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A Multidisciplinary Approach for the Development of a Supply Chain in Biomass Conversion of Agrifood Waste Mediated by Larvae of *Hermetia illucens* L.: From Rearing to By-Product Exploitation

Eleonora De Santis [1,2], Alberto de Iudicibus [1,3], Francesca Lecce [4], Massimiliano De Mei [2], Francesco Petrazzuolo [2], Angelo Del Giudice [1], Monica Carnevale [1], Francesco Gallucci [1], Claudio Beni [1], Alberto Assirelli [1], Enrico Santangelo [1,*] and Silvia Arnone [2,*]

1 Council for Agricultural Research and Economics (CREA), Research Centre for Engineering and Agro-Food Processing, Via della Pascolare 16, 00015 Monterotondo, Italy; eledes2106@gmail.com (E.D.S.); alberto.deiudicibus@crea.gov.it (A.d.I.); francesco.gallucci@crea.gov.it (F.G.)
2 Energy Technologies and Renewable Sources Department, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Via Anguillarese 301, 00123 Santa Maria di Galeria, Italy; massimiliano.demei@enea.it (M.D.M.)
3 Council for Agricultural Research and Economics (CREA), Research Centre for Plant Protection and Certification, Via C. G. Bertero 22, 00156 Rome, Italy
4 Department of Sustainability, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Via Anguillarese 301, 00123 Santa Maria di Galeria, Italy
* Correspondence: enrico.santangelo@crea.gov.it (E.S.); silvia.arnone@enea.it (S.A.); Tel.: +39-0630484656 (S.A.)

Abstract: Black soldier fly larvae (BSFL) can convert various organic substrates into high added-value biomass. In addition, the residue can be used as a soil conditioner. Several studies have been conducted on a laboratory scale that may not represent what happens on a prototype scale. Using fruit and vegetable waste as a basic substrate, mixing them with agro-industry by-products (called co-substrates), the Hermes project set up a process on medium (2 kg) and large (10 kg) scales with two different feeding regimes (1.25 g/BSFL and 2 g/BSFL). At the mature stage, larval biomass was separated from frass (the by-product of the larval rearing). The production of larval proteins and fats and the use of frass as soil conditioning were evaluated. The lowest feeding regime (1.25 g/BSFL) provided the best waste valorization. The shift towards higher production scales is not completely linear. The addition of co-substrates to fruit and vegetable waste, as they are provided by the large-scale retail trade, can help to standardize a process as part of an insect farm. The frass recovered from the residue of rearing (on the diet or on the agrifood leftovers) was composted and used in field to grow a processing tomato variety. The addition of composted frass assured a slightly lower yield than synthetic fertilizer but there was no statistically significant difference ($p > 0.10$). This suggests that partial replacement of synthetic fertilizer with composted frass has potential. Overall, the work demonstrated that, using a multidisciplinary approach, the interest and the value in building a supply chain based on bioconversion mediated by *Hermetia illucens* can be emphasized.

Keywords: circular economy; waste reduction; bioconversion; CORS; *Hermetia illucens*; frass; organic fertilizers

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1. Introduction

By 2050, the world population is estimated to grow to about 9.6 billion and the demand for animal protein to increase by 70% [1]. With this rapid population growth, economic development, and urbanization, meeting the demand for feed and quality food and organic waste management require substantial resources, often with great environmental impact [2].

The European Commission highlights organic waste as a key item of the circular and green economy, emphasizing its valorization into useful products. Several studies investigated their potential added value, mainly using anaerobic digestion and composting [3]. Among the organic biomass, fruit and vegetable waste (FVW), a mixture of edible and inedible leftovers, plays an important role. The Food and Agriculture Organization (FAO) reports about 45% of fruits and vegetables (FV) are discarded (as waste) across the food supply chain [2]. Furthermore, the United Nations predicts that 13% of all agricultural product is lost during harvest as well as retail, while 17% is wasted in houses, retail, and eating places [4]. In the context of FVW valorization, one promising strategy is its exploitation as a substrate for mass rearing of edible insects to be used as a protein source for aquaculture, chicken farming, or as a lipid source for biodiesel production [5,6].

Saprophagous insects represent a potentially valid solution to two problems: the recovery of the growing amount of organic waste and the increasing global demand for feed and food. In a circular economy perspective, the application of Conversion of Organic Refuse by Saprophages (CORS) technology mediated by the black soldier fly (BSF), *Hermetia illucens* L. (Diptera, Stratiomyidae, Hermetiinae), represents an important innovation that can significantly contribute to the development of supply chains that meet the principles of green economy and green chemistry. The species is distributed globally throughout temperate and tropical regions [7]. BSF larvae (BSFL) can grow on various organic substrates, i.e., vegetable matter, manure, and catering waste, transforming them into high added-value biomass. It was observed that mature larvae, fed on different organic waste, contain up to 44% of dry matter and reach 42% of protein and 35% of fat [8–10].

Larval performances, in terms of growth, size, biochemical composition, and life span, as well as the efficiency by which substrates are converted into larval biomass, vary considerably and are affected by the quality of the substrate [11–13], substrate supply [14], as well as temperature, oxygen availability, and moisture content [15,16].

Larvae can grow on substrate with a moisture content within the range of 60–90% [17], with the optimal at 70–75% [15]. For these reasons, the use of FVW as BSFL growth substrate may not be sufficient to create suitable conditions for the optimal larval development and the bioconversion of the waste into high added-value molecules, due to high moisture content of the substrate. Furthermore, in case of high moisture content of such a waste stream, the separation of BSFL from the frass might be challenging and time consuming [18].

Several works have investigated the feasibility of mixing FVW with other types of waste that would optimize the bioconversion process, like fermented "spent coffee grounds" or bread waste from catering [19,20]. Besides abiotic factors, such as substrate quality, temperature, and moisture content, the feeding regime intended as substrate amount per larva (sometimes stated as larval density, i.e., number of larvae per area unit) is an important factor that needs to be optimized for its ability to improve or hinder the BSFL growth [14,21]. At low larval densities, which means a great amount of substrate per larva, BSFL may not achieve sufficient substrate conditioning and co-operative digestion, while too high densities lead to providing a low quantity of feed per larva, causing competition over the nutrients, and may negatively influence the growth [22]. The density also influences other physical phenomena such as heat storage and evaporation, which change the feed properties and consequently affect the growth of the larvae [23].

The bioconversion process reduces the volume by 50–70% of waste, a result that could alleviate the environmental and economic cost of their disposal [24]. At the end of the bioconversion cycle, the larval biomass can be separated and used directly as feed or as raw material in a biorefinery conversion to produce protein meal, oil, biodiesel, and chitin [25]. The rearing residue (frass) is a mixture of uneaten substrate, feces, and exuviae, which has interesting characteristics for improving soil and crop productivity [26]. Several works have tested the frass as soil conditioner as such or after composting, demonstrating its potential as alternative organic fertilizer on different crops [26–29].

