaelis Menten like relationship. Accordingly, substrate load can be assumed as a design parameter for BSF treatment process, while substrate concentration might only influence potential resource recovery from larval biomass.

1. Introduction

Biological treatment process based on the use of Black Soldier Fly (BSF) larvae proved to be a highly promising technique for the treatment of high organic content (HOC) wastewater, efficiently removing organic substances and converting them into a valuable protein and fat-rich biomass suitable for resource recovery.

The process has recently been tested on MSW landfill leachate and encouraging results obtained after appropriate setting of the reactor system: an efficient physical support for larvae mobility (diving for eating and re-emerging for breathing) was provided to avoid high larvae mortality due to drowning; a reliable artificial wastewater was purposely studied and applied to keep under control wastewater quality and better analyse the different process variables. This is not feasible with real wastewater, as quality can vary dramatically with time even within the same generation process (e.g., landfill leachate, wineries, etc.) (Grossule et al., 2020, 2021; Grossule and Lavagnolo, 2020; Grossule and Cossu, 2021).

In line with previous studies, the advantages of BSF treatment compared to the conventional activated sludge process are the following:

- Higher organic removal efficiency, achieving up to 3-fold higher specific substrate consumption rate (mgCOD/mgVS/d) (Grossule et al., 2022).
- Avoidance of waste production in terms of excess sludge.
- Production of high valuable biomass, suitable either for direct use as animal food (i.a. Allegretti et al., 2018) or for production of biorefinery products, such as proteins and lipids (i.a. Franco et al., 2021), biodiesel (i.a. Leong et al., 2016), lubricants (i.a. Xiong et al.,

---

* Corresponding author.
E-mail address: valentina.grossule@unipd.it (V. Grossule).

https://doi.org/10.1016/j.jenvman.2023.117775
Received 15 November 2022; Received in revised form 11 March 2023; Accepted 19 March 2023

2020), chitin and chitosan (Triunfo et al., 2022), antimicrobial peptides (Di Somma et al., 2022).

On the other hand, BSF treatment process might be affected by constraints related to quality of the feeding substrate (organic composition, concentration and loads).

Grossule et al. (2022) previously investigated the influence produced by biodegradability and degree of oxidation of organic content on treatment performance, suggesting that the higher the BOD_5/TOC ratio in the feeding substrate (higher biodegradability and lower oxidation degree), the greater the process performance. However, the effect elicited by specific organic substrate load (mgC/larva) and concentration (mgC/L) on the treatment process remains to be clarified.

This aspect is investigated in the present study. Three different artificial wastewaters were used. They were prepared ad hoc in order to present same organic substances quality (degree of biodegradability and oxidation of the organic content measured respectively as BOD/COD and TOC/COD ratios) but different organic concentrations. Each wastewater was fed to larvae at four different loads. Treatment performance was assessed by monitoring both larvae growth (in terms of weight variation, mortality and prepupation), and wastewater quality and quantity variation to determine substrate consumption (measured in terms of Total Organic Carbon, TOC).

2. Materials and methods

2.1. Research program

The experiment was performed in batch reactors, where young BSF larvae (10-days-old, 28 mg as average wet weight per larva, SE 0.62) were in contact with the same volume of wastewater (150 mL). Larvae were physically supported by a patented plastic granular bed, which was fully saturated by wastewater (Grossule et al., 2021, 2022).

Three artificial wastewaters were prepared as an aqueous solution of chemicals, varying the preparation recipes (suggested by Grossule et al., 2021) to obtain different organic content concentrations, defined as low (L), medium (M) and high (H).

Each wastewater was tested under four different loads, obtained by varying larvae density in the reactors (20, 40, 80 and 160 larvae per reactor). Each test was conducted in triplicate.

A graphical description of the experimental set up is provided in Fig. 1.

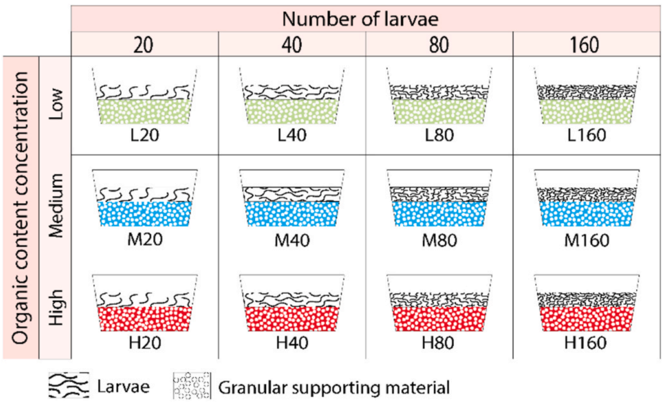

Fig. 1. Graphical description of the experimental set up.

