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MASTER THESIS

Scaling up of waste water treatment plant based on the use of BSF larvae: design optimisation, construction and operation.

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I dedicate this master thesis to my family, my friends and my supervisor, Dott.ssa Valentina Grossule.

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ABSTRACT

The use of Black Soldier Fly (BSF) larvae has been recently studied as a promising biological treatment process for high organic content wastewater. After proper setting of the operational conditions (providing efficient physical support for the larvae mobility), subject to a patent, several studies have been carried out aimed at achieving a better understanding of the process influencing variables and removal kinetics. Based on the results of previous studies, the research aimed at determining the optimising process operating conditions, in terms of feeding loads, larvae densities, solid supporting material and water depth. The final goal was the designing, building, and operating the first continuous semi pilot plant for the wastewater treatment using BSF larvae. The preliminary studies consisted in the operation of a small-scale continuous reactor to evaluate the hydraulic set up and the consistency of the removal kinetics with those obtained under batch test conditions. Moreover, a "Big Brother" test was carried out, watching larvae behaviour 24/7, to evaluate the maximum diving depth under different larvae densities and supporting material. Based on the preliminary tests and literature results, the semi pilot plant was designed, built and operated for four weeks.

1. STATE OF ART

In a circular economy context, the ultimate goal set by authorities and companies is to reach a stage of "zero emissions," whether in terms of solid, liquid or gaseous waste. The actions that are implemented to achieve this goal follow the 3R (Reduce, Reuse, Recycle) hierarchy, in which waste treatment is placed at the base of the pyramid. As technicians, we know that achieving an integrated management system in which no final residues are produced is scientifically impossible; therefore, it becomes essential to study and investigate methods to sustainably implement residue reuse and recycling steps. In particular, it is foundamental to study alternative methods for human-generated waste treatment. The term "sustainability" must be evaluated not only in terms of resources saving (raw materials and energy), but through a complete study of materials life cycle and how that particular treatment fits into the environmental and socioeconomic context in which it is to be used.
On the world scene, an important role is occupied by liquid waste, whether it comes from the civil discharges of residential settlements or from industrial activities. In a world situation in which authorities and private entities cannot afford to waste energy, water and land resources, studying systems that give the possibility to reduce resources and, at the same time, reuse liquid waste will play a key role in the future of environmental engineering.

Fig. 1 Schematic representation of "Circular Economy" (CE) for water concept.

The purpose of this thesis comes from this general overview. Studying the use of Black Soldier Fly larvae for the treatment of industrial wastewater could be an innovative and sustainable method as it reduces the use of energy, the GHG emissions and no sludge areproducted. In addition, larval biomass already knows a promising market, both for feed production and for the extraction of valuable chemicals, such as chitin.
Specifically, this thesis focuses on the study of the main kinetic parameters of pollutant removal by larvae, in constant comparison with the same parameters in conventional biological treatment plants. Starting from both the existing background in landfill leachate treatment and the concurrent tests carried out by Voltabarozzo reseach team on industrial wastewater, the thesis developed in 3 steps:

1. The study of kinetic parameters of removal, larval development and technical issues of an first small scale pilot plant "trial".
2. The study of the behavior of larvae within a fully saturated solid substrate, by the "Big Brother Test".
3. The study of the kinetic parameters of removal, larval development, and technical problems of a pilot plant capable of treating from 5 to 50 Lt/day of waste water.

1.1 BSF BACKGROUND

The Black Soldier Fly (BSF), *Hermetia illucens* (Linnaeus, 1758), is a fly (Diptera) belonging to Stratiomyidae family. It is native from the tropical, subtropical and warm temperate regions of the American continent (Newton *et al.*, 2005; Makkar *et al.*, 2014) but the international trade has contributed to a broad distribution (Makkar *et al.*, 2014; Lohri *et al.*, 2017); therefore, it is now widespread in tropical and warmer temperate zones worldwide between about 45° N and 40° S (Diener *et al.*, 2011).
The rapid spread of *Hermetia illucens* can be explained by its biological characteristics: high adaptability to different environmental conditions, to food shortages or to oxygen deficiencies (Diener *et al.*, 2011); resistance to insecticides and pesticides (Turchetto and Vanin, 2004); competition to other flies (Makkar *et al.*, 2014). In particular, Sheppard *et al.* (2002) demonstrate that the Black Soldier Fly Larvae (BSFL) can reduce the housefly (Musca domestica) population by 94-100%, inhibiting the oviposition (Makkar *et al.*, 2014).
Since the housefly is a key vector in spreading disease (Lalander *et al.*, 2013), the competition and the resulting reduction of the breeding of the housefly from BSFL is a positive hygiene aspect. Moreover, the Black Soldier Fly is not considered as a pest (Furman *et al.*, 1959) and a potential carrier of disease (Makkar *et al.*, 2014) because the adult fly does not feed but survives only on its body fat reserve (Diener *et al.*, 2009); therefore, there is not transmission of pathogens from faecal and organic waste to foodstuffs (Banks *et al.*, 2013). Studies by Erickson *et al.* (2004) and Lalander *et al.* (2013) demonstrate also that the Black Soldier Fly Larvae have a positive effect in sanitising the waste, potentially reducing harmful bacteria such as Escherichia coli 0157:H7 and Salmonella enterica (Makkar *et al.*, 2014).

1.1.1. Life cycle

The life cycle of a Black Soldier Fly consists of four stages: egg, larva, pupa and adult. According to Zhou *et al.* (2013) and Lohri *et al.*, (2017), at 28°C and with 75% relative humidity (RH), the development period from egg to adult stage lasts 20–35 days, but it could be prolonged up to several months if the conditions are not optimal and controlled (Makkar *et al.*, 2014). However, the life cycle of the BSF is most in the stage of larva and pupa (Sheppard *et al.*, 2002).
The description of the four stages are shown below:

• Egg stage

Females of BSF oviposit once in their lifetime (Holmes *et al.*, 2002) and, after the eggs are laid, females die within hours (Tomberlin *et al.*, 2002). Oviposition generally occurs 2 days after mating (Tomberlin *et al.*, 2009) with the laying of about 320-620 eggs (Tomberlin *et al.*, 2002; Park, 2016) in dry cracks near moist food sources (Li *et al.*, 2011b). Then, the eggs require about 4 days (4.3-4.5 days or 102-105 hours) to hatch at 24-27°C (Sheppard *et al.*, 2002; Myers *et al.*, 2008).

• Larval stage

Once the eggs hatch, the larvae start to consume the organic waste they find. The full maturity is reached in different times, depending on environmental conditions, such as breeding temperature (Tomberlin *et al.*, 2009), substrate composition, relative humidity and food availability (Ma *et al.*, 2017); in fact, the BSFL can extend their life cycle in unfavourable conditions (Park, 2016; Zurbrugg *et al.*, 2017). Therefore, in ideal conditions, the larval development requires 2-4 weeks (Sheppard *et al.*, 2002; Myers *et al.*, 2008; Tomberlin *et al.*, 2002; Tomberlin *et al.*, 2009; Makkar *et al.*, 2014; Diener *et al.*, 2015; Zurbrugg *et al.*, 2017) but the larval stage could last up to 4-6 months in hostile circumstances, such as in food shortage (Makkar *et al.*, 2014; Park, 2016).

The dimensions of the larvae can reach up to 27 mm in length and 6 mm in width with a weigh up to 220 mg (Makkar *et al.*, 2014; Park, 2016). The body has white colour and the head with their mouthparts is brown-black.
The final larval stage is called prepupal stage in which the exoskeleton starts to darken in pigmentation from white to dark brown (Tomberlin *et al.*, 2009; Banks *et al.*, 2013; Park, 2016). Moreover, the prepupa empties and evacuates the digestive tract and it uses the mouthparts to crawl out of the feeding moist mass, seeking a dark dry place to pupate (Newton *et al.*, 2005;
Diener *et al.*, 2009; Tomberlin *et al.*, 2009; Diener *et al.*, 2011; Lohri *et al.*, 2017; Zurbrugg *et al.*, 2017). From now on, the BSF seals its mouthparts and stops feeding; therefore, the metamorphosis is sustained by the fat stored during the previous stage (Newton *et al.*, 2005).

• Pupal stage

During the pupation, which occurs in a dark dry and sheltered site, the integument darkens, and the pupa develops inside the exoskeleton. The pupation period takes a minimum of 10-14 days (Newton *et al.*, 2005; Makkar *et al.*, 2014) but, depending on the environmental conditions, it could be prolonged (Furman *et al.*, 1959) and last up to 5 months (Makkar *et al.*, 2014).

• Adult stage

The BSF adult that emerges from the pupal case is black and about 15-20 mm long (Makkar *et al.*, 2014; Park, 2016). Adults are not provided with mouthparts and digestive organs, and they don't need food but only water (Tomberlin *et al.*, 2002). A few days (~2 days) after the emergence, BSFs mate and 2 days after females oviposit (Sheppard *et al.*, 2002; Park, 2016) in dry cracks near moist food sources (Diener *et al.*, 2011); according to Tomberlin and Sheppard (2002), 69% of mating occurs 2 days after the emergence, and 72% of oviposition occurs 4 days after the emergence. Adult flies have short lives of about 3-14 days (Furman *et al.*, 1959; Tomberlin *et al.*, 2002; Popa and Green, 2012; Park, 2016; Zurbrugg *et al.*, 2017) but Tomberlin *et al.* (2002) state also that females die within hours after the oviposition.

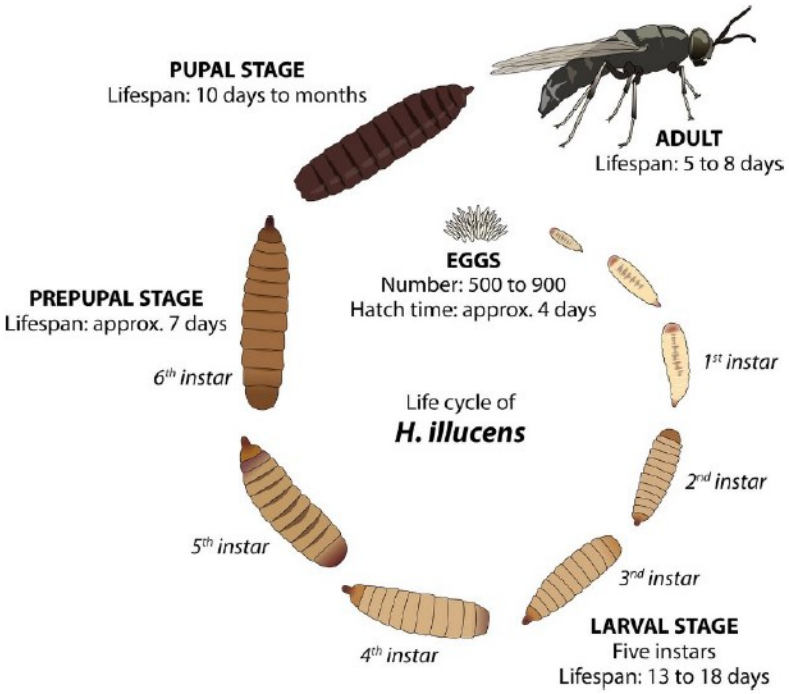


Fig.2 – BSF life cycle.

1.1.2. Optimal living conditions

Environmental conditions influence the success and the time of the development of the BSF. In particular, optimal temperature and moisture (Furman *et al.*,1959; Holmes *et al.*, 2002) are the most important variables to control; also, the food availability and good quality (Makkar *et al.*, 2014) and the ideal photoperiod (Zhou *et al.*, 2013) are fundamental to guarantee an optimal growth. Several authors (Tomberlin and Sheppard, 2002; Lalander *et al.*, 2013; Makkar *et al.*, 2014) demonstrate that the most proper temperature values for the entire life cycle of the BSF is in the range of 25-32°C. Park (2016) states that the longevity is at its most efficiency at 27°C, whereas the BSF development is acutely inhibited at temperatures higher than 30-36°C.
A successful mating and oviposition (hatching of ≈80% of the eggs) occurs with a temperature greater than 26°C (Tomberlin and Sheppard, 2002) and up to 40°C or more (Sheppard *et al.*, 2002). Moreover, in the winter months, the BSFs seem to do not mate (Park, 2016).
Makkar *et al.* (2014) shows that the ideal relative humidity (RH) ranges between 50 and 70 % but, according to Park (2016), increasing the humidity of the environment, the success of the BSFs development increases. A successful mating and oviposition (hatching of ≈80% of the eggs) occurs with a humidity greater than 60% (Tomberlin and Sheppard, 2002) but also a wide range (RH= 30-90%) is possible (Sheppard *et al.*, 2002). However, Lohri *et al.* (2017) state that, although BSF larvae are able to develop under pure liquid conditions, they prefer a moist or semisolid environment.
Considering the optimal pH of the feeding substrate, only Ma *et al.* (2017) deals with it, recommending an initial pH of 6-8 for a good bioconversion of organic waste.
Park (2016) observes that the natural direct sunlight promotes mating, with the 85% of mating activity happening with a light intensity of about 110 μmol m2 s-1. Moreover, a photoperiod of 12L:12D is considered by Diener *et al.* (2009) and Gobbi *et al.* (2013) and of 16L:8D by Myers *et al.* (2008) and Zhou *et al.* (2013).
The optimal living conditions suggested by the various authors are summarized in Table 1.

PARAMETER	OPTIMUM	REFERENCE
Food quantity and quality	125 mg/larva/d of biowaste (1-2 cm sized)	Zurbrugg et al. (2017)
100 mg/larva/d of chicken feed (60% moisture)		Diener at al. (2009)
Moisture (%)	65-80%	Lohri et al. (2017)
30-85%		Tomberlin et al. (2002)
30-90%		Sheppard et al. (2002)
60%		Myers et al. (2008)
70-80%		Zurbrugg et al. (2017)
Temperature (°C)	27	Tomberlin et al. (2009), Myers et al. (2008)
24.8-38.4		Tomberlin et al. (2002)
24-40		Sheppard et al. (2002)
25-32		Zurbrugg et al. (2017), Lohri et al. (2017)
Photoperiod (h)	18L:6D	Zhou et al. (2013), Myers et al. (2008)
12L:12D		Diener et al. (2009)
pH	>6	Ma et al. (2017)
Larvae age (days old)	5	Tomberlin and Sheppard (2002), Zurbrugg et al. (2017)
6		Diener et a. (2009)
7		Popa and Green (2012)

Tab.1 - Optimal conditions for BSFL development.