Historically, most of the studies concerning the knowledge on BSF feeding performance on different waste streams have been conducted at a laboratory scale (i.e., several

hundred larvae per replicate on hundred grams of substrate) showing how BSFL growth and performance can vary on different waste or with different bioconversion conditions (feeding rate, moisture of the substrate, larval density, and feeding regime). The shifting from laboratory to industrial scale is not necessarily linear; many authors indicated that the findings of small-scale trials could not be necessarily moved to large production systems. For example, small-scale compared to large-scale studies, both carried out in the same conditions on swine manure, gave different results for larval development time until the mature stage (less in the small scale) or survivorship (higher in the small scale) or for the prepupal weight (less in the small scale) [30]. The size of the pilot-level studies (also named large-scale or mass production or industrial-level studies) ranges from 10,000 to 20,000 larvae on 6–10 kg of substrate in a crate of about 30–40 L of volume, considering each crate as a bioconversion unit [31–34]. Gligorescu et al. [32] found the best feeding treatment testing the bioconversion efficiency in crates with 20,000 BSFL on 8–10 kg of food waste. Yang-Jie et al. [35] planned a 4-year full-scale study with containers filled with 6 kg of urban waste inoculated with 8000 BSFL. The containers were racked in rows and layers to treat 15 ton/day of waste. Over the 4 years, they could improve the production of larvae and the protein content modifying the frequency of pile turning or changing the fermentation agent of the initial substrate. Hence, the industrial bioconversion process must be developed by continuous technical optimization, according to the waste resource (type and quantity daily provided).

The HERMES project, funded within POR FESR Lazio 2014–2020 (Det. Reg. n. G09493 140721, 22/07/2021), aimed at making available a sustainable and environmentally friendly technology for the recovery and valorization of FVW from the large-scale retail trade by means of bioconversion mediated by BSFL. With a view of the likely seasonal variability of FVW, it was planned to carry out the bioconversion trials using waste provided by one supermarket and, since the large-scale tests required a longer time for the preparation and more space than the medium-scale laboratory tests, trials were diluted over time, within the time allowed for the completion of the Hermes project. Hence, a supermarket provided the mixture of FVW all over the year. To lower the moisture content of the FVW substrate, bakery waste bran, brewer's spent grain, and dry stuff (such as cellulose ramekins and straw) were added one at a time in different tests. All the by-products were chosen since they were locally available.

The experiments were planned in order to (i) compare two feeding regimes and two rearing scales on bioconversion efficiency parameters (larval biomass, added-value products, and BSFL waste reduction); (ii) verify the scale-up of the system from experimental proof-of-concept on a small-scale lab environment to the large scale; and (iii) evaluate the field behavior of the composted residue.

2. Materials and Methods

2.1. Insect Rearing

Specimens for the experiments were obtained from the *H. illucens* colony, established at the ENEA Casaccia Research Center of Rome, based on the rearing process described by Harnden & Tomberlin [36], which is a modified method of Sheppard et al. [37].

Adults were confined in a BugDorm cage (type 6M630) (60 cm × 60 cm × 180 cm), placed inside a climatic chamber maintained at 27 ± 1 °C, 70 ± 10% relative humidity (RH), and 16:8 (light/dark) photoperiod controlled by BEF Biosystems LED lights. The following were placed inside the cage: a Petri dish containing absorbent cotton soaked in a sugar-water solution, a beaker containing only water, and the oviposition site consisting of a beaker on the bottom of which "spent" diet, separated by a mesh, was placed to attract females. Strips of corrugated cardboard placed on the beaker's top edge were used by the flies for oviposition. Eggs were collected after 24 h exposure to oviposing females.

BSFL were fed on a standard Gainesville diet, consisting of 20% corn, 30% alfalfa meal, and 50% wheat bran, mixed with water in a 1:1.7 ratio (100 g diet:170 mL water) [38]. The larvae were reared in a climatic cabinet maintained at a temperature of 27 ± 1 °C,

70 ± 10% RH, and darkness. The rearing was carried out adopting a "semi-batch system", i.e., adding the substrate (~0.1 g/BSFL/day) necessary to complete the BSFL growing (about 1300 g/1000 BSFL) three times (instead of once at the beginning of the batch system). Once larvae reached the mature stage, they were separated from the residue and transferred into a new climatic chamber at 27 ± 1 °C, 50 ± 10% RH, and darkness for pupation. The frass of the rearing on the Gainesville diet was stored in the freezer until being used for composting tests and was named GD_{comp}.

2.2. The Substrates and the Experimental Trials

The basic substrate was a mixture of fruit and vegetable waste (FVW) provided by the Unicoop Tirreno supermarket located in Rome. With a view of the likely seasonal variability of FVW, it was planned to carry out the bioconversion trials by covering the entire year with waste collected in the spring–summer and fall–winter periods. For this purpose, an agreement was established with Unicoop for the continuing supply. As was expected, the composition of the basic substrate FVW varied with every supply. This variation has not been considered as a variable to follow a real-life scenario of a daily supply to a bioconversion plant. The parameter that addressed the choice about the addition of other residual biomass was the percentage of humidity of the total FVW. Since optimal larval growth requires 70–75% moisture content of the feeding substrate [15], different co-substrates have been added to lower the overabundant moisture content of FVW (95–98%). The bakery waste (BW) of the same supermarket has been firstly considered and was used for the first trial of this work (trial A). As a second step, bran (BR) (from a local seller), BSG (from two artisanal breweries in the province of Rome), and dry stuff (DS), such as straw or cellulose ramekins, were considered for a second experiment (trial B). Based on their moisture content, the residual biomasses were added to FVW at the following ratios: 80:20 (FVW:BW and BR), 60:40 (FVW:BSG), and 98:2 (FVW:DS). FVW and BD were chopped by hand before feeding the larvae; FVW, BW, BR, and BSG were carefully mixed; DS was placed in a layer of about 1–2 cm at the bottom of the box.

Table 1 shows the configuration of experiments A and B, in terms of scale (medium scale, MS, and large scale, LS), feeding regime (low feeding regime, LFR, and high feeding regime, HFR), and type of the substrate (1, 2, 3, and 4).

Table 1. The configuration of Trial A and Trial B.

Trial	Scale	Larval Feeding Regime	Subst.	Waste (%)				
				FW [1]	VW [2]		BW [3]	
A	MS [4]	HFR [5]	1	.5 30	50		20	
		LFR [6]	1	30	50		20	
	LS [7]	HFR	1	20	60		20	
		LFR	1	20	60		20	
				FW	VW	BR [8]	BSG [9]	DS [10]
B	MS	LFR	2		80	20		
			3	10	50		40	
			4		98			2
	LS	LFR	2	30	50	20		
			3	10	50		40	
			4	9	89			2

[1] FW, fruit waste; [2] VW, vegetable waste; [3] BW, bakery waste; [4] MS, medium scale (2 kg of substrate); [5] HFR, high feeding regime (2 g of substrate/BSFL); [6] LFR, low feeding regime (1.25 g of substrate/BSFL); [7] LS, large scale (10 kg of substrate); [8] BR, bran; [9] BSG, brewer's spent grains; [10] DS, dry stuff.