2.2. Tested wastewater quality and loads

Analytical composition of the tested wastewaters is provided in Table 1. Further to the BOD/COD (Biodegradability) and TOC/COD (Oxidation degree) ratios, Table 1 also reports the BOD/TOC ratio as a biotreatment index, as suggested by Grossule et al. (2022): the higher the value, the higher the concentration of BOD and the lower the Oxidation degree of the Organic Content in the leachate.

Table 1
Quality data of tested leachates.

Parameters	Leachate typologies		
	L	M	H
pH	8.1	8.2	8.0
TOC (mgC/L)	1952	3650	6940
COD (mgO_2/L)	6620	13,000	27,000
BOD_5 (mgO_2/L)	2791	5045	9785
VFA (as CH_3COOH) (mg/L)	521	1241	1965
N – Organic (mg/L)	148	235	693
Ptot (mg/L)	1.7	3	6.4
BOD_5/COD	0.42	0.39	0.36
TOC/COD	0.29	0.28	0.26
BOD_5/TOC	1.43	1.38	1.41

The artificial wastewaters were characterised by different organic parameter concentrations, which increased from L to M and H leachate with a ratio of approx. 1:2:4.

Similar BOD/COD, TOC/COD and BOD/TOC values were instead ensured across all tested wastewaters to maintain consistency of quality of organic content and were comparable with those tested in Grossule et al. (2022). M wastewater typology displayed virtually the same composition as the artificial synthetic leachate (named as H–S) tested in Grossule et al. (2022).

TOC concentrations and TOC loads applied in each test are illustrated in Fig. 2.

2.3. Equipment and growth conditions

Each testing reactor was made up of a plastic box (13.5 cm × 13.5 cm x 5.5 cm) containing granular plastic material (VALOX®, 2–3 mm diameter) completely saturated with wastewater. The granular material allowed larvae to move freely in the liquid substrate to meet their needs (feeding and breathing) (Grossule et al., 2022; Grossule and Cossu, 2021; Grossule et al., 2021).

Each box was covered by a permeable non-woven fabric (to avoid oviposition by other flies) and by a perforated plastic lid (to allow air recirculation). All tests were carried out in a thermally-insulated room under the same environmental conditions suggested by Grossule and Lavagnolo (2020): temperature range 25–30 °C; photoperiod Light/-Dark of 18/6 h.

All reactors were filled at the beginning of the test with 150 mL

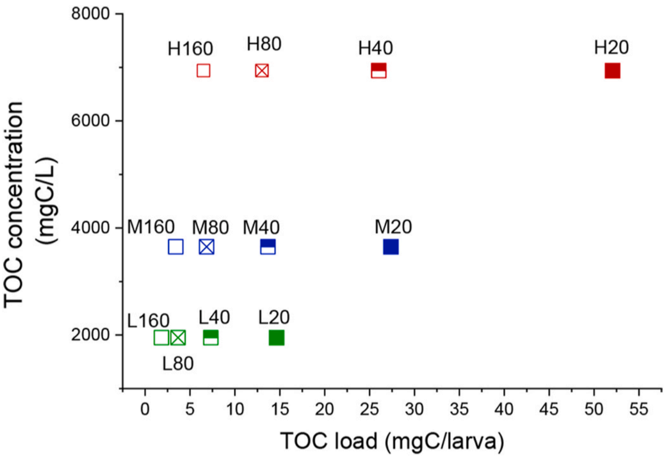

Fig. 2. TOC concentrations and TOC loads tested, resulting from the combination of different leachate typology (L = low-, M = medium-, H = high-organic content concentration) and larval densities (20, 40, 80, 160 larvae per reactor).

wastewater.

2.4. Monitoring

Twice weekly, wastewater volume was measured and 5 mL wastewater samples collected and analysed for TOC concentration; larvae were sampled, individually weighed and returned to the box. Prepupae, recognised by the darkening of their colour, were removed. At the end of the experiment, when all tests displayed a decrease in larval wet weight, residual wastewater was extracted, and volume and TOC concentration measured.