1.1.3 The use of larvae for waste treatment and resource recovery

Firstly, BSF larvae are robust, and can feed on many different types of waste: kitchen waste, poultry waste, dairy manure and human faeces (Singh & Kanchan, 2019). They also have the ability to significantly reduce the volume of waste they feed on (Singh & Kanchan, 2019). In a medium-scale field experiment, Diener, et al., 2011, achieved 68% of dry weight reduction. By decreasing the volume of organic waste, the transportation costs, and the landfill space requirements will be reduced as well (Diener, et al., 2015). They also are a low cost and low maintenance way of managing organic waste, particularly adapted to low- and middle- income countries due to its low capital investment. Indeed, the larvae are self-harvesting, which means that the operation and maintenance of these installations require few technical skills (Singh & Kanchan, 2019). Furthermore, they produce less greenhouse gases than usual biological decomposition because they capture and store carbon in the nutrient form and have a low ecological footprint (Singh & Kanchan, 2019). Besides, while feeding on waste, the BSF larvae produce an interesting biomass rich in protein with a high nutritional composition. They enable nutrient recovery: 85% of Nitrogen, and 75% of Phosphorous reduction from pig manure was achieved (Singh & Kanchan, 2019). When the larvae reach the prepupae stage, they have their maximum size, and are composed of 40% of crude protein, and 28% of crude fat (Liu, et al., 2017). Because BSF larvae do not accumulate pesticide (Singh & Kanchan, 2019), this biomass can then be used as animal or human feed and require less space than conventional animal feed. Then, as they lose their mouth apparatus when they become adults and they are not attracted to human houses or food, they do not propagate diseases, and are not considered as pest (Singh & Kanchan, 2019). Another interesting property of BSF larvae is that they transform the waste microflora during their feeding. In particular, they secrete bactericidal molecules that remove noxious bacteria like *E. coli*, and *Salmonella enterica* and reduce the risk of transmission of diseases between animals or between animals and humans when managing animal waste like animal manure or slaughterhouse waste (Singh & Kanchan, 2019) (Diener, et al., 2015). Finally, BSF can also be interesting from a socio-economic point of view. Indeed, the BSF treatment units can, in addition, act as a collection center for the informal sector. In these place, organic waste can be bought to waste pickers or farmers, generating an income, and at the same time improving the local waste management system (Diener, et al., 2015). When on-site BSF treatment facilities are implemented, in a farm for instance, the produced biomass can be sold by the farmer to produce animal feed and biodiesel, providing a new source of revenue.

1.1.4 Applications and product

Before 1950, the BSF was considered a pest. In the late 50s, researchers, and scientists started to realize the benefits that could be obtained from BSF. More specifically, the role BSF could play in organic waste management was first acknowledged in the 80s (Tomberlin & van Huis, 2020). Its potential in organic waste reduction and valorization has widely been studied in the last decades, and some large-scale facilities able to treat up to 200 tons of organic waste per day were implemented in some countries like Canada, the Netherlands, China or South Africa (Diener, et al., 2015). At a smaller scale, many individual BSF composting units are used by households or farmers (Diener, et al., 2015). BSF can feed on and convert a wide variety of organic wastes: kitchen waste, animal, and human manures, municipal organic solid waste, agricultural residues, milling, and brewery side streams, but also vertebrate remains (Singh & Kanchan, 2019), (Surendra, et al., 2020), (Gold, et al., 2018). SF larvae growing on organic waste serve a double purpose: organic waste reduction and treatment as well as the production of value-added products.

Two main products are obtained from the organic waste valorization by BSF larvae: a biomass rich in crude protein, and fats, and a residue (Surendra, et al., 2020). BSF biomass is produced with a high waste-to-biomass ratio of 20% (Diener, et al., 2015). The biomass can firstly be used as animal or to some extent human feed. The BSF biomass has already been successfully tested for fish, cattle and

poultry feed (Singh & Kanchan, 2019). As regard human consumption, the United Nation's Food, and Agriculture organization established the BSF as one of 6 insect species safe for human consumption (Singh & Kanchan, 2019). The extracted fat from the BSF biomass can be transformed into biodiesels via transesterification (Surendra, et al., 2020). Biodiesel obtained from BSF biomass respects international standards like ATSM D6751, and EN 14214 (Surendra, et al., 2020). Grossule & Lavagnolo, 2020, tested the predicted biodiesel quality obtained from BSF biomass lipids. They concluded that biodiesel from BSF satisfied limit values for cetane number, and iodine, but not for cold filter plugging point, similar characteristics to those of palm oil. The BSF biodiesel could not be used alone, but by mixing it with other biodiesels, optimal conditions could be reached (Grossule & Lavagnolo, 2020). The residue, meanwhile, has a high concentration of phosphorus, and nitrogen, and a pH around 7-8 which makes it usable as an organic fertilizer (Surendra, et al., 2020). Even if animal feed, organic fertilizer and biodiesels are the most common products obtained in this process, some other valuable products can be processed. For instance, the BSF biomass is made of 7% of chitin which is used in many industrial applications and in medicine (Surendra, et al., 2020) (Singh & Kanchan, 2019). The chitin obtained from BSF could be used in the textile industry or for some engineered tissues (Surendra, et al., 2020).

The following figure (figure 3) shows the main applications of BSF larvae:

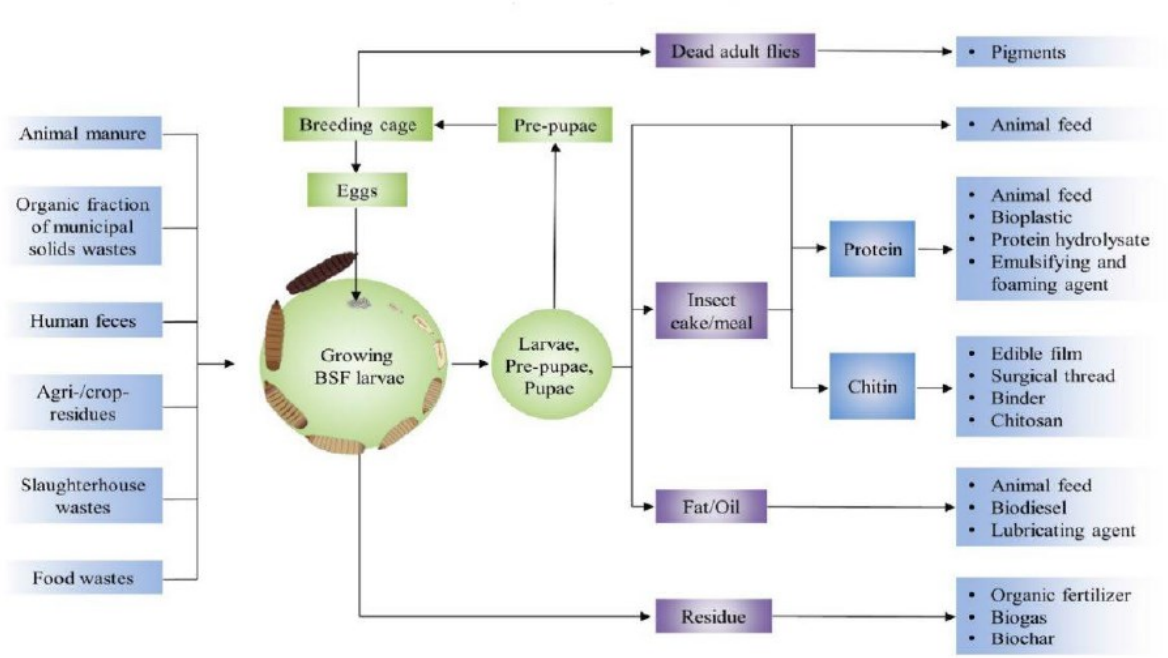


Fig. 3 – BSF larvae application and products scheme.

1.2 LIQUID WASTE APPLICATION BACKGROUND

1.2.1. Waste water data

A new study conducted by Utrecht University and the United Nations University (Jones, E.R., van Vliet, M.T.H., Qadir, M., and Bierkens, M.F.P., 2021) concludes that about half of global wastewater is treated, rather than the previous estimate of 20%. Despite this promising finding, the authors warn that treatment rates in developing countries are still very low.
Humans and factories produce vast quantities of wastewater per day. If not properly collected and treated, wastewater may severely threaten human health and pollute the environment.
Globally, about 359 billion cubic metres of wastewater is produced each year. 48 % of that water is currently released untreated (figure 4).
Particularly in the developing world, where most of the future population growth will likely occur, treatment rates are lagging behind. In these countries in particular, wastewater production is likely to rise at a faster pace than the current development of collection infrastructure and treatment facilities. This poses serious threats to both human health and the environment.
The main problem, especially in the developing world, is the lack of financial resources to build infrastructure to collect and treat wastewater. This is particularly the case for advanced treatment technologies, which can be prohibitively expensive. The most obvious reuse of treated wastewater is to augment freshwater water supplies. Treated wastewater reuse is already an important source of irrigation water in many dry countries, particularly in the Middle East and North Africa. However, only 11% of the wastewater produced globally is currently being reused, which shows large opportunities for expansion.
Wastewater also has large potential as a source of nutrients and energy. Recognition of wastewater as a resource, opposed to as 'waste', will be key to driving improved treatment going forward.

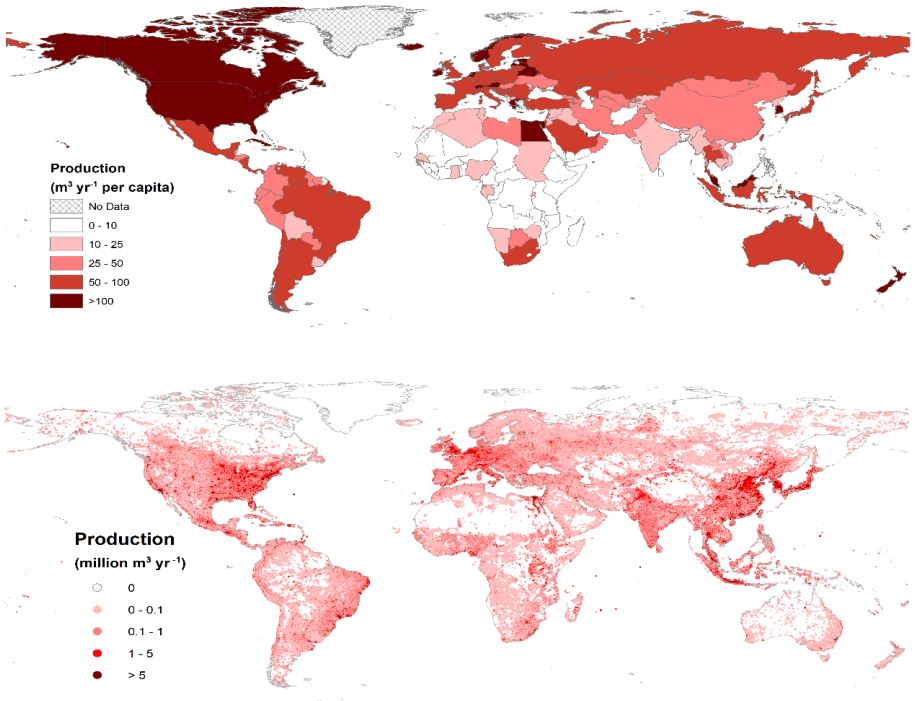


Fig. 4 – Worldwide waste water production data.

1.2.2 Liquid waste BSF larvae treatment background

Using BSF larvae in the treatment of solid waste has been studied and implemented for years by many countries around the world. However, their use in liquid waste treatment is a field that is still under development and deepening. In this sense, the first tests found were carried out by Popa & Green in 2012, which tested this system in organic compost leachate, finding good growth of the larvae, which in 4 weeks reached the prepupal stage. From the point of view of the removal of pollutants, the results showed a partial reduction of COD and VFA, simultaneously with an increase in the pH of the outgoing wastewater.

In 2019 Grossule & Lavagnolo tested this system on landfill leachate, monitoring the larval growth conditions. In particular, carrying out the toxicity test, the substrate was placed in PP plastic boxes with the following dimensions: L 13.5 x W 13.5 x H 5.5 cm, 0.6 L; perforated plastic lids are employed to allow air circulation and a permeable non-woven fabric is clamped between the box and the lid in order to avoid the oviposition by other flies and the contact with other insects. Two substrates are tested: the liquid one with different dilution of leachate and the semisolid one with 20% of wheat bran in 80% of different dilution of leachate.
The four different dilution of landfill leachate are named: 100%, 75%, 50% and 25% in which the 0%, 25%, 50% and 75% in volume of distilled water are present, respectively.
For each combination of substrate and dilution, 3 replicates containing also larvae and 1 control without larvae are provided.
Each box except the controls contains 10 larvae six-d-old and 200 mL of liquid substrate (in different percentage of landfill leachate and distilled water, according to the dilution) the semisolid set also contains 50 g of wheat bran (in order to guarantee about 80% of moisture).
The monitoring occurs two times per week (every 3.5 days): boxes with semisolid substrate are weighted by means of a technical balance and volume inside boxes with liquid substrate is measured; larvae are collected, washed and individually weighted by means of an analytical balance.
This series of tests demonstrated that landfill leachate cannot be considered toxic to larvae, as no LD_{50} was found. The semi-solid substrate performed better than the totally liquid but, being organic, it interfered with the removal processes as the larvae first degraded the bran and then the leachate.
Investigating the removal parameters, PP plastic boxes are used with the following dimensions: L 18 x W 12 x H 8 cm, 1.0 L; perforated plastic lids are employed to allow air circulation and a permeable non-woven fabric is clamped between the box and the lid in order to avoid the oviposition by other flies and the contact with other insects.
Four substrates are tested: the liquid one with 100% of leachate and three different semisolid substrates characterized by about 78% of moisture.
The three different solid substrates are sawdust,wheat bran and brewer's spent grain.
For each substrate, three replicates containing also larvae and control without larvae are provided.
Moreover, the liquid set is quite different from the semisolid ones because each replicate and the control are provided with a continuous recirculation system. Each box except the control contains 300 larvae six-d-old; boxes with liquid substrate contain 300 mL of 100% landfill leachate and boxes with semisolid substrate contain 163 mL of leachate and 47 g of solid substrate.
The monitoring occurs one time per week (every 7 days): boxes with semisolid substrate are weighted by means of a technical balance and volume inside boxes with liquid substrate is measured; larvae are collected and washed but only ten of them per box are randomly taken and individually weighted by means of an analytical balance.
For batch set bigger PP plastic boxes are used: Ø 18 x H 14.5 cm, 2.6 L; perforated plastic lids and a permeable non-woven fabric are provided as in the previous sets.
Only the three semisolid substrates with ≈78% moisture are tested and, for each substrate, only one replicate containing also larvae and one control without larvae are provided. Therefore, there are six boxes.