- Trial A was aimed to evaluate substrate 1 (FVW:BW), comparing two feeding regimes intended as the total amount of substrate divided per total number of larvae; the HFR consisted of 2 g/BSFL and the LFR of 1.25 g/BSFL, both applied at MS (2 kg of substrate) and LS (10 kg of substrate). At the end of the experiment, the effective feeding rates (g/BSFL/day) were calculated for each experiment, dividing the amount of provided substrate per number of days until larvae reached a mature stage.
- Trial B was aimed to evaluate the linearity of bioconversion efficiency in the shift towards higher production scales, comparing MS with LS trials. On the basis of the results of trial A, tests were carried out only adopting the LFR and were conducted using the other three substrate mixtures: FVW:BR (2), FVW:BSG (3), and FVW:DS (4). The data obtained in trial A with substrate 1 (MS- and LS-LFR1) were compared with data obtained with substrates 2, 3, and 4.

Plastic boxes of 36.5 cm × 25.5 cm × 14 H were used for MS trials; plastic boxes of 59 cm × 39 cm × 17 H were used for LS trials. All the boxes were covered with perforated lids lined with a mesh of 1 mm^2. For all the trials, 6-day-old larvae reared until then on a Gainesville diet were used and exposed to a batch feeding strategy. The experimental trials, carried out in triplicate, were kept in a dark climate chamber maintained at 27 ± 1 °C and 70 ± 10% RH.

2.3. Bionconversion Efficiency Parameters

Larvae were weighed at the beginning of the experiment and every 2–3 days during the trial. Three randomized subsamples of 30 larvae each were washed, dried on filter paper, weighed, and then returned to their respective container. The trials ended when the larval weight began to decrease. At this phase, mature larvae undertake the prepupal period with a nonfeeding phase, accompanied by weight loss and by darkening of the body color [34,39]. Once the larvae reached the prepupal stage, the larval biomass was separated from the residue (frass) that was stored in the freezer until being used for composting tests and was named FVW_{comp}.

Larvae were washed, dried on filter paper, and then weighed. Larvae and substrates were characterized as follows: MLW was based on fresh weight; dry matter, ash, protein, and lipid content of both initial substrate and mature larvae were determined using standard procedures [40]. Before starting each experiment, 100 g of fresh substrate was oven-dried at 105 °C for 24 h to determine dry matter. At the end of the bioconversion, 40 g of fresh larval biomass was boiled for 3 min [41] and then oven-dried at 60 °C for 48 h to determine dry matter. Ash content was determined in a muffle furnace at 550 °C for 4 h for all the samples. Crude fat content was determined on dry sample by means of an automatized Soxhlet extractor (Soxhtraction PBI International apparatus, Milano, Italy), using *n*-hexane as solvent [42]. Total nitrogen was determined on the defatted sample with the Kjeldahl system (Buchi 426 Digestion Unit and Buchi 323 Distillation Unit, Cornaredo, Italy) [43]. To determine protein content, total N was multiplied with the factor Kp 6.25 for the substrate and Kp 4.75 for the larval biomass to avoid an overestimation of proteins due to the presence of chitin in the exoskeleton [44]. All analyses were performed in triplicate.

Carbohydrate content (%) was estimated by difference, subtracting the sum of lipids, protein, and ash (g/100 gDM) from the total dry weight of the sample (100 gDM):

$$\text{Carbohydrates} = 100 - [(\text{crude lipid} + \text{crude protein} + \text{ash})\ \text{content}\ (\text{g}/100\ \text{g})] \quad (1)$$

Protein conversion ratio (% PrCR) was calculated on a dry matter basis following Lalander et al. [34]:

$$\text{PrCR} = \frac{\text{Pr}_{\text{BSFL}}}{\text{DM}_{\text{BSFL}}} \times \frac{\text{Pr}_{\text{IS}}}{\text{DM}_{\text{IS}}} \times 100 \quad (2)$$

where DM_{BSFL} and DM_{IS} are the total dry matter in the mature larvae and initial substrate, respectively, while Pr_{BSFL} and Pr_{IS} are the percentage of crude protein (% DM) in the mature larvae and initial substrate, respectively.

Waste reduction (% WR) was calculated on a wet weight basis as follows:

$$WR = \frac{\text{Initial substrate (g)} - \text{Residue (g)}}{\text{Initial substrate (g)}} \times 100 \quad (3)$$

2.4. Field Trial

In the case of an industrial plant processing heterogeneous material, as is the case of the present study, it is necessary to standardize the reproductive cycle of the insect using for larval development an artificial diet. The frass produced from the insect rearing starting from the Gainsville diet (GD) or from FVW were composted in two cylindrical composters (H 100, Ø 48 cm approx.) made of wire mesh covered with green shade cloth. Each composter had a capacity of approximately 25 kg and was intended for the dietary residue or the fruit and vegetable residue after bioconversion. Wheat straw was added to the frass in a 1:1 (*w*/*w*) ratio. The cylinders were irrigated for one hour periodically with a rotary sprinkler. The composting phase was conducted between the beginning of February and mid-June 2023, lasting for 100 days.

The final compost was analyzed for the content of the main elements and heavy metals. Before the characterization, 50 g of each substrate was dried for 24 h at 105 ± 2 °C (Memmert UFP800 drying oven, Schwabach, Germany) to determine the moisture content [45]. The dried biomass was treated with a Retsch SM 100 kneading mill and then with a centrifugal mill (Retsch ZM 200, Retsch, Haan, Germany) to shred and homogenize the matrix. To determine the content of carbon (C) and nitrogen (N), about 5 mg of each sample was analyzed by Costech ECS 4010 CHNS-O elemental analyzer (Costech International, Pioltello, Italy) [46]. The quantification limit (LOQ) for each sample was 0.05% *w*/*w*. For the macro- and trace element content, 0.5 g of the dehydrated sample was homogenized and solubilized with 6 ± 0.1 mL of HNO_3 65% and 3 ± 0.1 mL of H_2O_2 30%. The solution was digested in a microwave oven at 180 °C, 650 W, for 8 min and then for a further 15 min. At the end of digestion, the samples were filtered and diluted with MilliQ water. Two replicates and a blank were made for each sample. The calibration line was made in nitric acid on five points at increasing concentrations of internal standard. Element analysis was performed using ICP-MS (Agilent 7700, Agilent Technologies, Tokyo, Japan).

Tomato plantlets (*Solanum lycopersicum*, L.) of the Roma Nano F1 variety, at the 4–5th true leaf stage, were transplanted on May 2023 at the experimental field of the CREA Research Centre for Engineering and Agro-Food Processing, Monterotondo, Italy (42° N 05′056.86″, 12° E 37′026.23″). The soil, belonging to silty clayey loam USDA classification (Table 2), was left as fallow in the preceding year. In the recent past, the soil was fertilized with cow manure for at least three years.

Table 2. Soil physical and chemical properties.