The development of larvae and substrate consumption were monitored by measuring the variation of average larval wet weight, larvae mortality (% of dead larvae over the initial total number of larvae) and prepupation (percentage of prepupae formed throughout mean larval development time), variation of TOC concentration and wastewater volumes. Consequently, parameters relating to conversion efficiency of substrate into new biomass and specific substrate consumption rate were calculated.

Conversion efficiency of substrate into new biomass by anabolic processes is defined by the yield (Y), expressed as mg larvae/mgTOC consumed. Y values were calculated weekly during the growth phase (before weight decreasing phase) as follows (Eq. (1)):

$$Y = \frac{\Delta X}{\Delta TOC} = \frac{weekly\ larvae\ growth\ weight\ (mg)}{weekly\ substrate\ consumption\ (mgTOC)} \tag{1}$$

Specific substrate consumption rate (v_S), introduced as a simple design parameter by Grossule et al. (2022), was calculated in terms of TOC according to the following equation (Eq. 2):

$$v_S = \frac{dS}{X_0 dt} = \frac{S_0 - S}{X_0 (t - t_0)} \tag{2}$$

where:

S_0, S = substrate mass at the beginning and at the end of the monitoring period, measured as TOC

S_0–S = removed Substrate (mg TOC)

X_0 = initial number of larvae at time $t_0 = 0$

t = monitoring time (days)

2.5. Analytical methods

Wastewaters were analysed for the following parameters (Table 1): pH, TOC, COD, BOD_5, VFA, Ammonia nitrogen ($N–NH_4$) and Total Kjeldahl Nitrogen (N-TKN), Norg, Ptot.

TOC was determined using a TOC–VCSN Shimadzu Analyzer, COD and BOD were determined according to the standard Italian method IRSA-CNR (29/2003 vol 2 n. 5130; 29/2003 vol 2 n. 5120 B^2). VFA were analysed by acid titration between pH 5 and 4.4. Norg was calculated as difference between TKN and $N–NH_4$. Ammonia nitrogen was measured with a distillation-titration procedure and TKN was measured through a distillation-titration procedure after an acid digestion phase. Total phosphorus (Ptot) was determined using a UV–vis spectrophotometer (Shimadzu UV-1601).

3. Results and discussion

3.1. Variation of larval wet weight and substrate concentration

Fig. 3 illustrates the variation over time of average larval wet weight (w/w) and substrate concentration (mgTOC/L) observed with the different types of wastewater and larval densities.

In general, larval growth and TOC variation was greater and occurred faster when moving from L to M and H wastewaters.

In tests conducted with wastewater L, the low substrate concentration in the feed resulted in a clear and general larval starvation: larval growth and TOC variation displayed a similar pattern regardless of larval density, and consequently, of organic load. Larvae reached maximum weight at day 7 (approx. 39 ± 1.2 mg w/w) and started losing weight from day 17. Consistently, TOC concentration decreased significantly over the first 7 days below 1000 mg C/L, while it increased from the 17th day, when larvae stopped feeding and evaporation occurred.