Each box except the controls contains 300 larvae six-d-old; for each kind of semisolid substrate there are 489 mL of leachate and 141 g of solid.
The monitoring occurs only at the end of the experimentation: boxes with semisolid substrate are weighted by means of a technical balance and sampled; larvae are collected and washed but only ten of them per box are randomly taken and individually weighted by means of an analytical balance. Taking into account the removal efficiency, the larvae contributed 10-15% in the removal of TOC and 25-36% in the removal of organic nitrogen. No effect was found in the removal of ammoniacal nitrogen.

Further tests carried out by Grossule & Lavagnolo in 2021 focused on studying the behavior of BSF larvae as regards the influence of liquid waste biodegradability (BOD/COD) and the degree of oxidation of the wastewater (TOC / COD).
Experiment was performed by placing young BSF larvae (six days old, 13mg as average wet weight per larva, 20 x boxes) in batch reactors. Larvae were physically supported by a patented plastic granular bed, which was fully saturated by leachate.
Three typologies of leachates featuring similar COD values, were used:

- real leachate (R), from MSW landfill;
- artificial synthetic leachate (S), prepared as an aqueous solution of chemicals only;
- artificial mixed leachate (M), prepared by mixing chemicals with artificial food waste eluate.

These three typologies were characterized by a degree of oxidation (TOC/COD) decreasing from R to S to M leachate (Grossule et al., 2021). Two samples of each typology were tested, each displaying varying degrees of biodegradability in terms of BOD/COD values: high (H) and low (L).
Real leachates were sampled from landfill sectors of different ages. For artificial leachates, BOD/COD ratio was adjusted by varying preparation recipes (Grossule et al., 2021).
Test results showed that larvae growth was greater and occurred faster as the BOD/TOC ratio increased, displaying higher maximum average wet weight, $Xmax$ and maximum specific growth rates, μmax. Also survival rate and prepupation increased proportionally to BOD/TOC ratio. In addition, similar substrate consumption rate values were obtained under the same oxidative conditions of organic content (TOC/COD), with lower values occurring at lower TOC/COD ratios. $kCOD$, VS ranged between 0.49 and 1.37 mgCOD / mgVS / d, up to 3-fold the values reported in literature for activated sludge. Although under constant feeding conditions, growth rate rx, specific growth rate μ and biomass yield Y changed in line with larvae aging. No significant differences were detected in larvae protein and lipid contents, including the profiling of fatty acids.

A further step was also carried out by Grossule & Lavagnolo (2021), this time testing larval growth and removal parameters as the organic load and wastewater concentration vary. The experiment was performed accommodating in batch reactors young BSF larvae (10-day-old, 28 mg as average wet weight per larva). Larvae were physically supported by a patented plastic granular bed, which was fully saturated by leachate (Grossule et al., 2021 and 2022).
Three artificial leachates were prepared as an aqueous solution of chemicals, varying the preparation recipes (suggested by Grossule et al., 2021) in order to obtain different organic content concentrations, defined as low (L), medium (M) and high (H).
Each leachate was tested at four different loads, by feeding with the same volume 20, 40, 80 and 160 larvae. Each test was conducted in triplicate.
Each testing reactor was made of a plastic box (13.5cm x 13.5cm x 5.5cm), containing granular plastic material (VALOX®, 2-3 mm diameter) completely saturated with leachate. The granular material allowed to larvae free movement in the liquid substrate to meet their needs (feeding and breathing) (Grossule el 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 thermal insulated room under the same environmental conditions suggested by Grossule and Lavagnolo (2020): temperature range 25-30 ° C; photoperiod Light / Dark of 18 / 6h.
The tests showed that higher the organic content concentrations in feeding substrate, the greater and faster the larval growth and TOC (mgC/L) consumption; larvae starvation was observed in all tests when TOC concentrations reduced approx. below 1000mgC / L. High organic content concentration had a greater positive effect on larval growth, resulting in higher maximum wet weight, prepupation and survival rate. And, finally, the specific daily substrate consumption values (vS, mgC/larva/day) linearly increased as the organic substrate loads increased, regardless the substrate concentration.

The results of the previous tests are therefore summarized in table 2:

Author	Liquid waste type	Substrate	Objective	Materials & methods	Conclusions
Green and Popa (2012)	Compost leachate.	/	Larvae development and removal parameters in liquid waste.	4 weeks test.	Partial reduction in COD and VFA. pH increase.
Grossule & Lavagnolo (2019)	Landfill leachate.	Both liquid and semisolid organic (Spent grain, sawdust, wheat bran).	Adaptability and resource recovery testing.	Batch test 4 degrees of leachate dilution (25,50,75,100%) 10 larvae x box.	No LD_{50} deteceted.
Grossule & Lavagnolo (2020)	Landfill leachate.	Both liquid and semisolid organic (Spent grain, sawdust, wheat bran).	Performance under different feeding conditions.	Batch test + batch with recirculation 300 larvae x box.	Better development in wheat bran. Partial TOC & N removal No ammonia removal.
Grossule & Lavagnolo (2021)	6 different leachates (real/artificial/ mixed).	Patented plastic granular bed.	Treatment of wastewater using BSF larvae, under different degrees of biodegradability and oxidation of organic content.	Batch test 20 larvae x box.	Higher BOD_5 load, better removal performance. COD removal ratios 3 times faster than activated sludge.
Grossule & Lavagnolo (2022)	3 different leachates (real/artificial/ mixed).	Patented plastic granular bed.	Treatment of wastewater by using BSF larvae: effect of organic content load and concentration.	Batch test 20, 40, 80, 160 larvae x box.	Faster larval growth in with high organic load Larvae starvation under 1 gTOC/L. Specific daily substrate consumption vS not affected by concentration. Linear relation between organic load and vS.

Tab. 2 – Background BSFL test overview.

Summarizing the tests conducted previously, useful data were obtained to develop the research program of this thesis.
First, it was found that black soldier fly larvae can live in liquid environment and feed on the organic matter present in solution, with excellent results in terms of larval development; in particular, in liquid waste considered as "difficult to treat" such as landfill leachate. In addition, they prefer environments with high concentrations of organic matter, as shown in the study by Grossule & Lavagnolo (2021), which demonstrate that development and removal performance increases proportionally with respect to the Biotreatment Index (BOD5/TOC). Finally, the first sizing parameters were produced, including the key finding of specific substrate removal vS.
The objective of the research was to verify, through three different tests, that the data previously found in batch tests were confirmed; in addition, new parameters and technical arrangements were studied for the application of this treatment to a continuous plant.
The thesis thus aims to address the new issues posed, such as maximum larval depth, mass development, and removal kinetics of continuously fed larvae.

1.3 RESEARCH PROGRAM AND PARAMETERS DEFINITION

1.3.1 Research program

The laboratory experience consists in three different tests:

1 – Small scale continuous semi pilot plant,"Trial", 500 ml/d, built by connecting five trays in series filled with plastic filling material and fed in continuous with liquid substrate, aimed primarily at addressing technical and engineering problems such as the hydraulic seal of the piping, clogging problems and operation of the peristaltic pumps. Also, larval development dX and maximum larval mass Xmax were monitored, in addition to the main removal parameters such as the specific organic load F/L and the specific removal rate vS.

2 - "Big Brother Test", to verify the maximum liquid depth Hmax beyond which the larvae do not dive and consequently set a constant reactor height. The test was carried out by monitoring the behavior of the larvae 24/7, inside closed boxes, by means of a video camera, by varying larval density and the solid support.

3- Continuous pilot plant from 5 to 50 L/d, built by connecting in series three trays filled with plastic support material and fed in continuous with liquid substrate, aimed to monitor the larval development and define the removal design parameters such as the specific organic load F/L and the specific removal rate vS. The further step was to interpolate the relation between F/L and vS in order to fix a replicable relationship between the organic load provided by the larva and the removal efficiency. All this in order to generate a first sizing model for a large-scale BSF larvae treatment system.

Data collected by the three tests were constantly analyzed and compared with the ones coming from the batch tests carried out in parallel by the research team. They mostly focused on verifying the best solid support to be used within the reaction volume, the role of the wastewater biodegradability and the best suitable larval density. For this reason, the research program achieved is the result of the optimization of the sizing parameters that led to the choice, for the "trial" test, of a synthetic wastewater already used with a known and replicable recipe and which had given excellent results in batch tests.
Later, for a technical-economic question (the daily volume to be replicated was substantial), milk diluted 1:10 was used, which was also previously tested with good results. Furthermore, this choice replicate the wastewater from the dairy, thus making the pilot plant test as similar as possible to a real case.
During the analysis and the definition of sizing parameters, it was carried out a constant comparison with the corrispetive of the traditional activated sludge. It was considered important not only to verify the differences between one system and the other, but also to try to make the reading and interpretation of the data relating to the BSF treatment as clear and understandable as possible.

1.3.2 Parameters definition

The main parameters used, mentioned in 1.3.1 "research program" will be defined below:

- *Hmax* [cm]: average diving-depth of larval mass inside a container packed with filling material, completely saturated by the liquid substrate. Height used to fix the liquid head inside the reaction tanks in such a way as to maximize the volume and, at the same time, to make the substrate accessible, avoiding areas where the wastewater was not treated.

- *Cls* [larvae/cm^2] | [lar/cm^2]: larval surface concentration, defined as the number of larvae per square centimeter of reactor tank.

- *Clv* [larvae/L] | [lar/L]: larval volumetric concentration, defined as the number of larvae per liter of reactor.

- *Xmax* [mg]: maximum larval wet weight reached by the single larva during the sampling period.

- *dX* [mg]: net increase in larval mass, dX = X1-X0, with X0 and X1 defined as larval mass at sampling 0 and 1. dX can be expressed in%, $dX\% = \frac{X1-X0}{X0} \times 100$.

- *rx* [mg/d]: larval grow rate, calculated as: $rx = \frac{dX}{dt}$.

- *F/L* [mg/larva/day]: food-larvae ratio, daily specific organic load per larva, calculated as:

  $F/L = \frac{Si \times Qd}{No.\ larvae}$, where:
  Si = organic substrate concentration entering the plant [mg/L]
  Qd = daily inlet flow [L/d],
  No. larvae = number of larvae present in the reactor.

- *F/Lm* [mg/mglarva/day]: food-larval mass ratio, daily specific organic load per unit of larval mass, calculated as:

  $F/Lm = \frac{Si \times Qd}{N\ larvae \times Xi}$, where:
  Xi = initial mean larval weight.

- vS [mgremoved/larva/day]: specific daily substrate consumption, which defines the amount of substrate degraded by the single larva per day, calculated as:

  $vS = \frac{(Sout-Si) \times Qd}{No.\ larvae}$, where:
  Sout = concentration of organic substance in the outgoing wastewater.

- vSm [mgremoved/mglarva/day]: specific mass daily substrate consumption, which defines the amount of substrate degraded by the larval mass unit per day, calculated as:

  $vS = \frac{(Sout-Si) \times Qd}{No.\ larvae \times Xi}$.

- η: Substrate removal efficiency, calculated as:

$\eta = \frac{DeltaS}{Si}$ where:
DeltaS: Si-Sout.

- *HRT* [d]: Hydraulic Retention Time, residence time of wastewater inlet inside the reactor volume, calculated as:

$HRT = \frac{DeltaS}{Vs \times Clv}$

- Organic Load [mg/d]: mass quantity of organic substance that enter inside the reactor every day, calculated as:

$O.Load = Qd \times Si$

- *Vtot* [ml]: total BSFL reactor volume, calculated as:

$Vtot = No.Boxes \times Vww/box,$ where:
Vww/box= volume of wastewater per box.

- Tot. Larval mass [mg]: total larval mass quantity inside the reactor volume, calculated as:

$Tot.larval\ mass = Tot.no.larvae \times Xi$

2. CONTINUOUS PILOT TEST "TRIAL" PAPER

2.1 RESEARCH PROGRAM AND OBJECTIVE

After analyzing existing literature data regarding the use of BSF larvae for liquid waste treatment, it was decided to construct a first small scale continuous pilot plant, formed by connecting five trays in series, and fed continuously with synthetic leachate. The test came into being with two basic objectives, the first practical, the second theoretical.
From the practical point of view, there was a need to verify several issues to be addressed; first, since no previous continuous test had ever been carried out, the piping connecting the trays was tested. In particular, we monitored: the hydraulic tightness of the materials used for the connections, the adequate diameter of the holes and passage piping, the possible protection of these from larvae intrusion, and the possibility of clogging caused either by entrainment of the filling material, by the larvae themselves, and/or by effluent residues.
From a theoretical point of view, this experiment was essential to verify that background data were respected. In addition, the results obtained were compared to the counterparts of batch tests carried out simultaneously by colleagues in the team. Another important objective was to define an initial treatment plant sizing system that would be as consistent and reproducible as possible, as well as comparable with existing biological treatments. The two main parameters monitored were:

- Larval development, in terms of increase in mass dX and maximum larval mass Xmax. In this regard, the objective was to verify literature data, using same synthetic leachate and same support material as liquid substrate. The only parameters varied were Cls and, consequently, F/L, food-larvae ratio. The graph below (figure 5) shows the larval mass development from the experiment carried out by Grossule & Lavagnolo (2021), with the same liquid substrate.