Soil Properties	U.M. [1]	
Sand	%	25
Silt	%	38
Clay	%	37
pH		7.9
Organic matter	%	2.6
Total nitrogen (N)	%	0.16
Assimilable phosphorous (P)	mg/kg	14.1
Exchangeable potassium (K)	mg/kg	365.3
Cation Exchange Capacity	Meq/100 g	31.8

[1] Unit of measurement.

The soil composition was studied by Bascietto et al. [47], which outlined that the endowment in organic matter and total nitrogen (N) was moderate, while potassium (K) was well represented. Overall, N and K concentrations were widely above the levels of sufficiency for an average demanding crop, so their supply was not required. On the other

side, the available phosphorus was low and, hence, it needed to be added with specific fertilizations.

Both composts were used in an open field test. The study compared three fertilization treatments and a control group: mineral fertilization, using diammonium phosphate (18–46); compost from residue recovered from the rearing on a Gainsville diet (GD_{comp}); compost from residue recovered from the rearing on fruit and vegetable waste (FVW_{comp}); and unfertilized control. The amounts of compost and mineral fertilizer were calculated to provide 130 kg/ha of nitrogen as required for processing tomato [48] and provided near the plants just after transplanting.

The experimental design was a 4 × 4 Latin square. Each plot included 5 plants spaced 40 cm apart. A buffer zone of 80 cm was left between the plots. The distance between the rows was 1 m. The total plot area was 1.6 m^2, which corresponds to approx. 31,250 p/ha. To reduce evaporation losses and weed infestation, the area was mulched immediately with wheat straw after transplanting (Figure 1). Plants were drip irrigated with lines placed near the plants and the water supply was scheduled according to the Integrated Production Regulations for processing tomato [48].

Figure 1. Tomato plants at different stages of vegetative growth: 15 (**above**) and 30 (**below**) days after transplant. The picture shows the irrigation system and the mulching on the area.

The same regulations were applied for controlling late blight and insect pests (southern green shield bug and tomato pinworm) affecting the plants during growth. Meteorological data were collected by the Arsial control unit of Monterotondo (RM), location: Grotta Marozza (92 m asl) [49].

Three central plants of each plot were used for field measurements: chlorophyll content (SPAD units) on at least three leaflets of the composed leaf, using a Konica Minolta, Europe SPAD-502 reader; fruit set (ratio between the number of flowers per inflorescence and the number of fruits) of the first two basal inflorescences of each plant.

Fruits were collected after 98 days from transplanting when around 80% of the fruits were at the red-ripe stage. The number of marketable, unripened, and damaged fruits per plant was recorded and weighed using a digital balance (Mettler PC8000, Mettler-Toledo S.p.A., Milan, Italy). Ten marketable fruits for each plot were chosen to determine the average length and the polar diameter. The ratio of length/diameter was used for the calculation of the fruit shape index (FSI).

Finally, five fruits per plot identified as marketable were used for the destructive analyses. The fruits were squeezed and the juice was used to determine the pH with the SensION pH25+ portable pH meter (Crison Instruments, Alella, Spain) and the soluble solids (°Brix) with the OPTech digital refractometer (Optical Technology, Hannover, Germany). pH and soluble solids were measured in triplicate.

2.5. Statistical Analysis

All the statistical analyses were performed using PAST software version 4.13 [50]. The data concerning the combination between feeding rate and rearing scale were compared using two-way ANOVA; the remaining data related to substrate and larval biomass characterization, maximum larval weight, waste reduction percentage, and fertilization study were analyzed by one-way ANOVA. Before performing the ANOVA test, data were checked for normality by Shapiro–Wilk test and the Levene's test for the homogeneity of variance. Differences among the means were tested according to the Tukey's honestly significant difference (HSD) test ($p < 0.05$). Differences in composition between two types of compost were analyzed by Student's *t*-test. Principal component analysis (PCA) was performed using the same software to visualize the differences between the fertilization treatments.

3. Results

3.1. Trial A: Feeding Regimes Comparison

This study compared two feeding regimes (HFR and LFR) at a medium (MS) and large scale (LS) and was performed with the substrate FVW:BW, identified as number 1. Although the general ratio among the residues was the same (FVW:BW 80:20 in trial A), each substrate supplied to BSFL had specific composition (Table 3). Significant differences among the values of the substrate nutritional components were found for DM, ash, crude lipid, and carbohydrate content. In all the cases, only one type of substrate differs from the others and, with the exception of crude lipids, all the values were within a narrow range. The crude protein content did not significantly vary among the different substrates.

Table 3. Characterization of the substrate (mean ± SD) used in the experiment at high feeding rate (HFR) and low feeding rate (LFR) with substrate 1 (FVW:BW) in both the medium (MS) and large (LS) scales. Different letters indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

Parameter	U.M. [1]	Scale	Trial	
			HFR1	LFR1
DM [2]	%	MS	25.4 ± 5.9 a	27.3 ± 3.6 a
		LS	31.7 ± 1.7 a	21.9 ± 1.5 b
Ash	g/100 gDM	MS	4.8 ± 0.2 a	4.9 ± 0.3 a
		LS	5.2 ± 0.2 a	3.8 ± 0.6 b
Crude lipid	g/100 gDM	MS	1.8 ± 0.1 b	1.6 ± 0.3 b
		LS	4.7 ± 0.3 a	2.3 ± 0.6 b
Crude protein	g/100 gDM	MS	13.2 ± 0.3 a	12.8 ± 0.6 a
		LS	13.1 ± 0.5 a	14.6 ± 0.9 a
Carbohydrate	g/100 gDM	MS	80.5 ± 0.5 b	81.2 ± 0.2 a
		LS	78.0 ± 0.9 b	79.3 ± 0.5 b
P:C [3] ratio		MS	1:6	1:6.5
		LS	1:6	1:5.5

[1] U.M., unit of measurement; [2] DM, dry matter; [3] P:C, protein/carbohydrate.

The variability in substrates is due to the variability in FVW itself and in the type of BW, supplies that have not been chosen, coming directly from the supermarket. With regard to BSFL nutritional needs, all the substrates had a low lipid content (range 1.6 ± 0.3–4.7 ± 0.3 g/100 gDM), a medium–low protein content (range 12.8 ± 0.6–14.6 ± 0.9 g/100 gDM), a very high content of carbohydrates (range 78.0 ± 0.8–81.2 ± 0.2 g/100 gDM), and, consequently, a very unbalanced P:C ratio (range 1:5.5–1:6.5).

The addition of BW was effective in obtaining a suitable substrate for larval growth in terms of moisture content (range 68.3 ± 1.7–78.1 ± 1.5 g/100 gDM).