In M and H tests, larval density (organic loads) significantly

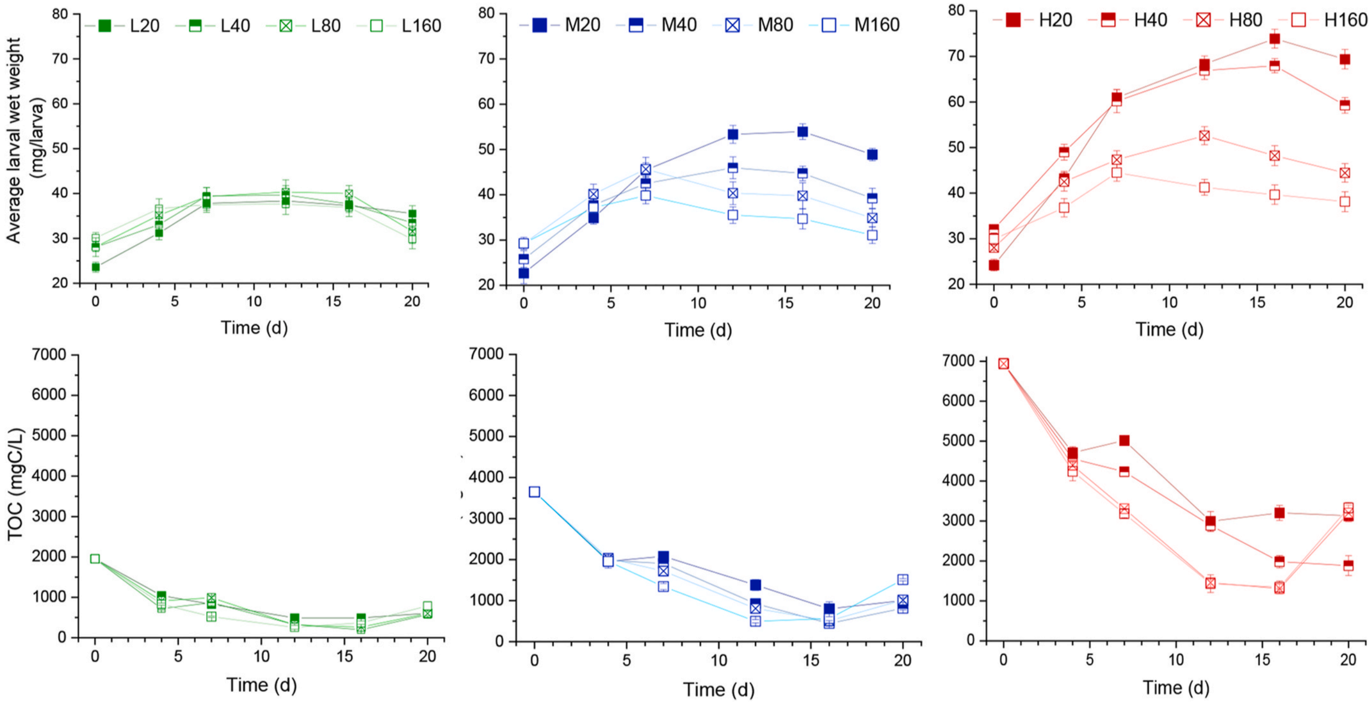

Fig. 3. Variation of average larval wet weight (w/w) and substrate concentration (measured as TOC) under different leachate feeding typologies (L = low-, M = medium-, H = high-organic content concentration) and larval densities (20, 40, 80, 160 larvae per reactor).

influenced larval growth and substrate consumption. On the one hand, higher larval densities (lower organic loads) resulted in a greater and faster TOC variation, whilst on the other, larval growth was lower and max wet weight was achieved earlier. In M tests, maximum weight occurred at days 16, 12 and 7, respectively, in tests M20, M40, and both M80 and M160. In H tests, larval growth stopped at day 16, in both H20 and H40, and at days 12 and 7 respectively in tests H80 and H160. At the end of the experiment, all tests displayed a decrease in larval wet weight due to starvation and/or to onset of prepupation. In the majority of tests, the decrease in larval wet weight was due to starvation, observed when TOC concentration dropped below approx. 1000 mg C/L (in correspondence to the first larval weight value in the decreasing phase). In H20 and H40 tests, the decrease in larval wet weight was mainly due to onset of prepupation.

3.2. *Effect of organic content load and concentration on larval growth*

The relationship between growth performance parameters (max wet weight, mortality and prepupation), substrate concentration (L, M, H wastewater) and organic loads (mgC/larva) is illustrated in Fig. 4a–c. In general, substrate concentration produced a greater positive effect on larval growth in line with increase in organic load. TOC loads below 10 mgC/larva (L40, L160, L80, M80, M160, H160) resulted in lower max larval wet weight (between 35 and 45 mg/larva), lower prepupation percentages (between 0 and 2%), and higher mortality percentages (between 2,5 and 7%), regardless of substrate concentration. Conversely, above 10 mgC/larva, the higher the substrate loads, the higher the influence of substrate concentration. In L20, M40 and H80 tests, characterised by similar TOC loads (varying between 13 and 14.5 mgC/L), max larval wet weight was respectively 38.3 ± 1.5 mg/larva, 46 ± 2.4 mg/larva, 52.6 ± 1.9 mg/larva. In M20 and H40, at higher and similar loads (27.4 and 26 mgC/larva, respectively), the difference in larval weight was even more evident, being 53.9 ± 1.9 mg/larva and 67.9 ± 1.5 mg/larva, respectively (Fig. 4a).