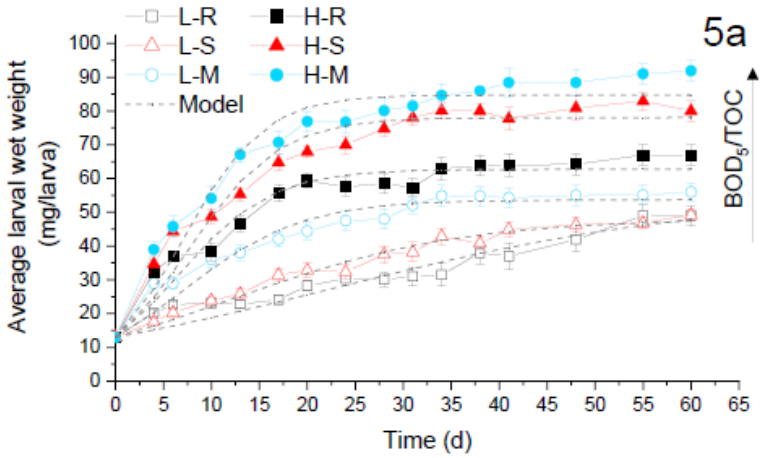


Fig. 5 – Larval mass development from Grossule &Lavagnolo 2021. Line: H-S (Red line with triangles).

Moreover, the following graph (figure 5) shows larval mass development obtained on the same liquid substrate from batch tests carried out by Voltabarozzo research team (2022).

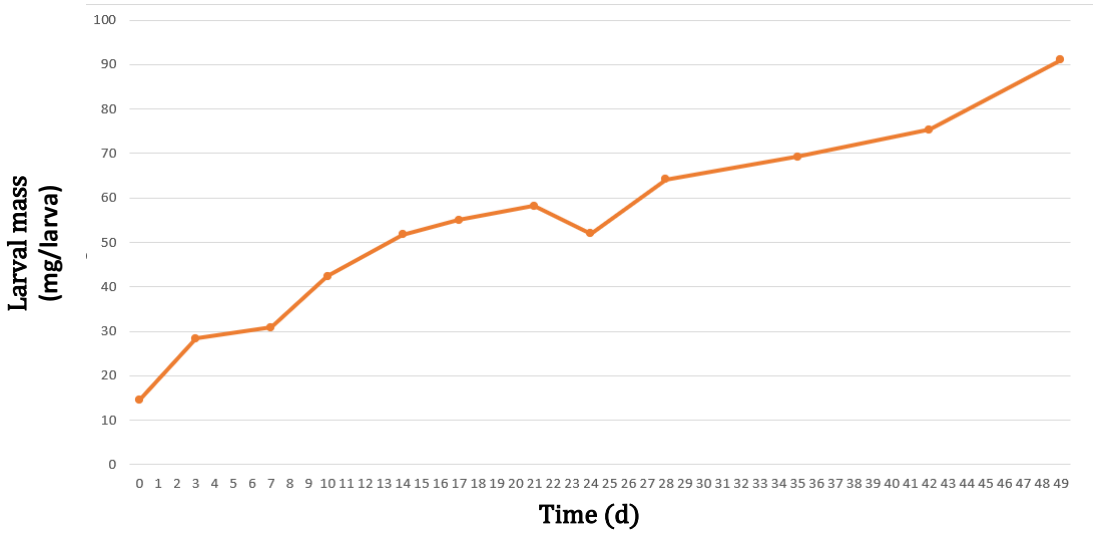


Fig. 6 – Larval mass development in batch test, by Voltabarozzo research team (2022).

Therefore, the objective of the test was to verify that the previously obtained data were met.

- The removal kinetics vS and vSm in terms of TOC. Specifically, the continuous system was sized starting from a vS comng from the studies of Grossule & Lavagnolo (2021) (figure 7), equal to vSTOC= 2 mgTOCremoved/lar/day.

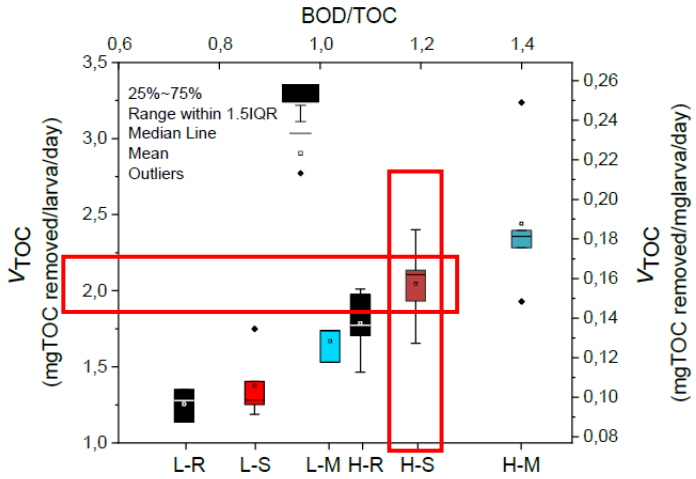


Fig. 7 – $vSTOC$ obtained by Grossule & Lavagnolo (2021).

The choice of liquid waste and support material remained the same of previous application, in order to work with as few variables in play as possible. The No. of boxes, and consequently also the reactor volume, was decided a priori. Number of larvae per box was fixed, so the sizing was developed around the HRT retention time formula, assuming that all incoming organic matter was degraded by the larvae, thus vSTOC = F/L:

$$HRT = \frac{Si}{Clv \times \text{vSTOC}}.$$

From the retention time, Qd, i.e., the daily inlet flow rate, Qd = Vtot/HRT, was then calculated.

The spreadsheet for sizing the continuous "trial" pilot plant, complete with design data, is proposed below.

Qh (ml/h)	Qd [ml/d]	TOC (mg/d)	Vtot [L]	Vtot [ml]
53	426	1600	0,75	750
DESIGN PARAMETERS				
vS [mg/larva]d] TOC	Si [mg/l]	Clv [larve/L]	HRT [h]	N giorni
2	3760	1067	42,3	20
F/L [mg/mgd]		Clv [larve/ml]	HRT [day]	
2		1,07	1,8	
F/Lm [mg/mgd]		Cl x box [larve/box]		
0,16		160		
Xi [mg]		V wastewater/box [ml]		
15		150		
		Cl x 150 ml [larve/150ml]		**Vww TOT [L]**
		160		9
		No. Box	TOT LARVAE	**Vww tot [ml]**
		5	800	8511

Fig. 8 – 1st "trial" sizing model.

The objective of the test was to verify that the results were suitable with the design data.

2.2 MATERIALS AND METHODS

2.2.1 Equipment and growth conditions

The test made use of No. 5 trays used as BSF larval treatment reactor, each consisting of a platic box (13.5cm x 13.5cm x 5.5cm), containing granular plastic material (VALOX®, 2-3 mm in diameter) completely saturated with 150 mL of leachate (Grossule and Cossu, 2021; Grossule et al., 2021). A total of 160 larvae per box, 6 days old and average Xi weight of 15 mg, were inserted. The granular material allowed larvae to move freely in the liquid substrate to meet their needs (feeding and respiration).
Each box was covered with a permeable nonwoven fabric (to prevent oviposition by other flies) and a perforated plastic lid (to allow air recirculation) (figure 9).

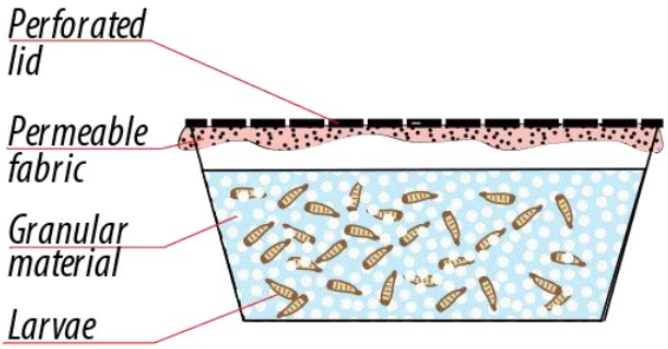


Fig. 9 – "Trial" test reactor box scheme

A cylindrical plastic container was used as effluent storage volume, with a capacity of 2 000 ml, closed at the surface by a plastic lid, in which was drilled a hole of peristaltic pump suction line size, so that the accumulated effluent would not be contaminated by external agents. The peristaltic pump was used to dose the inlet load, with adjustable flow rate 10-100%, timed by daily clock with a minimum trip of 15 min. The suction, formed by flexible silicon tubing of 0.3 cm diam., took the liquid from the storage container and dosed it to the first reaction tank by means of a hose connector. This, attached with bonding resin to a meshed tap gasket, ensured constant dosing of the effluent, preventing larvae from escaping. The five tanks were connected in series via bottom connections, applying a hole of 0.3 cm, on which the flexible silicone tubing of was inserted. Then made watertight by application of external silicone. A plastic net was placed inside the trays near the inlet and outlet holes to prevent the intrusion of larvae into the connecting pipes. The last container was connected to a sixth tray with same characteristics, which was used with the function of both waterhead regulator and sampling the effluent at the outlet. From the latter, by drop, the outflow effluent was accumulated in a cylindrical plastic container with a capacity of 2 000 ml.
The flow diagram of the plant is proposed below, (figure 10):

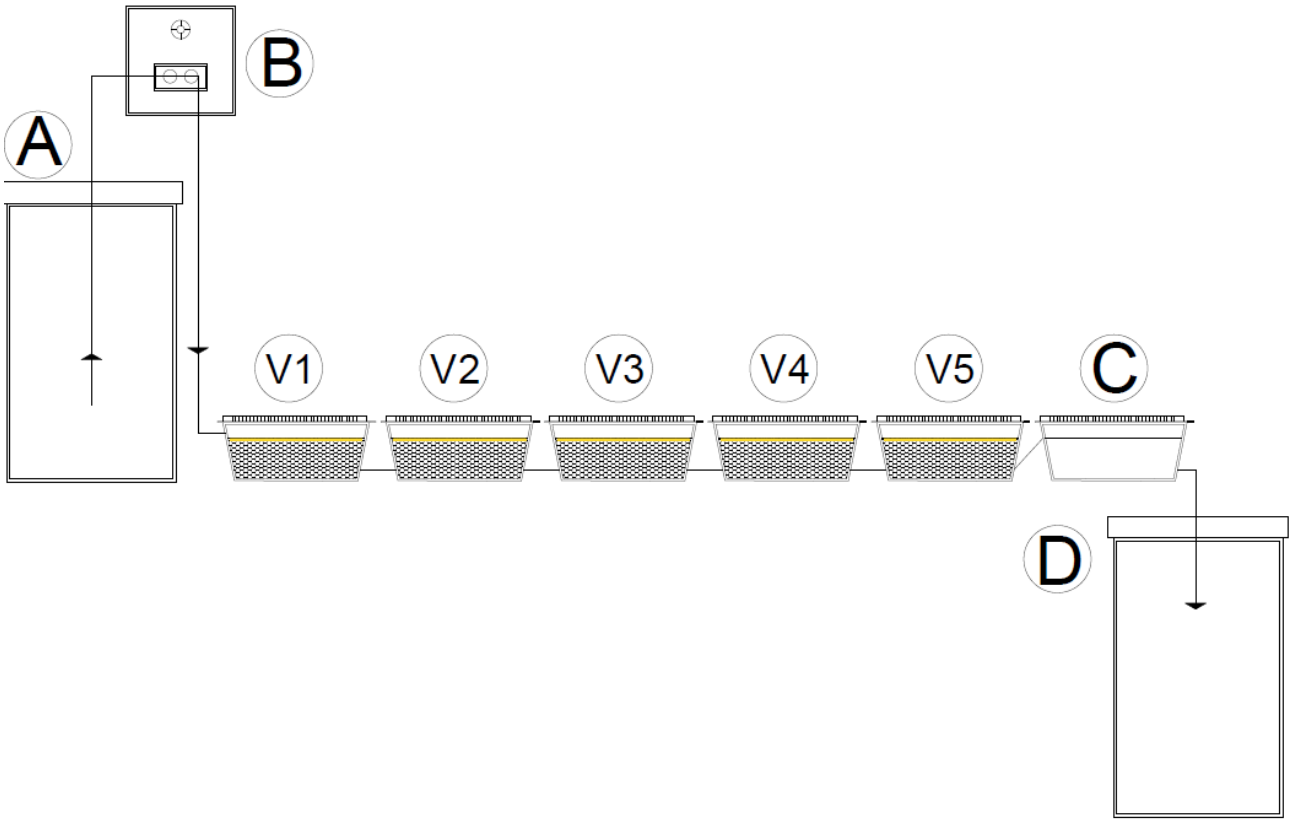


Fig. 10 – Continuous pilot test "trial" flux scheme: A: storage tank wwIN, B: peristaltic pump for ww dosing, V1..V5: reactor boxes, C: sampling box, D:storage stank wwOUT.

At t=0, larvae were placed inside the trays filled only with plastic support.
The storage container was filled with 2 000 ml of leachate, and the peristaltic pump was set, timed for 8 doses/day, each with a flow rate of 53 ml, for a daily dose rate of Qd= 426 ml/d.
All tests were conducted into a thermally insulated room under the same environmental conditions suggested by Grossule and Lavagnolo (2020): temperature range 25-30 °C; light/dark photoperiod of 18/6h.
Leachate was sampled 6 times in 14 experimental days and analyzed for TOC, COD and BOD_5 concentration.

Larvae were collected 4 times in 14 experimental days, washed, weighed individually with an analytical balance and returned to the box.
Larval development and substrate consumption were monitored by measuring the following parameters: average wet weight of larvae, growth kinetics, substrate removal, and average daily consumption of TOC and COD.

2.2.2 Liquid waste quality

The quality of the liquid waste used is reported in table 3:

Parameters	Synthetic artificial leachate
	H-S
pH	8
TOC (mgC)	3760
COD (mgO2)	13100
BOD_5 (mgO2)	4489
VFA (as CH_3COOH)	1241
N - Organic	401
$N\text{-}NH_4$ (mg/L)	3197
N-TKN (mg/L)	3598
Cl^- (mg/L)	9350
Ca (mg/L)	87,3
Na (mg/L)	3233
K (mg/L)	1497
Mg (mg/L)	65,3
BOD_5/COD	0,34
TOC/COD	0,29
BOD_5/TOC	1,19

Tab. 3 – Liquid waste quality

2.2.3 Analytical methods

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 B2). VFA were analysed by acid titration between pH 5 and 4.4. Ammonia nitrogen was measured with a distillation-titration procedure and TKN was measured through a distillation-titration procedure after an acid digestion phase.
Metals were measured using an ICP-OES analyser.