Larval weight increment is shown in Figure 2 and in Table 4. The trend of larval growth for all the trials looks like a part of a sigmoidal curve [51]. The highest MLW was reached at day 13 in MS-HFR1 and was equal to 368.2 ± 22.0 mg/BSFL value that is significantly different from the MLW reached in 14 days in MS-LFR1 (256.8 ± 21.9 mg/BSFL). In the LS trials, BSFL have longer larval lifetime until mature stage. The MLW was reached at day 17 in LS-HFR1 (347.7 ± 12.8 mg/BSFL) and at day 19 in LS-LFR1 (247.3 ± 1.2 mg/BSFL). MLW values at HFR were significantly higher than at LFR1 in both scales. The shift from MS to LS seems to be linear. Regarding the feeding regime, the batch feeding strategy does not allow the quantity of feed provided to a single larva to be exactly modulated. At the end of the experiment, based upon the time necessary to achieve the MLW, we could verify the effective feeding rate intended as g/BSFL/day. The range was 0.066–0.089 mg/BSFL/day for LFR against 0.12–0.15 mg/BSFL/day for HFR (Table 4).

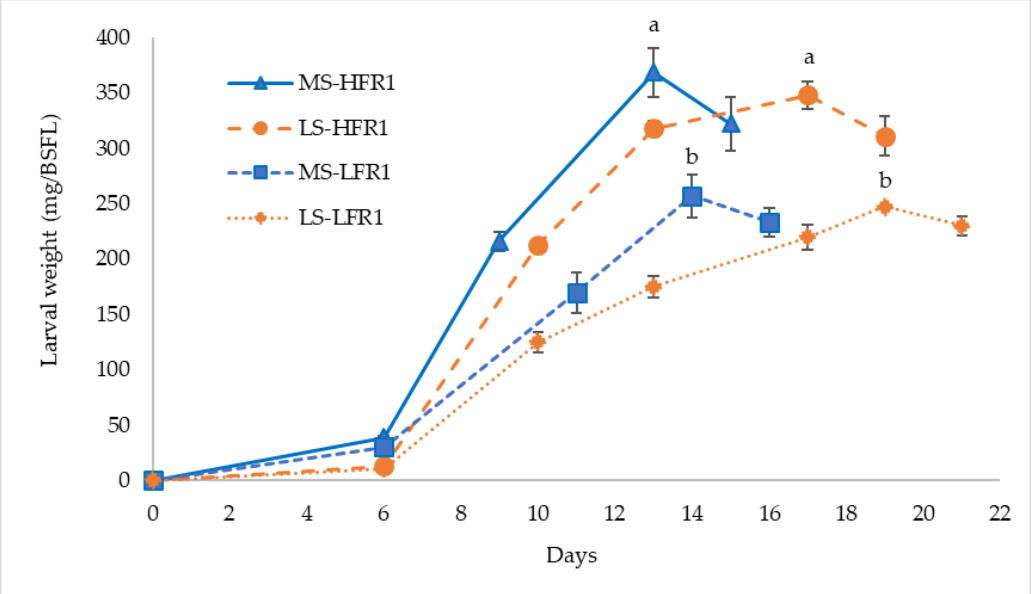

Figure 2. Trend of larval weight (mg/BSFL) during larval lifetime until mature stage (days) comparing medium scale (MS) with large scale (LS) and high feeding regime (HFR) with low feeding regime (LFR) using substrate 1 (FVW:BW). Bars indicate standard deviation. Within MS or LS condition different letters indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

Regarding waste reduction (Figure 3), statistically significant highest values were obtained in MS-LFR1 (79.3 ± 1.2%) and in LS-HFR1 (77.4 ± 3.0%). In this case, the shift toward higher production scales was opposite and nonlinear; the waste reduction increased significantly passing from MS to LS with the HFR, while decreasing likewise significantly from MS to LS using the LFR.

Table 4. Maximum larval weight (mean ± SD), larval lifetime until mature stage, and effective feeding rate: comparison between high feeding rate (HFR) and low feeding rate (LFR) with substrate 1 (FVW:BW) in both the medium (MS) and large (LS) scale. Different letters beside the maximum larval weight indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

Parameter	U.M. [1]	Scale	Trial	
			HFR1	LFR1
Maximum larval weight	mg/BSFL	MS	368.2 ± 22.0 a	256.8 ± 19.3 b
		LS	347.7 ± 12.2 a	247.3 ± 1.2 b
Time until mature stage	days	MS	13	14
		LS	17	19
Effective feeding rate	mg/BSFL/day	MS	0.15	0.089
		LS	0.12	0.066

[1] U.M., unit of measurement.

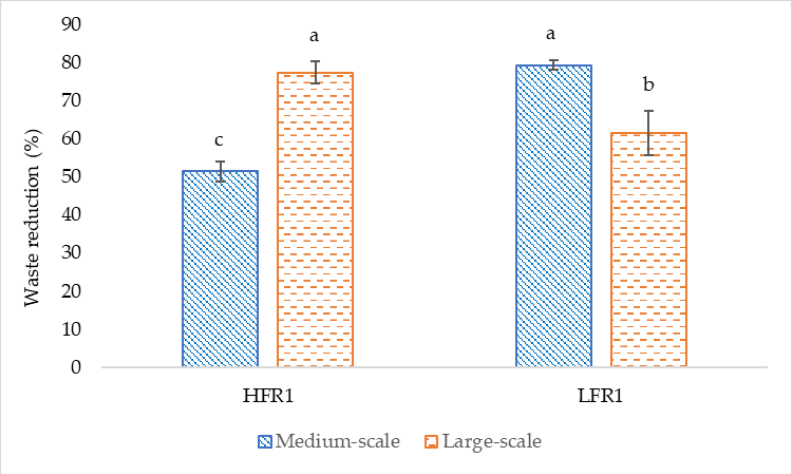

Figure 3. Waste reduction (% on wet waste): comparison between high feeding rate (HFR) and low feeding rate (LFR) with substrate 1 (FVW:BW) in both medium (MS) and large (LS) scale. Bars indicate standard deviation. Different letters indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

The characterization of BSFL biomass (Table 5) showed statistically significant differences between HFR1 and LFR1 at both scales. In detail, the significant highest values were obtained, both in MS and LS, at HFR1 for the percentage of DM and the crude protein content, while for the percentage of ash and crude lipid content at LFR1. Evident linearity between MS and LS has been detected in HFR1 for DM, ash, lipid, protein, and carbohydrate content per larva. Differently, at the same feeding regime, none of the values related to the other parameters (PrCR, total larval biomass, total lipids, and proteins) showed linearity in the passage from MS to LS. In contrast, at LFR1, biomass and protein production maintains linearity. With regard to PrCR, the highest value was obtained in MS-HFR1 (58.8 ± 5.1%), which was significantly different from LS-HFR1 (37.7 ± 2.3%). At HFR, for the parameters associated to global production (biomass, lipids, and proteins production), the values obtained at MS are significantly higher than those obtained at LS. In both the scales, LFR1 achieves good results in terms of biomass and lipid production comparable or significantly better than HFR1 (Table 5). At LFR, moving from MS to LS did not appear to significantly change biomass and protein production. At the same time, the choice of LFR at LS condition gave a significant increase in lipid content. LFR appeared to have a buffering effect in scaling up from MS to LS. Keeping in mind the objective of scaling up the system at an affordable cost and considering the results with LFR, trial B was set to compare the bioconversion efficiency in MS and LS with other substrates at LFR condition.