H tests alone, at higher substrate concentration, displayed higher prepupation rates as substrate loads increased (Fig. 4b), achieving prepupation values of approx. 17% in H20. Consistently, mortality reduced as substrate loads increased: mortality values were similar (3–7%) in all tests at low TOC loads (<20 mg C/L), while decreased to 0% as TOC loads increased in M and H tests.

Yield values were calculated for the growth phase up until achievement of max wet weight (Fig. 4d). In general, yield values decreased as substrate loads increased, suggesting that under food shortages, most of the available food is used for anabolic processes and converted into new biomass, while under high food availability, the fraction of food dedicated to catabolic production of bioenergy increases. Variation of yield values in line with substrate loads was significantly influenced by organic content concentration. In particular, the decrease of yield values as substrate loads increased was more evident under low substrate concentration.

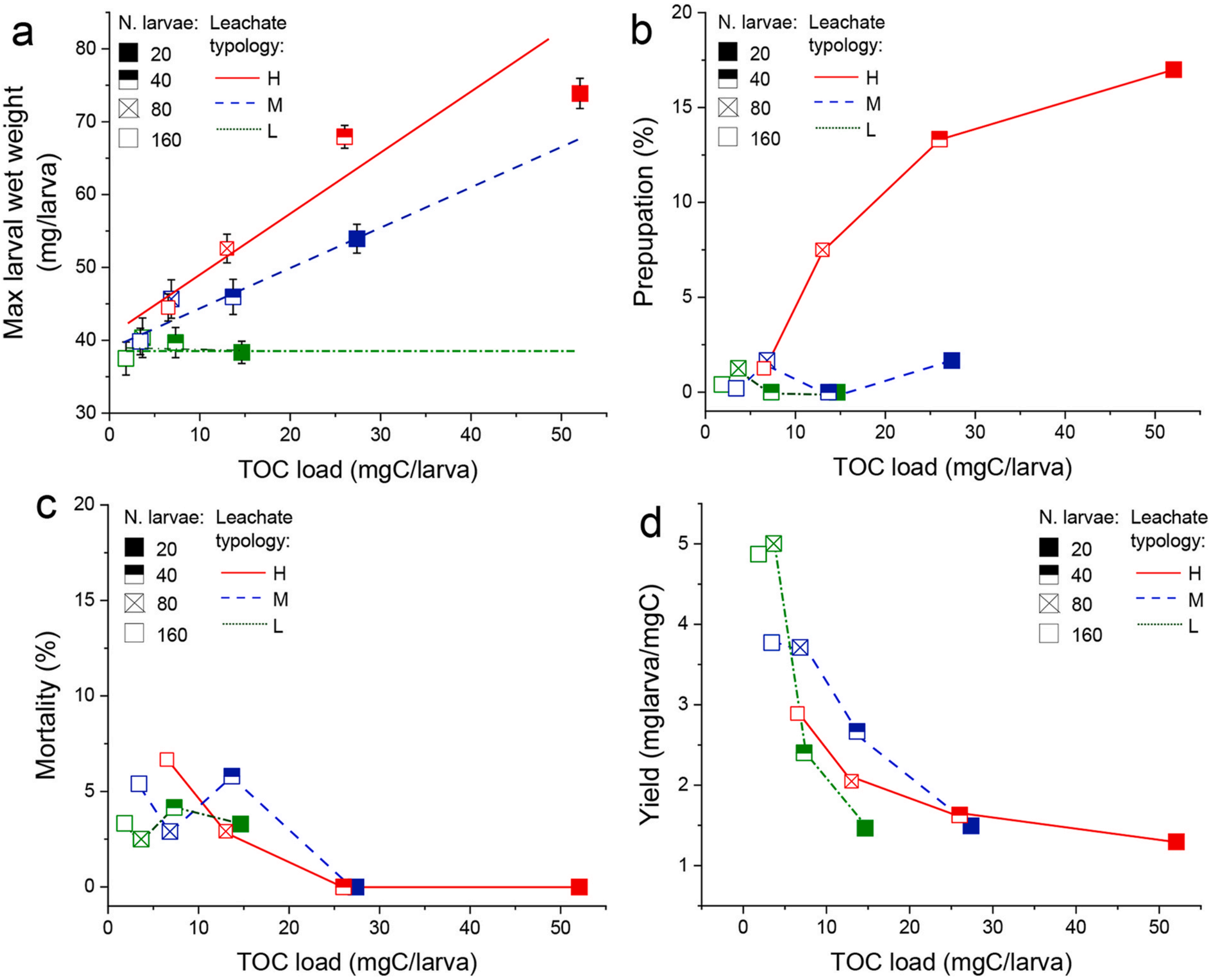

Fig. 4. Variation of larval growth performance (in terms of maximum larval wet weight, prepupation, mortality and yield values) with TOC loads, under different substrate concentration. (L = low-, M = medium, H = high-organic content concentration; 20, 40, 80, 160 = number of larvae per reactor).