2.3 RESULTS AND DISCUSSION

2.3.1 Technical issues

The test was stopped on day 15, the period when the plant came up to steady state. During the course of the test, however, technical issues occurred, confirming the usefulness of a "trial" in order to optimize materials and working methodologies. In particular, the main issue encountered was dealing with the attempted escape of the larval population from the reaction tanks; they bypassed the nets put in place to protect the connecting pipes, with subsequent clogging caused by the larvae themselves and the support material dragged with them. The clogging caused the rise of the first V1 basin to rise, causing all the larvae in it to drown and the effluent leakeage from the holes applied on the lid to ensure aeration of the basin.
The technical arrangements that resulted from this first experiment were:

- Create a more effective inlet and outlet hole protection system.
- Provide more aeration space, that is, a greater distance between the substrate surface and the reactor lid. This will have a positive repercussion both for larval development, aided by greater oxygen recirculation, and to prevent larvae from spreading over the surface of the nonwoven fabric, causing it to deteriorate.
- Larger connection hole diameters, as, working with a reduced liquid head, pressure drops increase and with it the risk of deposits in the pipes. In addition, increasing the diameter of the passage holes decreases the possibility of clogging by larvae getting past the protection systems.
- The hydraulic tightness of connections should be improved, avoiding the use of siliconized closures that make the system one-piece.

2.3.2 Larval mass development

Three larval weighing operations were carried out, resulting in the data expressed in the graph (figure 11):

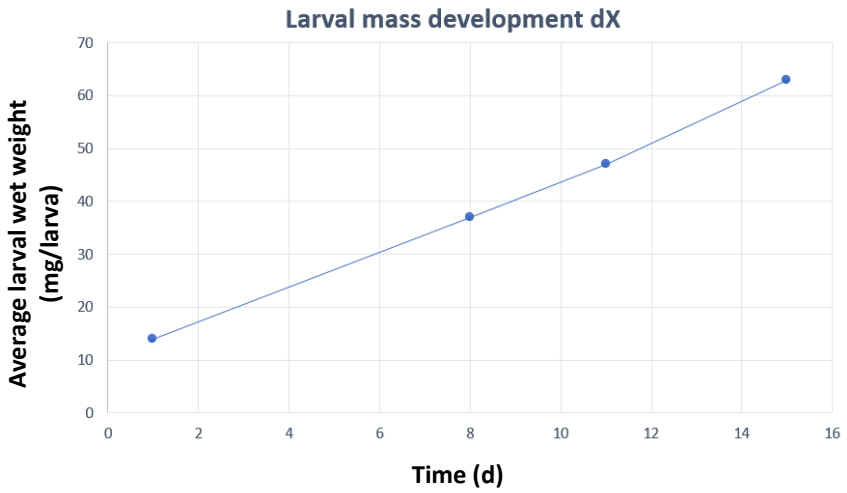


Fig. 11 – Average larval mass development during pilot test "trial".

The graph demonstrates how the larval development monitored in the pilot test "trial" verified the design data from the previous tests. In fact, if we compare larval mass of the three tests examined - Grossule & Lavagnolo (2021), Voltabarozzo research team (2022), "trial" test -, after 16 days we find, respectively, X_{16d}= 60 mg/larva, X_{16d}= 55 mg/larva, X_{16d}= 63 mg/larva, symptom that, from the

point of view examined, the continuous feeding did not create different situations from the previous batch tests.
Having connected the reactors in series, increasing values of Xi were monitored from V1 to V5, as shown in figure 12:

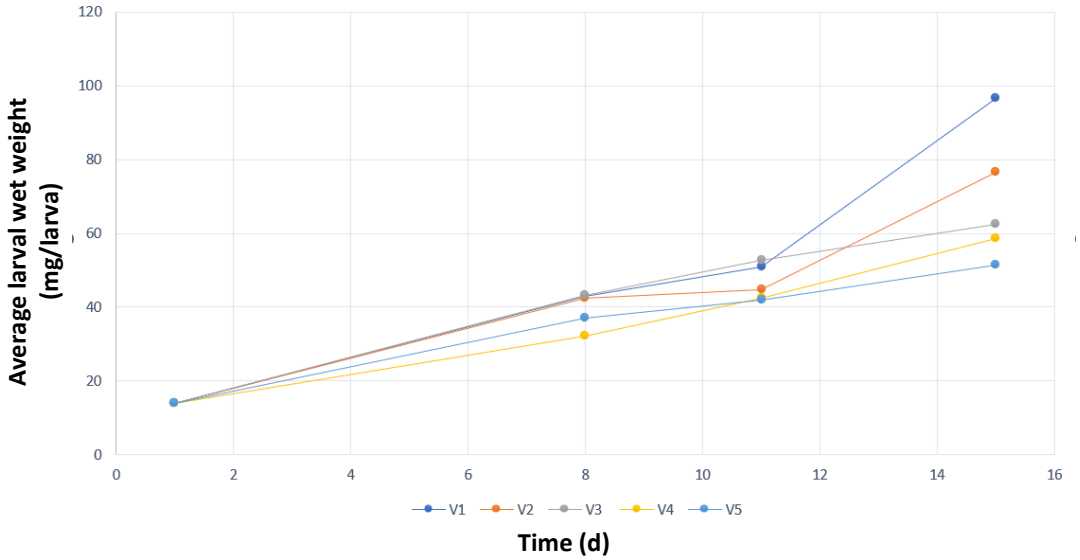


Fig. 12 – Larval mass development during pilot test "trial" from V1 to V5.

In fact, the plant receives a gradually decreasing F/L organic load from V1 to V5. The mass larval development graph gives us information about the health status of the larval population, which, in other words, results in a indication on the best F/L provided. If, for example, we found net mass increase dx=0 in the last tank, it would mean that wastewater coming in the last stage is already degraded or with a concentration below the minimum larval treatment threshold. In this case, in each tank dX was > 0.
Taking into account maximum larval mass, it was found a Xmax= 165.9 mg in the weighing on 22.07, in tank V1. This value, obtained after only 15 days, denotes excellent larval growth, a sign that the liquid habitat may be favorable for their development.

2.3.3 Substrate removal performance

The test was sized from the specific substrate removal of vSTOC = F/L= 2 mgTOCremoved/larva/day, assuming 100% removal. The design data were not verified, showing an overestimation of larval population removal performance, as summarized in table 4 and demonstrated in figure 14 and 15.

SAMPLING	**Sout** [mgTOC/L]	**vSTOC** [mgTOCremoved/lar/day]	**Sout** [mgCOD/L]	**vSCOD** [mgCODremoved/lar/day]
11.07	2980	0,415	9900	1,702
12.07	2760	0,532	9800	0,744
13.07	2690	0,569	9600	1,861
15.07	2570	0,633	9000	2,180
18.07	2460	0,695	8580	2,404
19.07 = steady condition	2400	0,723	7860	2,787
AVERAGE	2643	0,594	9123	1,946

Tab. 4 – "Trial" test output parameters

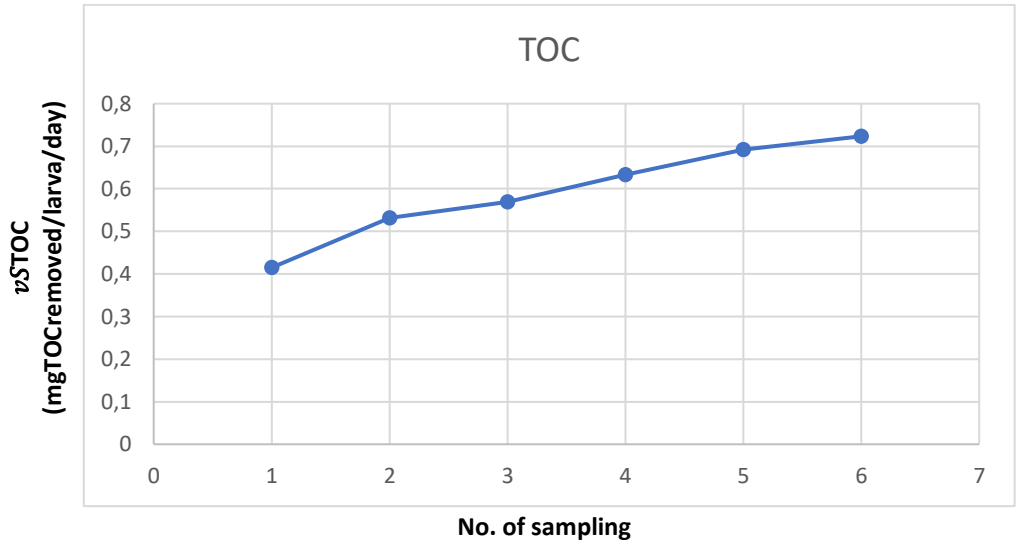


Fig. 13 – "Trial" TOC substrate removal rate

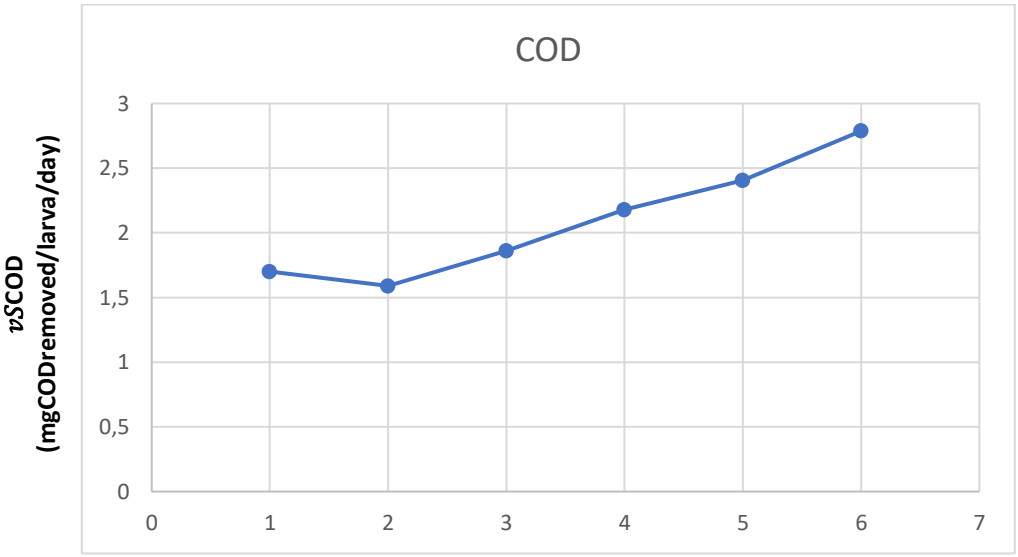


Fig. 14 –"Trial" COD substrate removal rate

The results show an average specific TOC and COD removal vSTOC= 0.594 mgTOCremoved/larva/day, vSCOD= 1.946 mgCODremoved/larva/day, respectively; the obtained removals do not confirm the design data (vSTOC = 2 mgTOCremoved/larva/day); this result can be explained by the fact that we compare two test imposing same substrate removal rate starting from different F/L, i.e., in the two tests, larvae were not provided with the same "food" per capita. In addition, literature data on vSTOC were probably overestimated, as they considered other side phenomena such as bacterial oxidation, adsorption and precipitation.

The total removal efficiency, in percentage, was 30%, both in terms of TOC and COD, a similarity that confirms the validity of the data obtained. The effluent came out with an average TOC concentration STOCout = 2643 mg/L compared to a STOCin= 3760 mg/L. In terms of COD, SCODout= 9123 mg/L, compared with a SCODin= 13100 mg/L.

2.4 CONCLUSIONS

Finally, from the semi pilot continuous "trial" test, we can draw the following conclusions:

- Important technical arrangements were noted in view of the final pilot plant, including the choice of appropriate diameters for passage holes, more effective anti-intrusion systems, and increased aeration space to create the most comfortable habitat possible for larval population.
- From the point of view of larval development, data from previous tests were confirmed, with similar larval growths after 16 days, respectively, X_{16d}= 60 mg/larva, X_{16d}= 55 mg/larva, X_{16d}= 63 mg/larva for Grossule & Lavagnolo (2021), Voltabarozzo research team (2022) and "trial" test.
- As for the maximum larval mass, $Xmax$= 165.9 mg was found, which attests an excellent growth environment.
- From the standpoint of substrate removal, against a predicted vSTOC of 2 mgTOCremoved/larva/day, a vSTOC= 0.594 mgTOCremoved/larva/day was found; in terms of COD, vSCOD= 1.946 mgCODremoved/larva/day.
- This overestimation of removal data suggests that F/L vs vS correlation for this particular type of wastewater should be studied in future sizing.

3. “BIG BROTHER” TEST PAPER

3.1 RESEARCH PROGRAM

The "Big Brother Test" aimed at studying the behavior of larvae within a solid support material fully saturated with liquid substrate to determine their maximum immersion depth. From previous tests, it was difficult to figure out how much liquid headroom could be exploited within the reactor, assuming that larvae would only take advantage of the first 1-2 cm of available effluent. Determining the parameter Hmax was of paramount importance, as it allowed a sizing constant to be set, making equivalent to talk about surface larval density Cls and volumetric larval density Clv, just multiplying the former by the height - constant - of the reactor. Compared with traditional biological treatments, where the reactor height can vary within a certain range of values, being able to say that the liquid head inside the trays is constant is a major design simplification for BSF larvae treatment. It also fits within the context of uniformity and interpretability of parameters already mentioned in the introduction of the thesis. The variables included within this test were two:

(a) The support material (Vaalox, Kaldness, Geomat).
(b) The Cls surface larval density (2, 4, 8, 16 lar/cm^2).

In this way, useful data could be recorded to cross-reference with tests from other team experiments, specifically comparing the various Hmax obtained with the larval removal and development performance obtained by colleagues. Doing so provided a range of information useful in choosing the best solid media and larval density to use in the pilot plant. It was important, therefore, to determine the influence of the two variables on the obtained Hmax.