Table 5. BSFL biomass characterization and total amount of BSFL biomass and its added-value products (mean ± SD): comparison between HFR1 (high feeding rate) and LFR1 (low feeding rate) with substrate 1 (FVW:BW) in both medium (MS) and large (LS) scale. Different letters indicate statistically significant differences for the Tukey's HSD test ($p < 0.05$).

Parameter	U.M. [1]	Scale	Trial	
			HFR1	LFR1
DM [2]	%	MS	34.5 ± 0.8 a	28.7 ± 0.6 b
		LS	33.8 ± 0.6 a	27.6 ± 0.8 b
Ash	g/100 gDM	MS	4.8 ± 0.1 b	5.7 ± 0.5 a
		LS	4.2 ± 0.3 b	4.9 ± 0.3 a
Crude lipid	g/100 gDM	MS	38.1 ± 0.5 b	39.7 ± 2.9 b
		LS	41.7 ± 1.0 b	45.7 ± 1.0 a
Crude protein	g/100 gDM	MS	31.7 ± 0.5 a	27.4 ± 2.2 b
		LS	29.2 ± 0.4 a	23.7 ± 0.5 c
Carbohydrate	g/100 gDM	MS	25.7 ± 0.5 a	27.1 ± 1.2 a
		LS	24.9 ± 0.4 a	25.8 ± 0.7 a
PrCR [3]	%	MS	58.8 ± 5.1 a	46.5 ± 2.9 b
		LS	37.7 ± 2.3 c	42.4 ± 1.5 bc
Biomass production	g/kgWW [4]	MS	61.7 ± 5.3 a	57.3 ± 2.0 a
		LS	49.5 ± 3.1 b	57.3 ± 1.1 a
Lipid production	g/kgWW	MS	23.5 ± 2.3 ab	22.8 ± 2.4 ab
		LS	20.7 ± 1.4 b	26.0 ± 0.3 a
Protein production	g/kgWW	MS	19.6 ± 1.7 a	15.7 ± 1.0 b
		LS	14.5 ± 0.9 b	13.6 ± 0.5 b

[1] U.M., unit of measurement; [2] DM, dry matter; [3] PrCR, protein conversion ratio; [4] WW, wet waste.

3.2. Trial B: Medium-Scale and Large-Scale Comparison

Trial B aimed to compare MS with LS using all the substrates described in Table 1 (FVW:BW, FVW:BR, FVW:BSG, and FVW:DS) at LFR. Substrate characterization is shown in Table 6.

Table 6. Characterization of the substrates in the experiment with low feeding rate (LFR) in both medium (MS) and large (LS) scale. Numbers 1, 2, 3, and 4 refer to the type of substrate described in Section 2.2. and Table 1 (mean ± SD). Different letters within each parameter indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

Parameter	U.M. [1]	Scale	Trial			
			LFR1	LFR2	LFR3	LFR4
DM [2]	%	MS	27.3 ± 3.6 a	25.3 ± 0.5 ab	16.9 ± 1.0 c	8.9 ± 0.6 d
		LS	21.9 ± 1.0 b	26.8 ± 0.6 a	15.6 ± 1.1 c	11.5 ± 1.3 d
Ash	g/100 gDM	MS	4.9 ± 0.3 bc	8.4 ± 0.1 a	5.2 ± 0.0 bc	9.2 ± 0.0 a
		LS	3.8 ± 0.6 c	6.2 ± 1.2 b	8.3 ± 0.0 a	9.5 ± 0.0 a
Crude lipid	g/100 gDM	MS	1.6 ± 0.1 d	2.9 ± 0.1 c	4.4 ± 0.1 b	2.8 ± 0.1 c
		LS	2.3 ± 0.2 cd	2.6 ± 0.3 c	6.0 ± 0.4 a	6.3 ± 0.5 a
Crude protein	g/100 gDM	MS	12.4 ± 0.0 d	20.9 ± 1.3 a	17.8 ± 0.5 b	21.3 ± 1.4 a
		LS	14.6 ± 0.9 c	17.5 ± 0.5 b	15.2 ± 0.2 c	14.9 ± 0.2 c
Carbohydrate	g/100 gDM	MS	81.2 ± 1.2 a	67.8 ± 1.3 c	72.5 ± 0.4 b	66.7 ± 1.4 c
		LS	79.3 ± 0.5 a	73.7 ± 0.7 b	70.6 ± 0.2 bc	69.7 ± 2.7 bc
P:C [3] ratio		MS	1:6.5	1: 3	1:4	1:3
		LS	1:5.5	1: 4	1:4.5	1:4.5

[1] U.M., unit of measurement; [2] DM, dry matter; [3] P:C, protein/carbohydrate.

From the observation of the data of Table 6, it appears that the addition of BW and BR (LFR1 and LFR2, respectively) to the FVW was effective in obtaining a substrate with a moisture content ranging from 72.7 ± 3.6% to 78.1 ± 1.2%, suitable for larval growth. In contrast, the addition of BSG (LFR3) and DS (LFR4) absorbed only a small part of the leachate, making a substrate with a moisture content ranging from to 83.2 ± 1.3% to 91.1 ± 0.9%.

Regarding crude lipids, the difference was significant in LFR3 and LFR4, while, for protein content, there was a statistical difference in all substrates,

The carbohydrate content was comparable within each substrate except for LFR2. However, the range between the substrates of the MS and LS tests was very narrow. Moreover, owing to the BW presence in LFR1 the carbohydrate content was statistically lower in substrates 2, 3, and 4. As a consequence, P:C ratio lowered proportionally.

In general, considering the nutritional needs of BSFL, all the substrates had an excess of carbohydrates content. The higher protein content of substrate 2, 3, and 4 compared to substrate 1 contributed to increasing the P:C ratio ranging from 1:3 to 1:4.5.

The differences within the same substrate are due, as already described, to the variability in FVW itself and to the use of different by-products mixed with FVW.

The MLWs in substrates 2, 3, and 4, in both MS and LS trials, were significantly lower than values reached on substrate 1 (Figure 4) and the lowest ones were those of FVW:DS (LFR4). Within each substrate, no statistically significant differences were observed between MS and LS, except in FVW:BR (LFR2). The MLW in the LS test was over 200 mg in both LFR2 (216.0 ± 5.3 mg/BSFL) and LFR3 (206.7 ± 3.2 mg/BSFL). Except LFR2, the larval lifetime until the mature stage was longer in LS than MS; for the first, it ranged from 16 to 19 days, while, at MS, the range was 10–14 days. The feeding rate resulted in being effectively at a low level (ranged from 0.066 in LS-LFR1 to 0.125 mg/BSFL/day in MS-LFR4), which was the condition we wanted to test. Waste reduction at MS condition (Figure 5) showed a decreasing trend according to the order LFR1 > LFR2 > LFR4 > LFR3. The same pattern was partially confirmed for the LS where LFR1 and LFR2 had a comparable waste reduction, higher than the waste reduction observed for LFR3 and LFR4. Waste reduction in MS was significantly higher (LFR1), lower (LFR3), or comparable (LFR2 and LFR3) compared to the LS condition.