3.3. Effect of organic content load and concentration on substrate consumption

The specific substrate consumption rate (v_S) was calculated in terms of mg TOC/larva/day (according to Eq. (2)) throughout the larval growth phase (i.e. up until achievement of max wet weight). Relationship between v_S and organic loads is plotted in Fig. 5. At variance with larval growth performance (Fig. 4), substrate consumption rate was not influenced by organic substrate concentration in the feed (L, M, H). v_S values increased nonlinearly with organic loads, regardless of substrate concentration, fitting a Michaelis Menten like relationship (Eq. (3)):

$$v_S = \frac{v_{S_{max}} * TOC_{load}}{K_s + TOC_{load}} \qquad \text{Eq. 3}$$

Where:

v_S = specific substrate consumption rate (mg TOC/larva/d)
$v_{S_{max}}$ = max specific substrate consumption rate (mg TOC/larva/d)
K_s = half saturation constant (mg TOC/larva)
TOC_{load} = TOC load (mgC/larva)

By fitting the experimental data and Eq. (3) the following parameters values can be obtained (Fig. 5):

$$v_{S_{max}} = 3.8 \text{ (mg TOC / larva / d)}$$

$$K_s = 34.3 \text{ (mgC / larva)}$$

The v_S value obtained in test M20 is in line with the result obtained by Grossule et al. (2022) (approx. 2 mg C/larva/d as average in test H–S, operated under same leachate quality and TOC load) under the same larval density and leachate quality conditions.

A substrate attenuation due to mechanisms other than larvae metabolism, such as bacterial oxidation, adsorption, precipitation, etc, might be possible. This could be more evident in a batch test due to the long retention time and might lead to an overestimation of v_S values, while this should be negligible under shorter retention time, as typically occurring under continuous feeding operation. In addition, these side effects would lose any relevance as larval density increases.

4. Conclusions

The effect of organic concentration and load on treatment of wastewater using BSF larvae was investigated. The research was carried out in a series of batch tests where treatment performance was assessed by monitoring both larval growth (in terms of weight variation, mortality and prepupation), and variation of wastewater quality and quantity under different operational conditions.

Based on the results obtained, the following conclusive remarks can be drawn:

- The higher the organic content concentrations in the feeding substrate, the greater and faster the larval growth and substrate consumption.
- The use of BSF larvae process is appropriate for the pretreatment of high organic concentrated wastewaters. Indeed, larval starvation was observed in all tests when TOC concentrations dropped below approx. 1000 mg C/L, regardless of substrate loads. Accordingly, this value could be assumed, for the tested wastewater, as the limit concentration value for adopting BSF larvae process.
- High substrate concentration produced a significantly positive effect on larval growth when organic load increased beyond 10 mg C/larva, resulting in higher maximum wet weight, prepupation and survival rate.
- Yield values decreased as substrate loads increased, suggesting that under food shortages, most of the available food is used for anabolic processes and converted into new biomass, while under high food availability, the fraction of food dedicated to the catabolic production of bioenergy increases.
- The specific substrate consumption rates (v_S, mgC/larva/day) increased nonlinearly with organic loads, regardless of 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 removal rate. v_S values can be used in treatment unit design, based on organic loads, regardless of organics concentration.
- Substrate attenuation phenomena other than larval metabolism (such as bacterial oxidation, adsorption, precipitation, etc) might lead to a overestimation of the specific substrate removal rate under long retention times of batch tests and low larval densities; the latter should however be negligible under shorter retention times, as typically adopted under continuous feeding operation and when increasing larval density.