3.2 MATERIALS AND METHODS

The test was carried out using N.4 PIREX glass laboratory as vessels, size: diam. 5 x 15 H cm, area 78 cm^2, filled with N.3 different support materials:

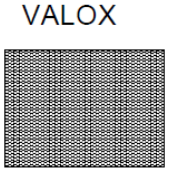
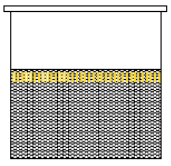
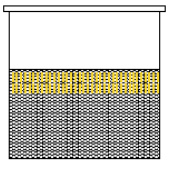
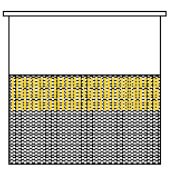
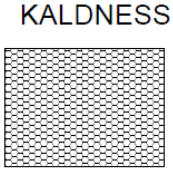
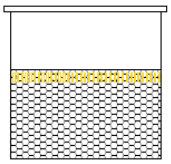
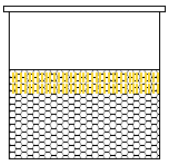
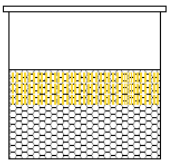
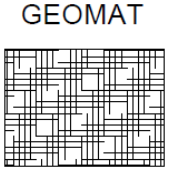
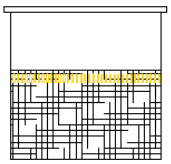
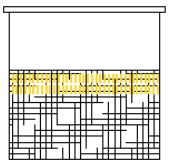
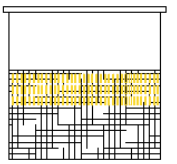
- VAALOX plastic filling material (2-3 mm diam.) (figure 15 A).
- KALDNESS plastic backfill material (4 mm diam.) (figure 15 B).
- GEOMAT backfill material. (figure 15 C)

Fig. 15 A Filling material VALOX – Fig. 15 B Filling material KALDNESS – Fig.15 C Filling material GEOMAT

These 4 containers were placed on top of a table, in front of a white sheet of paper to highlight the image, filled with the selected filling material for 8 cm, leaving 8 cm for aeration of the larvae. Later, this material was completely saturated with milk diluted 1.10 with distilled water. Each container

received the same amount of diluted milk since, at this stage of experimentation, the specific organic load per larva was not taken into account. Inside the vessels were placed 6-day-old larvae, average weight 14.5 mg, with Cls of 2, 4, 8, 16 lar/cm^2. Accordingly, beacuse of the containers fixed area of 78 cm^2, the No. total larvae per container were: 156, 312, 624, 1248. They were covered with nonwoven fabric attached by elastic to the edge of the vessel.
Monitoring was done by video camera installed in front of the containers, capable of producing "time lapse" videos, sped up to 29 fps. Monitoring occurred, for each type of support material, for 48 hours.

Test modality are shown in table 5:

Cls / FILLING MATERIAL	2 LAR/CM2	4 LAR/CM2	8 LAR/CM2	16 LAR/CM2
VALOX				
KALDNESS				
GEOMAT				

Tab. 5 – "Big Brother Test" setup.

3.3 RESULTS AND DISCUSSION

Video monitoring shows that, initially, regardless to the Cls and support material used, larvae attempted to leave the vessel, distributing themselves mainly in the dry walls separating the liquid substrate from the nonwoven cover. After an initial acclimation period, larval population begins to settle on the surface of the substrate, starting to become familiar with the new habitat.
Preferring the dark environment and being on the lookout for food, they begin to deepen, fairly evenly, for the first centimeter of substrate. At this stage, the first differences, albeit slight, between the different monitored situations start to be noticed. When analyzing only the different Cls, it is immediate to note that, with increasing larval concentrations, attempts to deepen the larval population increase, as they are physically searching for their living space and, at the same time, they found less food per capita. Another important observation is that, despite testing far higher larval concentrations than in all previous tests, the response of the maximum population (1248 larvae) was comparable to the minimum (156 larvae): in fact, no particular signs of discomfort were noted in any vessel,

particularly those with the highest number of larvae. This is a sign that high larval concentrations could be use in final test without risking compromising their habitat.
Evaluating the support material, again the first differences, albeit small, are seen in the post-acclimation phase, where the larval population goes in search of food. Due to higher vacuum index, the best performance seems to be achieved by KALDNESS, which allows larvae to deepen while making less effort, thanks to the various pathways made available by the numerous burrows created by this type of material. In addition, the particular shape seems to create voids in which microbubbles of air are generated, thus hypothesizing that the larvae can find trapped oxygen even at depth.
However, there are no scientific data to support this thesis.
In contrast, the support material GEOMAT, with its lattice structure, gave the worst performance as larvae had difficulty exploiting it as a medium, finding themselves mostly submerged in the liquid, wich resulted in a bad living environment.
By monitoring Hmax, the main parameter of the research purpose, analyzing the video contents we can state that most of the larval population settles in the first 4 cm of substrate. Although attempts at deeper penetration could be seen, they can be traced back to occasional episodes caused by the formation of preferential pathways. Therefore, it is not possible to consider them suitable for an assessment of the maximum height of a treatment reactor, as there would be a risk of overestimating the action of the larval population, creating areas of untreatable substrate.
Monitoring images are proposed below:

Fig. 16 – Video frame in wich we could notice the Xmax = 4 cm.

3.4 CONCLUSIONS

The conclusions deduced from the analysis of the results of the "Big Brother Test" will be presented and summarized below:

- No substantial differences were noted about the behavior of the larval population when varying the three filling materials used, evaluating their deepening and adaptation to environmental conditions. KALDNESS material gave the best responses in terms of deepening. GEOMAT gave the worst performance because larvae had difficulty exploiting it as a medium, finding themselves mostly submerged in the liquid, wich resulted in a bad living environment.
- No particular criticalities were noted in exponentially increasing the Cls larval density inside the vessels.
- The maximum depth Hmax in which most of the larval population is stationed is estimated at 4 cm; this Hmax will be used as a sizing parameter for the pilot plant.

4. CONTINUOUS PILOT PLANT TEST PAPER

4.1 RESEARCH PROGRAM AND OBJECTIVES

The final result of the experimentation was the construction of BSF larvae continuous wastewater treatment pilot plant.
Three plastic tanks were connected in series, filled with larvae, plastic support material and diluted milk with increasing flow rates according to the research program that will be outlined later, for a daily treatment flow rate Qd varying between 5 and 50 L/d.
The first objective of the research was to make the plant structurally solid, taking advantage of the results obtained from "trial" test. The diameter of the passage holes, the distance between the substrate and the reactor cover for air circulation, and the larvae anti-intrusion systems were increased, but the structure and flow pattern remained unchanged from the first continuous trial. The KALDNESS plastic support material was chosen based on the performance obtained from "Big Brother Test," along with the height of liquid head Hmax= 4 cm. In addition, prepupal shelters were added by drilling holes on the vertical part of the second tray and connecting an L-shaped outer tube. The idea was to create a dark and dry environment, following the literature indications, which suggest that prepupae autonomously seek a habitat with these characteristics to settle and continue their path to become flies. The danger of this tube turning out to be a "trap" was avoided since, not finding food in the tube, they would return to the substrate-rich reactor. This shelter structure was fitted with a cap at the bottom so as to make it convenient to extract the prepupae. It should be noted that this "nursery" function was not discussed in the thesis, but it represents a small step forward in treatment design according to the philosophy of Circular Economy.
For techno-economic reasons, the liquid substrate was changed from the "trial" pilot plant, as it would not have been possible to produce more than 2 L/d of the leachate previously used. The choice therefore fell on milk, as it had already been tested in the "Big Brother Test," economically accessible and traceable to a dairy effluent, in order to make the experimentation as likely as possible to a real case.
From larval development point of view, although there were no literature data on diluted milk, the objective of the research was to verify that the weight changes obtained traced those of previous trials conducted on effluent with similar quality characteristics. Specifically, milk diluted to 1.10 exhibits similarities in terms of TOC and COD concentrations with the leachate used in the pilot plant "trial," as discussed in the next chapter. As in the previous continuous pilot test, dX and Xmax larval development parameters were monitored.
From the standpoint of substrate removal efficiency, again there were no literature data to verify. The "trial" test yielded a steady-state vSTOC= 0.72 mgTOCremoved/larva/day, compared with an F/L= 2 mgTOC/larva/day. The research strategy focused on obtaining as much data as possible that related specific substrate removal vS and specific organic load F/L; then, study a correlation between these two parameters so that future plants could be sized by predicting the desired output data by entering known input parameters. In this direction, an analysis was conducted that could extrapolate a relationship giving vSTOC as OUTPUT, entering η as INPUT. Three more batch trials were set up, fed with milk diluted to 1.10, in which F/L was varied by entering larval populations with Cls of 4, 8, 16 lar/cm^2, respectively, to have sufficient data supporting the interpolation,. The vS related to each trial were monitored, with the following results (in which those from the "trial" trial were also included) (table 6).

TEST	Cls [lar/cm^2]	F/L [mgTOC/larva/day]	$vSTOC$ [mgTOCremoved/larva/day]	η
"Trial"	1,1	2	0,720	0,36
Batch 1	4	1,43	0,660	0,46
Batch 2	8	0,71	0,430	0,61
Batch 3	16	0,2	0,187	0,94

Tab. 6 – Final test fitting values

Data interpolation resulted in two relationships between the parameters studied. The first, which is a linear one, compares $vSTOC$ and η (figure 17); the second, which follows Michaelis Menten's law, describes the trend of $vSTOC$ as a function of F/L (figure 18):

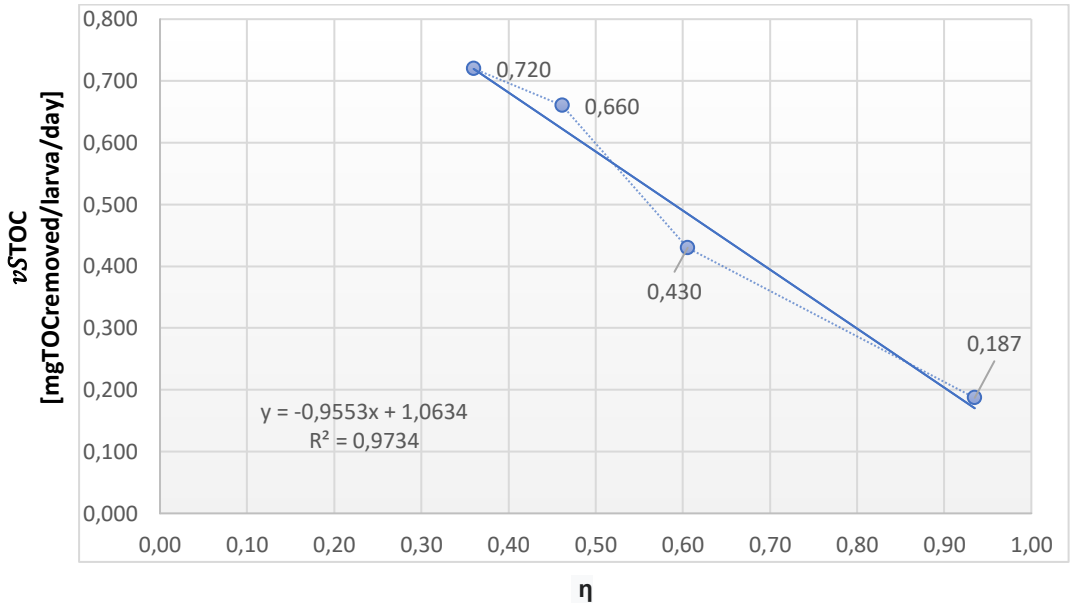


Fig. 17 – $vSTOC$ vs η values and fitting equation.

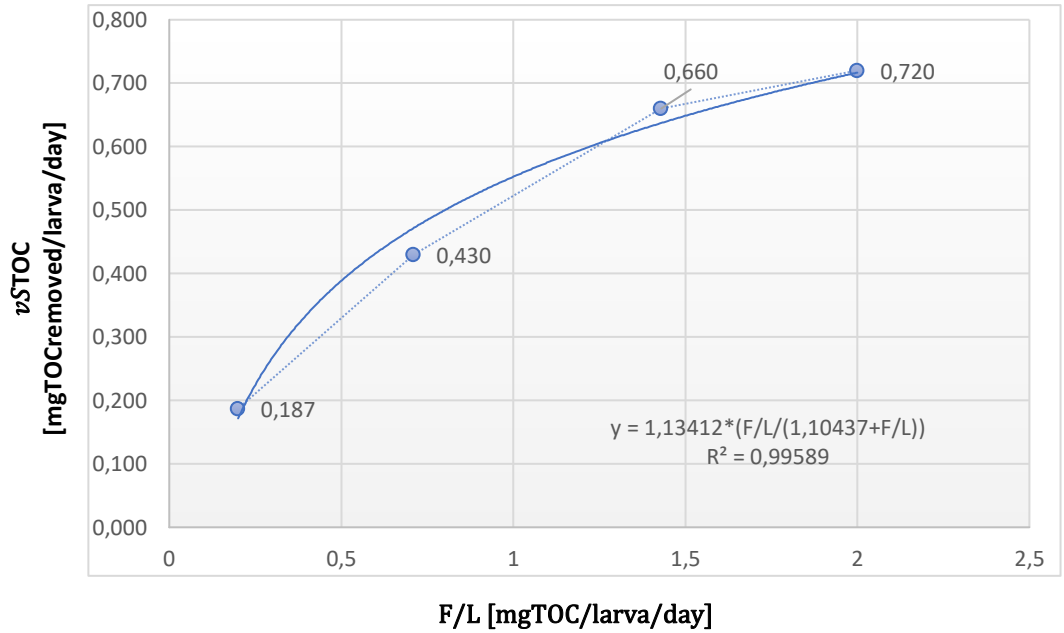


Fig. 18 – F/L vs $vSTOC$ values and fitting equation.

The results of this fitting proved to be excellent, showing a linear correlation between specific substrate removal rate vS and removal efficiency η. Studying the relationship between vS and F/L, it was hypothesized to follow Michaelis Menten's law, as literature:

$vs = vsmax \frac{F/L}{km+F/L}$ where:

- $Vsmax$: max substrate removal velocity [mgTOCremoved/lar/day]
- Km: semi-saturation constant [mg/L]

Altough the INPUT values came from different effluents, the R^2 found in the two equations of 0.9734 and 0.99589, respectively, shows that the fitting has a very high statistical significance, confirming that the interpolations can be used for future sizing.
Accordingly, the sizing scheme used for the pilot was updated by inserting the correlations between F/L, vS and η obtained, so that all other sizing parameters such as HRT, Qd_{in} could be obtained according to the new relationships.
The total volume of the reactor, as in the case of the "trial," was decided a priori, multiplying the surface area of the available tanks by the Hmax obtained from the "Big Brother Test", as well as the Cls, choosing Cls= 16 lar/cm^2 in order to achieve the maximum removal rate.