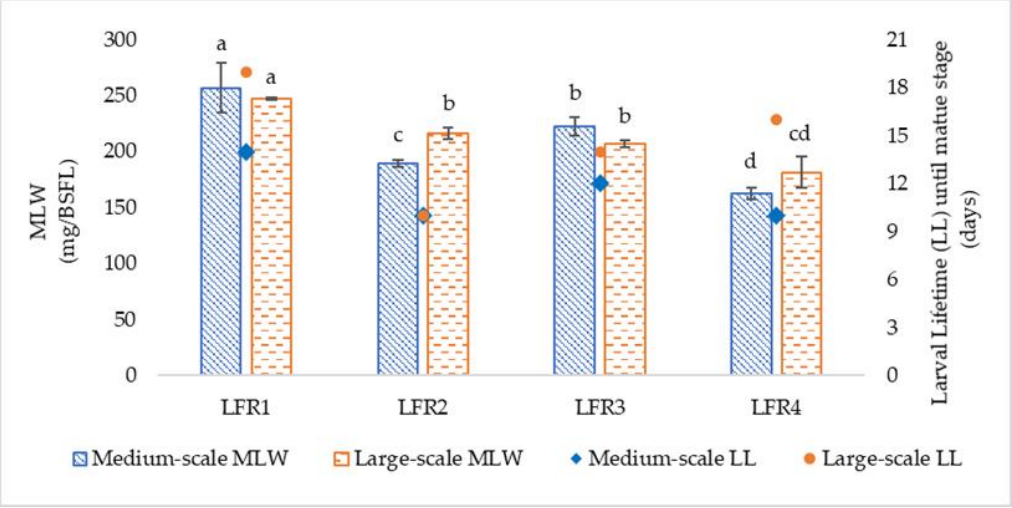

Figure 4. Maximum larval weight (MLW) (mg/BSFL) and larval lifetime (LL) until mature stage (days) in the experiment with low feeding rate (LFR) in both medium (MS) and large (LS) scale. Numbers 1, 2, 3, and 4 refer to the type of substrate described in Section 2.2 and Table 1. Bars indicate standard deviation. Different letters indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

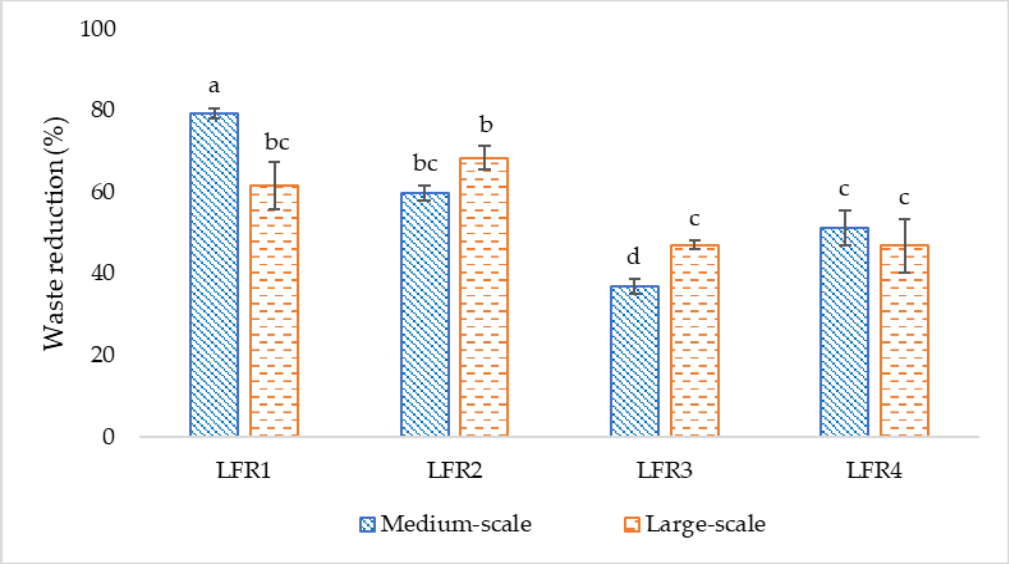

Figure 5. Waste reduction (% on wet waste) in the experiment with low feeding rate (LFR) in both medium (MS) and large (LS) scale. Numbers 1, 2, 3, and 4 refer to the type of substrate described in Section 2.2 and Table 1. Bars indicate standard deviation. Different letters indicate statistically significant differences for Tukey's HSD test ($p < 0.05$).

LFR1 and LFR2 showed the highest values of DM, independently of the rearing scale (Table 7). LFR4 had the lowest DM. Interestingly, LFR1 was the substrate allowing the highest accumulation of crude lipid in the larvae both in MS (39.7 ± 2.8%) and LS (45.7 ± 1.0%). The difference with the other substrates was very impressive, ranging from 1.5 (MS-LFR3) to almost fourfold (MS-LFR4) compared to the highest value (LS-LFR1). On the other side, the content of crude protein and carbohydrate in LFR1 were lower than the remaining three substrates. Specifically, the percentage of crude protein was abundantly above 30% in LFR2, LFR3, and LFR4, while, in LFR1, the BSFL crude protein was just below this threshold in MS (27.4 ± 2.2%) and even significantly lower in LS (23.7 ± 0.5%). LFR2 and LFR4 were instead the substrates allowing for the highest accumulation of carbohydrates in BSFL. Except LFR1, where MS and LS were comparable, the highest values of the PrCR value were obtained in the LS condition, with a difference statistically significant compared to the MS.

Table 7. BSFL biomass characterization: comparison between medium- and large-scale trials with the FVW-based substrates (mean ± SD) in the experiment with low feeding rate (LFR) in both medium (MS) and large (LS) scale. Numbers 1, 2, 3, and 4 refer to the type of substrate described in Section 2.2 and Table 1. Different letters indicate statistically significant differences for the Tukey HSD test ($p < 0.05$).