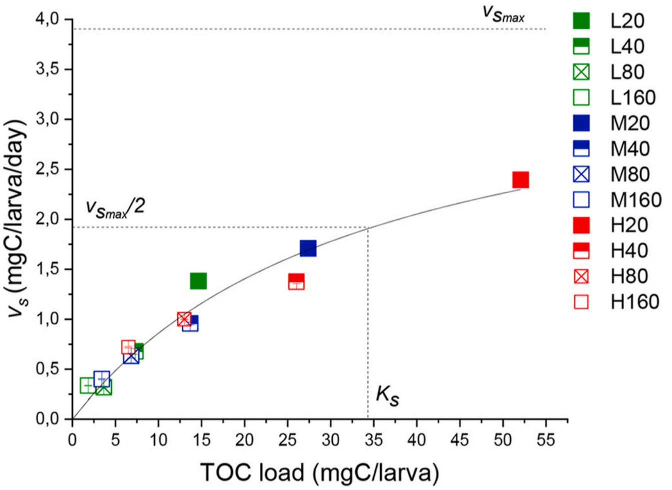

Fig. 5. Variation of specific substrate consumption rates (v_S, mgC/larva/day) with TOC loads, under different substrate concentration. (L = low-, M = medium-, H = high-organic content concentration; 20, 40, 80, 160 = number of larvae per reactor).

Credit author statement

Valentina Grossule: Conceptualization, Methodology, Laboratory activities, Data elaboration and interpretation, Writing Original draft. **Ding Fang**: Methodology. **Maria Cristina Lavagnolo**: Review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P.C., Ortega, E., 2018. Insect as feed: an emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. J. Clean. Prod. 171, 403–412. https://doi.org/10.1016/j.jclepro.2017.09.244.

Di Somma, A., Moretta, A., Cané, C., Scieuzo, C., Salvia, R., Falabella, P., Duilio, A., 2022. Structural and functional characterization of a novel recombinant antimicrobial peptide from hermetia illucens. Curr. Issues Mol. Biol. 44, 1–13. https://doi.org/10.3390/cimb44010001.

Franco, A., Scieuzo, C., Salvia, R., Petrone, A.M., Tafi, E., Moretta, A., Schmitt, E., Falabella, P., 2021. Lipids from hermetia illucens, an innovative and sustainable source. Sustain. Times 13. https://doi.org/10.3390/su131810198.

Grossule, V., Cossu, R., 2021. IT Patent 102021000016700.

Grossule, V., Fang, D., Yue, D., Lavagnolo, M.C., Raga, R., 2021. Preparation of Artificial MSW Leachate for Treatment Studies: Testing on Black Soldier Fly Larvae Process. Waste Management & Research, pp. 1–11. https://doi.org/10.1177/0734242X211066702. Waste Management & Research.

Grossule, V., Fang, D., Yue, D., Cristina, M., 2022. Treatment of wastewater using black soldier fly larvae , under different degrees of biodegradability and oxidation of organic content. J. Environ. Manag. 319, 115734 https://doi.org/10.1016/j.jenvman.2022.115734.

Grossule, V., Lavagnolo, M.C., 2020. The treatment of leachate using Black Soldier Fly (BSF) larvae: adaptability and resource recovery testing. J. Environ. Manag. 253, 109707 https://doi.org/10.1016/j.jenvman.2019.109707.

Grossule, V., Vanin, S., Lavagnolo, M.C., 2020. Potential treatment of leachate by Hermetia illucens (Diptera, Stratyomyidae) larvae: performance under different feeding conditions. Waste Manag. Res. 38, 537–545. https://doi.org/10.1177/0734242X19894625.

Leong, S.Y., Kutty, S.R.M., Malakahmad, A., Tan, C.K., 2016. Feasibility study of biodiesel production using lipids of Hermetia illucens larva fed with organic waste. Waste Manag. 47, 84–90. https://doi.org/10.1016/j.wasman.2015.03.030.

Triunfo, M., Tafi, E., Guarnieri, A., Salvia, R., Scieuzo, C., Hahn, T., Zibek, S., Gagliardini, A., Panariello, L., Coltelli, M.B., De Bonis, A., Falabella, P., 2022. Characterization of chitin and chitosan derived from Hermetia illucens, a further step in a circular economy process. Sci. Rep. 12, 1–17. https://doi.org/10.1038/s41598-022-10423-5.

Xiong, J., Mao, J., Wang, T., Feng, W., Wang, W., Yang, C., Miao, X., Wang, C., 2020. Refining and sulfurization of oil from black soldier fly and its application as biodegradable lubricant additive. JAOCS, J. Am. Oil Chem. Soc. 97, 1243–1251. https://doi.org/10.1002/aocs.12403.