In the first phase of the test, the research objective was to confirm that the system was working from a technical hydraulic point of view, to monitor that larval development was suitable for the premises, and to verify that the design data for specific substrate removal were consistent with those previously obtained. We started by setting a removal efficiency of 95% as the starting INPUT data. Than we first derived the vS via the first linear interpolation and, at the same time, the Sout assumed. Having vS and Sout, the HRT retention time, Qd and, finally, F/L were calculated.
The sizing scheme is shown in figure 19.

SIZING INPUT	TECHNICAL OUTPUT	SIZING OUTPUT
Si [mg/l]	Vtot [ml]	vS [mgTOCremoved/lar/d]
2750	15000	0,1559
Removal efficiency n	Vtot [L]	DeltaS [mg/l]
0,95	15	2612,5
Cls [larve/cm2]	No. Lar x box [lar/box]	Sout [mg/L]
16	27200	137,5
Hmax [cm]	Clv [larve/ml]	HRT [day]
4	5,44	3,08
TECHNICAL INPUT	Clv [lar/L]	HRT [h]
No. boxes	5440	73,95
3	Tot. no. Larvae	Qd [L/d]
V ww/box [ml]	81600	4,87
5000	Tot. Larval mass [mg]	TOC load (mg/d)
Box surface [cm2]	1060800	13388
1700	Tot. Larval mass [g]	F/L [mgTOC/lar/d]
Xi [mg]	1060,8	0,164
13	Tot. Vww [L]	F/Lm [mgTOC/mglar/d]
No. Days	97	0,013
20		

Fig. 19 – 1st phase sizing model.

In the second phase of the test, it was decided to increase the specific organic load F/L by 10-times, while increasing the inlet flow rate Qd, because of two reasons. First, to expand the range of data available that related F/L, vS and removal efficiency, in order to improve interpolations fitting. In addition, it was considered important to collect data for high organic matter loads, in order to have informations about both low- and high-load performance, as in traditional activated sludge plants. In

addition, since it has been shown that the inlet substrate concentration S is not a factor affecting removal rate, increasing the loading by 10 times means collecting data for a wide range of different effluents.
In this case, the initial INPUT data was F/L (multiplying by 10 F/L from the first step). Consequently, the sizing logic also changed, thus starting from F/L to obtain vS via Michaelis Menten interpolation; From vS, the removal efficiency (vS/F/L) was derived and successively the other parameters.
The sizing scheme then became as exhibited in figure 20.

SIZING INPUT	TECHNICAL OUTPUT	SIZING OUTPUT
Si [mg/l]	Vtot [ml]	vS [mgTOCremoved/lar/d]
2750	15000	0,6777
F/L [mgTOC/lar/d]	Vtot [L]	Removal Efficiency n
1,640	15	0,41
Cls [larve/cm2]	No. Lar x box [lar/box]	DeltaS [mg/l]
16	27200	1136
Hmax [cm]	Clv [larve/ml]	Sout [mg/L]
4	5,44	1614
TECHNICAL INPUT	Clv [lar/L]	HRT [day]
No. boxes	5440	0,31
3	Tot. no. Larvae	HRT [h]
V ww/box [ml]	81600	7,40
5000	Tot. Larval mass [mg]	Qd [L/d]
Box surface [cm2]	1060800	48,66
1700	Tot. Larval mass [g]	TOC load (mg/d)
Xi [mg]	1060,8	133824
13	Tot. Vww [L]	F/Lm [mgTOC/mglar/d]
No. Days	973	0,126
20		

Fig. 20 – 2nd phase sizing model.

4.2 MATERIALS AND METHODS

4.2.1 Equipment and growth conditions

The test consists in three trays used as the reactor of the larval BSF treatment, each consisting of a platic box (45 cm x 38 cm x 13cm), containing plastic support material (KALDNESS®, 4-6 mm in diameter) completely saturated with 5 000 mL of diluted milk each.. A Cls of 16 lar/cm^2, 27 200 larvae per box, 6 days old and average Xi weight of 13 mg, were inserted. The filling material allowed larvae to move freely in the liquid substrate to meet their needs (feeding and respiration).
Each box was covered with a permeable nonwoven fabric (to prevent oviposition by other flies) and a perforated plastic lid (to allow air recirculation) (figure 21).

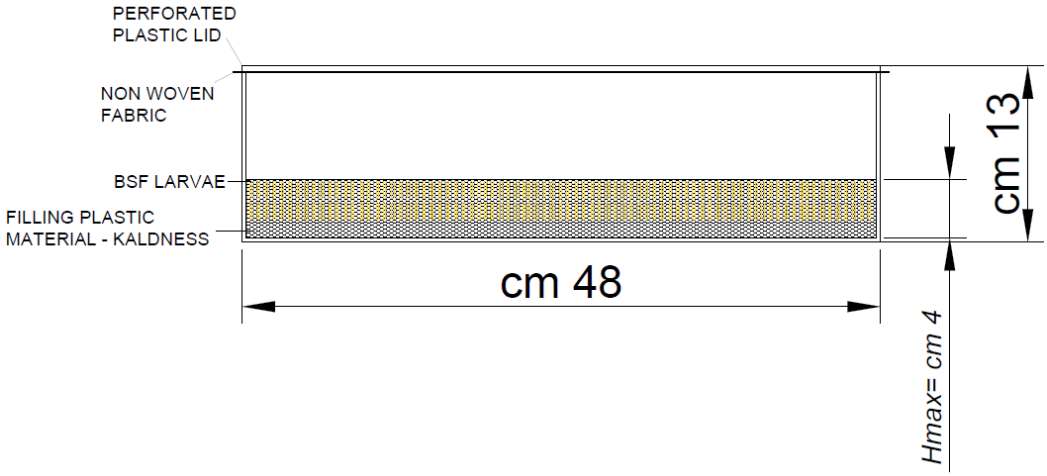


Fig. 21 –Pilot plant box scheme.

In the first phase of experimentation, following flow sequence, the effluent was stored in 30 L volume tank, from which the peristaltic pump sucked the diluted milk with daily flow rate Qd= 5 L/d, divided into 10 daily dosages of duration equal to 15 min and flow rate equal to 500 ml.
Proper suction of the effluent was ensured by attaching the suction line to a rigid rod immersed in the liquid, so that it always occurred from the bottom of the tank to prevent it from working in vacuum.
The delivery line of the peristaltic pump was connected to the first tank via a 3/8 inch PVC hose holder with, inserted into the vertical wall via a hole with a hole cutter, and secured by screwing a sleeve of the same diameter internally. An anti-intrusion larval net was applied at the head of the sleeve and secured by clamping. This scheme was repeated for the series connection of all the trays. Hydraulic tightness was ensured by inserting a double seal, both inside and outside the tray, between the hose holder and the sleeve (figure 22 A). The three trays were connected from the bottom, with the outlet pipe resting on a wooden support calibrated to ensure an Hmax = 4 cm inside the reactor (figure 22 B) with the effluent discharged into a tank of volume equal to 20 L.

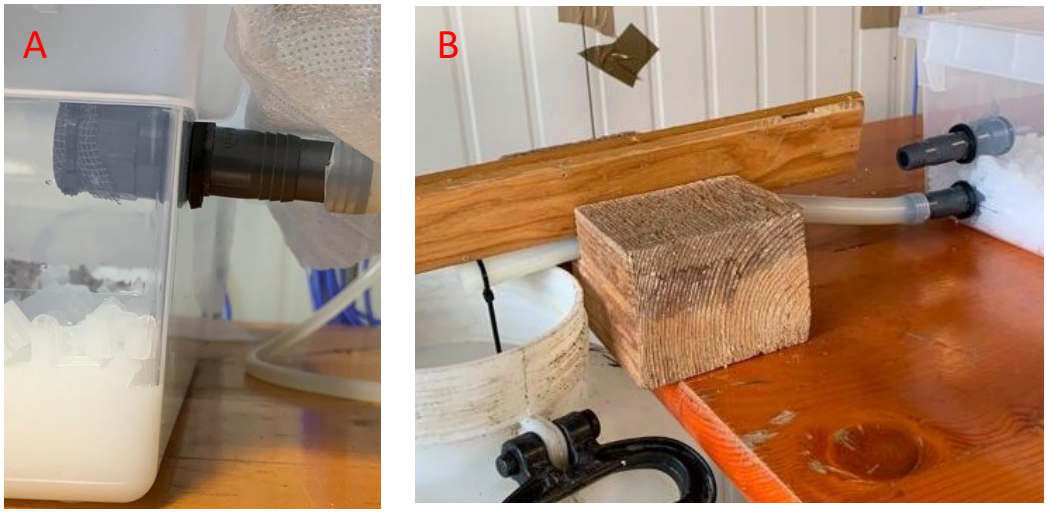


Fig. 22 A– Inlet connecting pipe fittings– Fig. 22 B Exit piping.

In the second phase of experimentation, the daily inlet flow rate was increased from 5 to 50 L/d, with the intention of increasing the F/L load by 10 times; in this case, the storage volume was moved through the use of a second peristaltic pump whose function was to recirculate the milk internally to the storage tank to avoid lump formation and make the inlet substrate as homogeneous as possible. The flow diagram of the pilot plant is proposed below figure 23:

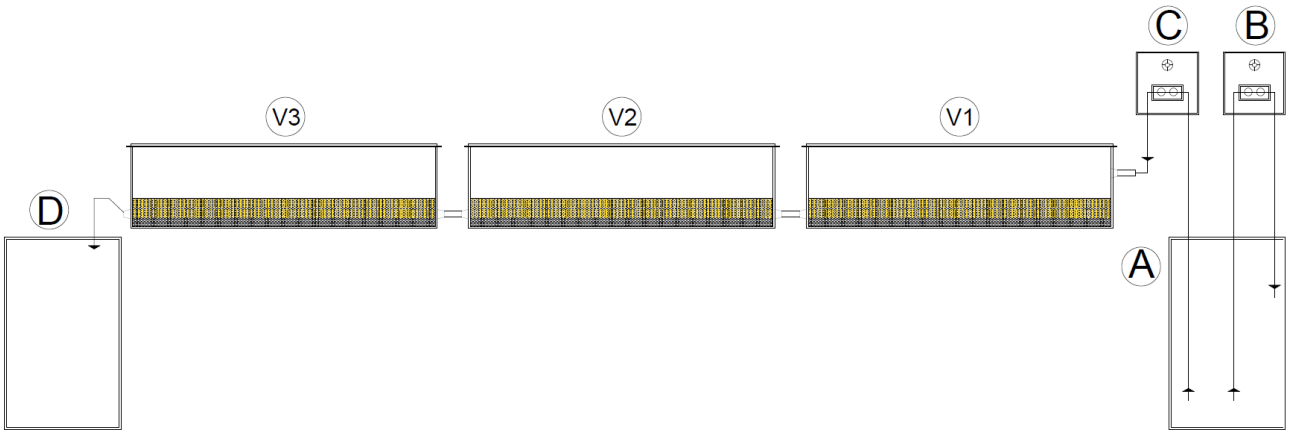


Fig. 23 – Continuous pilot test flux scheme: A: storage tank wwIN, B: peristaltic pump for storage mixing, V1..V3: BSF reactor boxes, C: peristaltic pump for ww dosing, D:storage stank wwOUT

Regarding sampling and weighing operations, the test was designed with a duration of 25 days, 15 dedicated to the first phase, 10 to the second phase. 4 samplings and 3 weighings were carried out in the first phase, 3 samplings and 3 weighings for the second phase. The first week was not investigated as it is evaluated as acclimatization phase. Concerning weighing operations, 50 larvae were extracted from each tray; they were then washed and dried, before all 50 were weighed to assess larval development dX, then weighed 10 individually to identify Xmax.

4.2.2 Liquid waste quality

The quality of the liquid waste quality used in this test is reported in table 7:

PARAMETERS	MILK 1.10
TOC [mg/L]	2 750
COD [mg/L]	15 300
BOD_5 [mg/L]	13 800

Tab. 7 – Diluted milk quality parameters

4.2.3 Analytical methods

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 B2).

4.3 RESULTS AND DISCUSSION

4.3.1 Technical issues

From a technical point of view, the assumptions outlined in the research program were confirmed. The three tanks held hydraulically, as did the connecting piping. The decision to increase the piping diameter proved successful and helped to avoid clogging problems verified in the "trial" test. In this regard, the anti-intrusion structures also worked, blocking the escape of larvae from every possible route. The outlet piping, adjustable in height as designed, ensured that the Hmax within the tanks could be set as hydraulic conditions and pressure drops changed. The mixing of the storage tank, generated by the second peristaltic pump, prevented stratification by sending homogeneous feeding to the system. The increased distance between the substrate and the nonwoven fabric created a more favorable habitat for the development of such a large number of larvae, ensuring adequate oxygenation and a comfortable living space.

4.3.2 Larval mass development

3 larval weighing operations were carried out for each test phases, resulting in data expressed in the graph (figure 24):

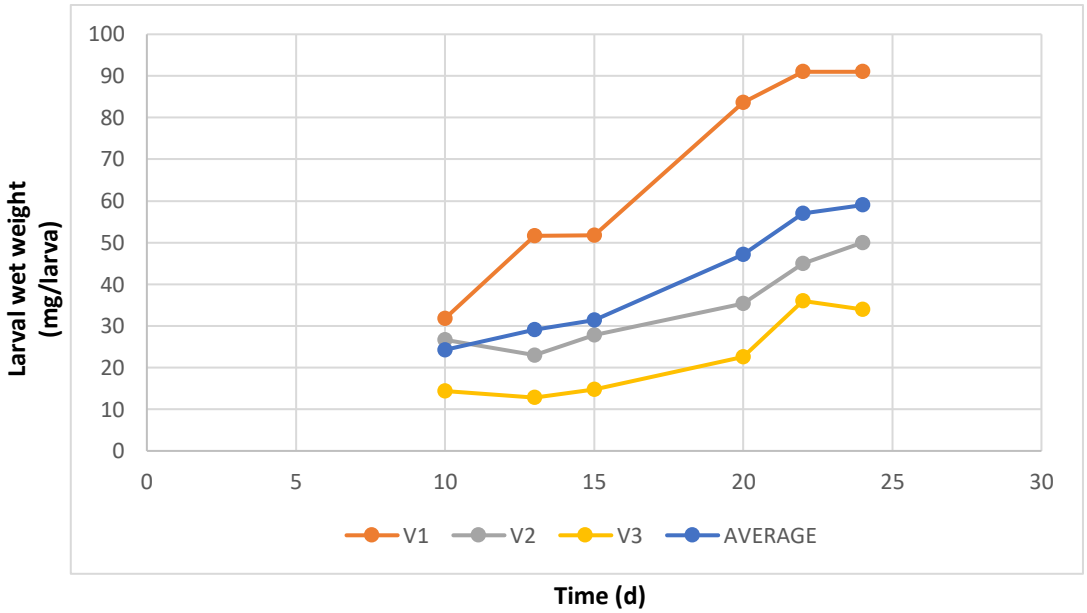


Fig. 24 – Larval mass development during final pilot test from V1 to V3 and average value.

The graph shows the evolution of the larval mass within the reactor, both in the specific data of the three trays and as an overall plant mean. The result does not seem to be in line with the data previously found; in fact, if we compare the X_{15d}= 30 mg/larva obtained with those summarized on p. 30, (X_{16d}= 60 mg/larva, X_{16d}= 55 mg/larva, X_{16d}= 63 mg/larva for Grossule & Lavagnolo (2021), Voltabarozzo research team (2022) and "trial" test (2022)), we can state that the average larval weight of the pilot test is about half of the previous ones. This result can be explained by two reasons; the first is that we worked with an F/L about ten times lower than the compared tests. In addition, the average larval mass was strongly influenced by the zero-trending growth rate found in the last two tanks in the first phase of the experiment.
As the graph highlights, in the first tank, receiving the highest F/L loading, larval mass growth followed the trend previously found, with an X_{15d}= 52 mg/larva.

After day 15, in the second part of the test, a net increase in larval mass can be seen in the last two tanks and graphically as well, concomitant with the abundance of food per capita generated by the 10-fold increase in input load.
This situation suggests that probably the best plant layout would have been with parallel feeding, ensuring the same F/L for the entire larval population, carrying forward a life cycle advance common to all tanks.
Taking into account maximum larval mass, it was found a Xmax= 211.2 mg in the weighing on 18.11, in tank V1. This value, obtained after 25 days, denotes excellent larval development, a sign that the liquid habitat may be favorable for their growth.

4.3.3 Substrate removal performance

The first stage of the test was sized by setting a removal efficiency of 95% and, consequently, a vSTOC= 0.156 mgTOCremoved/larva/day. Starting from an inlet concentration Si= 2750 mgTOC/L, a Sout= 137 mgTOC/L was expected.
Table 8-9 shows the Sout and removal efficiencies η found in the first phase of experimentation:

SAMPLING	**Sout [mgTOC/L]**	**removal efficiency η**	**vSTOC [mgTOCremoved/lar/day]**
02.11	257	0,91	0,149
04.11	145	0,95	0,1559
07.11	141	0,95	0,156
09.11	145	0,95	0,155
AVERAGE	172	0,94	0,154

Tab. 8. – 1st phase TOC output parameters.

SAMPLING	**Sout [mgCOD/L]**	**removal efficiency η**	**vSCOD [mgCODremoved/lar/day]**
02.11	1530	0,90	0,8215
04.11	1320	0,91	0,8341
07.11	1080	0,93	0,8484
09.11	760	0,95	0,8675
AVERAGE	1162	0,92	0,8429

Tab. 9. – 1st phase COD output parameters.

The average removal efficiency obtained was 94%, not confirming the 95% assumed but still achieving a very good result. The vSTOC= 0.154 mgTOCremoved/larva/day average obtained is slightly lower than expected, as it is the result of an initial sizing based on an interpolation performed on a limited number of data, resulting in possible approximation errors.

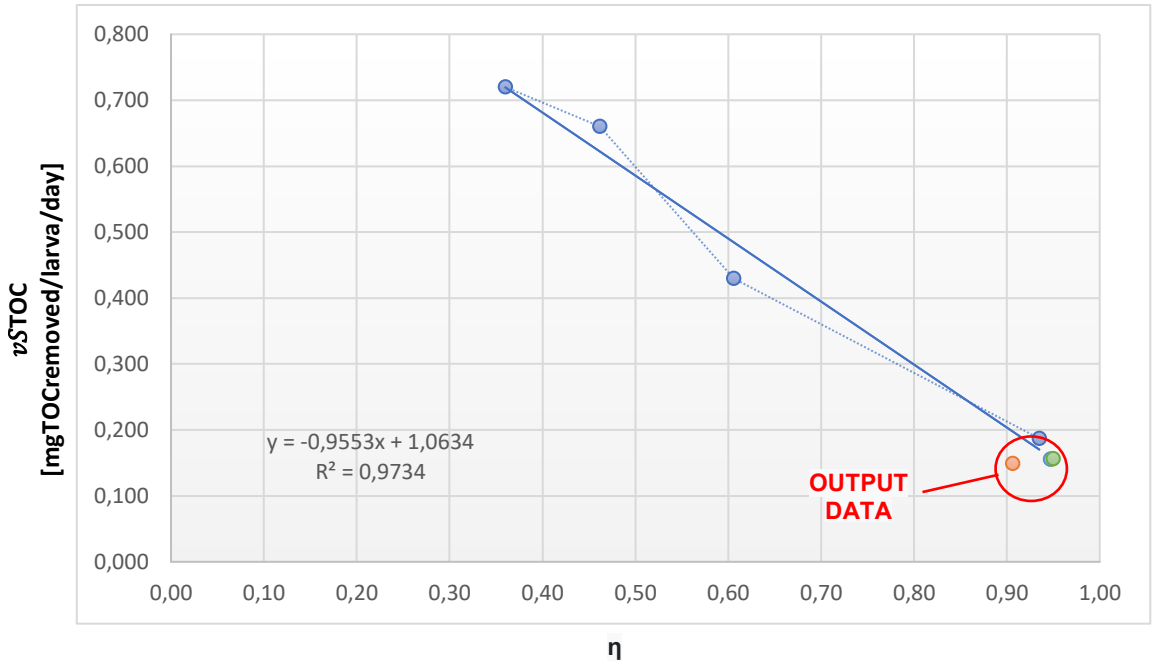


Fig. 25. – Interpolation data vs output data

The minimal distance between the obtained and predicted data, therefore, confirms the validity of the previous analysis that led to the construction of the first sizing scheme for continuous BSF reactors. Moreover, the results attest to this type of treatment a significantly higher organic substrate removal rate than other traditional treatments. If we consider, for example, the HRT retention time HRT= 3.1 d, we can state that it is at least 4-5 times lower than a conventional activated sludge biological treatment. In other words, in terms of organic matter TOC removal, the BSFL biological treatment system removes the pollutant 4-5 times faster than a traditional activated sludge.
The same conclusions can be reported in terms of COD, with an average removal found of 94% for this parameter.
Another aspect that can be extrapolated from the analysis of these first data is that BSF treatment suffers of a treatment threshold below which it is not possible to go; that is, as also found in previous tests, below a certain concentration of substrate S, larvae are no longer able to feed on the dissolved organic matter. This threshold limit probably varies with the type of effluent and suggests that it is difficult to expect removal efficiencies above 95 % for this type of treatment. This information may indicate the application target for this technology, which is unlikely to be able to disregard other treatments in order to discharge the effluent complying with law limits.

In the second phase of the test, the organic load was increased 10-fold to F/L= 1.64 mgTOC/larva/day. The sizing model thus calculated a vSTOC= 0.677 mgTOCremoved/larvae/day and a removal efficiency of 41%.
Table 10-11 summarizes the parameters monitored in the second phase of the test:

SAMPLING	Sout [mgTOC/L]	removal efficiency η	vSTOC [mgTOCremoved/lar/day]
14.11	1640	0,40	0.686
16.11	950	0,65	1.11
18.11	795	0,71	1.20
AVERAGE	1128	0,59	0,998

Tab. 10. – 2nd phase TOC output parameters.

SAMPLING	Sout [mgCOD/L]	removal efficiency η	vSCOD [mgCODremoved/lar/day]
14.11	9540	0.38	3.5607
16.11	5661	0.63	5.9587
18.11	4743	0.69	6.5261
AVERAGE	6648	0.57	5.3485

Tab. 11. – 2nd phase COD output parameters.

The first sampling seems to confirm the accuracy of the sizing model, as found in the first part of the test, achieving a TOC removal efficiency of 40%, compared to the 41% assumed. The second and third sampling, however, deviated from the predicted data, achieving a removal of, respectively, 63 and 69%.
Same conclusions can be drawn from the COD analysis, which also aligns with the calculation model in the first analysis and then exceeds expectations in the last two.
In the following graph we can visualize the comparison of the interpolated data against the output data, in the last testing period.

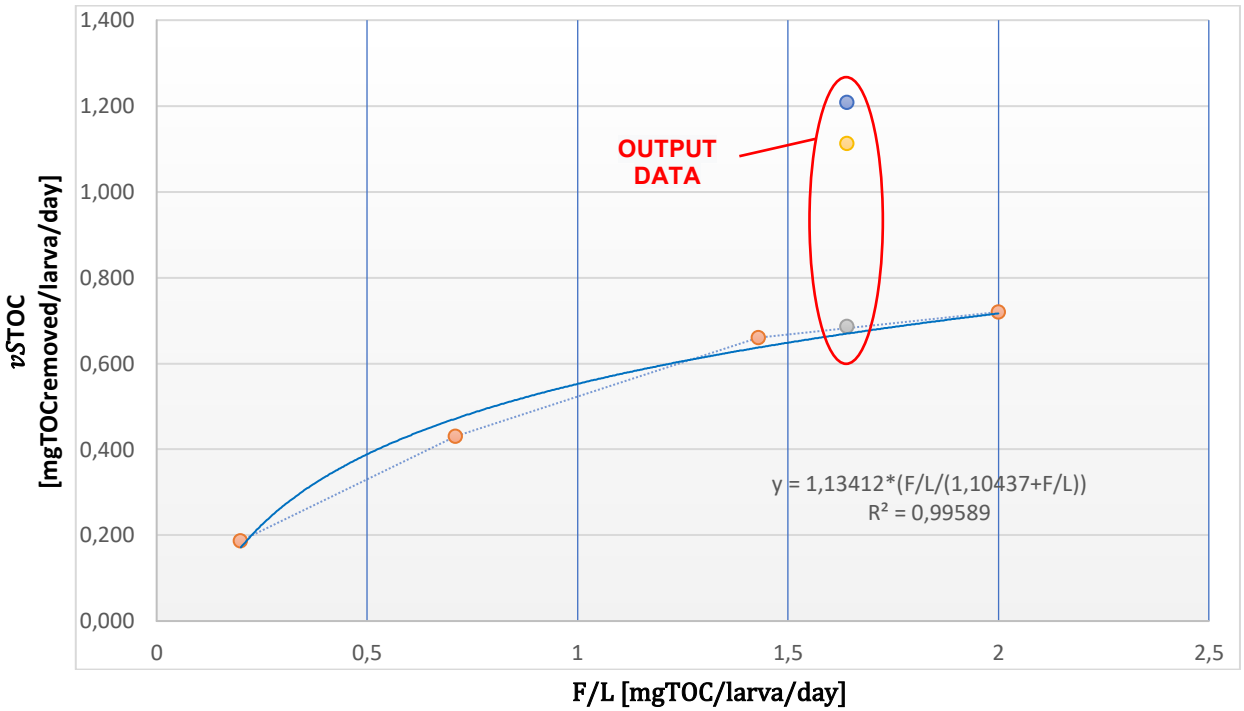


Fig. 26. – Interpolation data vs output data.

Although the data obtained denote a higher effluent quality output than expected, they do not satisfy the research program because of the significant deviation from the interpolation performed. The fact that the first point is perfectly in line with the premise raises the doubt that there were external agents influencing the test performance on the last sampling days. One hypothesis could be that issues related to the hydraulic flow of the effluent changed the retention times, granting larvae more time for degradation than predicted. Another hypothesis may be that the sudden increase in larval mass in the last two tanks has both related to an increase in voracity of the larval population resulting in a rise of specific removal capacity. Although it hardly explains a doubling of vSTOC in just two days, the correlation between Xi and vS is a finding that will be crucial to investigate in future studies.

Fig. 27. – Final pilot plant test with open lids.

4.4 CONCLUSIONS

Finally, from the continuous pilot plant test we can draw the following conclusions:

- From a structural point of view, the changes made in the practical implementation of the facility ensured proper operation. Problems related to hydraulic flows and larval habitat management have been avoided. Future studies will focus on increasing the scale of the reactor and implementing the "nursery" function.
- From larval mass development point of view of, data from previous tests were confirmed only for the 1st tray X_{15d}= 52 mg/larva, with similar larval growths after 15 days, respectively, X_{16d}= 60 mg/larva, X_{16d}= 55 mg/larva, X_{16d}= 63 mg/larva for Grossule & Lavagnolo 2021, Voltabarozzo team 2022 and "trial" test. This results suggest that the trays would may be connected in parallel in future tests to increase mass larval development in all the reactors.
- As for the maximum larval mass, Xmax= 211.2 mg, was found in the weighing on 18.11, in the V1 tank, which attests an excellent growth environment, comparable to solid waste feeding one.
- From the standpoint of substrate removal, taking into account low organic load, F/L= 0.164, sizing model worked properly, with high estimation accuracy. Against a predicted vSTOC of 0.1560 mgTOCremoved/larva/day and a η= 0.95, a vSTOC= 0.154 mgTOCremoved/larva/day and a η= 0.94 were found;
- In terms of COD, vSCOD= 0.8429 mgCODremoved/larva/day and a η= 0.92 were found, demonstrating that BSFL biological treatment remove the organic substances 4-5 times faster than activated sludge.
- Taking into account high organic load, F/L= 1.64, sizing model understimate larval specific removal rate. Against a predicted vSTOC of 0.677 mgTOCremoved/larva/day and a η= 0.41 a vSTOC= 0.998 mgTOCremoved/larva/day and a η= 0.59 were found.
- This results suggest that hydraulic problems may occur. Otherwise, another hypothesis could be that larval mass development heavily influenced vS; in this case, more data occurs to evaluate this phenomenon, improving the sizing model.
- In terms of COD, vSCOD=5.3485 mgCODremoved/larva/day and a η= 0.57 were found, demonstrating that BSFL biological treatment remove the organic substances 4-5 times faster than activated sludge.
- Future test will focuse on TKN and BOD_5 monitoring.

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