Parameter	U.M. [1]	Scale	Trial			
			LFR1	LFR2	LFR3	LFR4
DM [2]	%	MS	28.7 ± 0.6 a	24.8 ± 0.2 b	25.0 ± 0.2 b	16.5 ± 0.2 d
		LS	27.6 ± 0.8 a	27.3 ± 0.3 a	24.1 ± 0.9 b	20.8 ± 0.7 c
Ash	g/100 gDM	MS	5.7 ± 0.5 d	8.9 ± 0.2 c	7.5 ± 0.3 c	17.1 ± 1.0 a
		LS	4.9 ± 0.3 d	5.5 ± 0.5 d	8.6 ± 0.5 c	10.5 ± 0.3 b
Crude lipid	g/100 gDM	MS	39.7 ± 2.9 b	21.1 ± 1.8 e	30.6 ± 1.2 c	12.8 ± 0.9 f
		LS	45.7 ± 1.0 a	27.9 ± 1.3 cd	26.8 ± 1.1 cd	24.8 ± 0.3 de
Crude protein	g/100 gDM	MS	27.4 ± 2.2 c	37.1 ± 1.3 a	34.0 ± 0.2 ab	34.4 ± 0.7 ab
		LS	23.7 ± 0.5 d	35.8 ± 0.4 ab	34.5 ± 0.8 ab	32.9 ± 0.8 b
Carbohydrate	g/100 gDM	MS	27.1 ± 1.2 d	33.0 ± 0.3 b	27.9 ± 1.6 cd	35.8 ± 0.9 a
		LS	25.8 ± 0.7 d	30.8 ± 1.4 bc	30.1 ± 0.3 c	31.8 ± 0.3 bc

Table 7. *Cont.*

Parameter	U.M. [1]	Scale	Trial			
			LFR1	LFR2	LFR3	LFR4
PrCR [3]	%	MS LS	46.5 ± 2.9 bc 42.4 ± 1.5 cd	27.6 ± 0.8 f 34.9 ± 1.2 e	46.3 ± 5.1 ac 53.0 ± 2.4 ab	36.5 ± 1.6 de 53.8 ± 1.8 a
Biomass production	g/kgWW [4]	MS LS	57.3 ± 2.0 a 57.3 ± 1.1 a	39.4 ± 2.5 c 46.1 ± 1.4 b	40.9 ± 4.3 bc 36.5 ± 0.8 c	20.2 ± 1.2 e 27.8 ± 0.9 d
Lipid production	g/kgWW	MS LS	22.8 ± 2.4 a 26.0 ± 0.3 a	8.3 ± 1.2 c 12.8 ± 1.0 b	12.6 ± 1.7 b 9.78 ± 0.2 bc	2.6 ± 0.3 d 6.9 ± 0.3 c
Protein production	g/kgWW	MS LS	15.7 ± 1.0 ab 13.6 ± 0.5 c	14.6 ± 0.4 bc 16.5 ± 0.3 a	13.9 ± 1.5 bc 12.6 ± 0.6 c	6.9 ± 0.3 e 9.2 ± 0.3 d

[1] U.M., unit of measurement; [2] DM, dry matter; [3] PrCR, protein conversion ratio; [4] WW, wet waste.

When the results were expressed as grams per kilogram of wet waste, the data emphasized the positive effect of the FVW:BW in LFR1 compared to the other substrates (Table 7). The values of biomass and lipid production were statistically different from those shown by the other substrates, while, for protein production, the highest values were measured for MS-LFR1 (15.7 ± 1.0 g/kgWW) and LS-LFR2 (16.5 ± 0.3 g/kgWW).

3.3. Field Trial

The weather showed a typical trend of the area (Figure 6). Substantial rainfall occurred until early June, gradually reducing in the summer months until harvest. Transplanting at the end of May made it possible to escape the downy mildew infestations that have damaged early tomato transplants in Italy. Gradually increasing temperatures through July mirrored the trends observed for central Italy by requiring proper water supply at different stages of plant development.

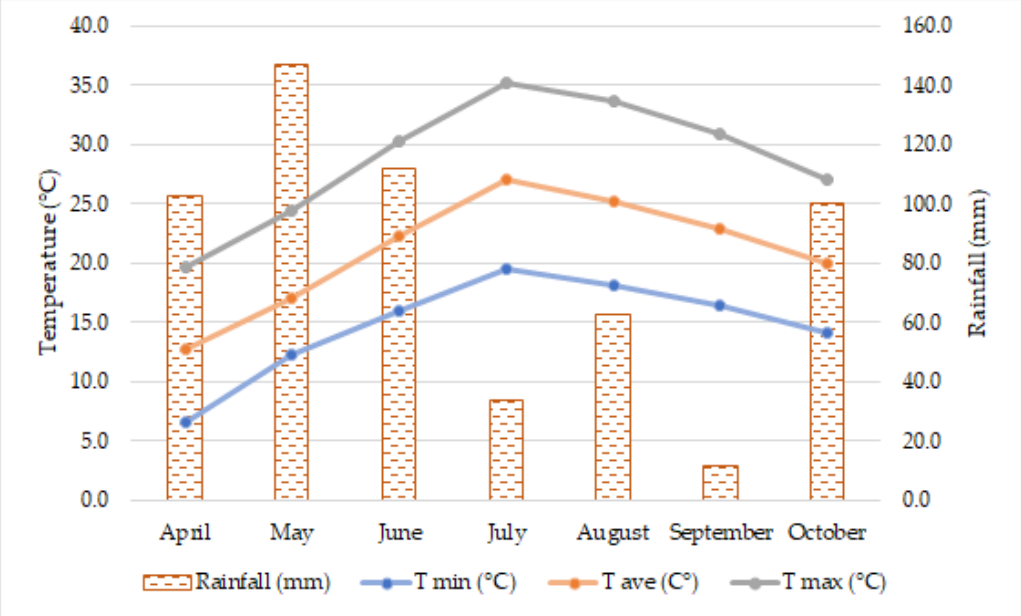

Figure 6. Data on minimum, average, maximum temperatures, and monthly rainfall of the period April–November 2023 were collected by the Arsial control unit of Monterotondo (RM), location: Grotta Marozza (92 m asl) [49].

The two types of compost were quite different (Table 8). The final moisture and pH of the FVW_{comp} were significantly higher than those of GD_{comp}. Although the carbon (C) and hydrogen (H) content were similar in both types, the nitrogen (N) content was significantly lower in FVW_{comp}. As a result, the C/N ratio in FVW_{comp} was significantly higher than that

of GD_{comp}. It should be noted that, in the straw added during the composting process, the average C content was 47.4 ± 1.6% and that of N 0.6 ± 0.1%, with a C:N ratio of 76.8 ± 8.6. The data measured in the mature compost indicated an effect of composting in obtaining a C:N ratio acceptable for agronomic use. Except for iron (Fe), the content of some macro- and microelements was significantly higher in FVW_{comp}.

Table 8. Characterization (mean ± SD; n = 3) of GD and FVW compost.

Value	U.M. [a]	GD_{comp} [b]	FVW_{comp}	t-Value	*p* [c]
Moisture	%	16.9 ± 4.1	48.2 ± 5.7	7.73	**
pH		6.9 ± 0.2	8.3 ± 0.4	6.17	**
C	%	34.9 ± 4.5	39.8 ± 1.0	1.86	ns
H	%	7.1 ± 1.7	7.6 ± 1.6	0.36	ns
N	%	2.8 ± 0.3	1.9 ± 0.3	3.70	*
C/N		12.4 ± 0.4	21.7 ± 2.6	6.22	**
Na	g/kg	0.9 ± 0.2	1.5 ± 0.3	2.82	*
Mg	g/kg	6.6 ± 0.5	8.3 ± 0.4	4.54	*
K	g/kg	1.4 ± 0.3	2.1 ± 0.7	1.47	ns
Ca	g/kg	5.4 ± 0.5	6.2 ± 1.3	0.95	ns
Fe	g/kg	0.8 ± 0.1	0.5 ± 0.0	3.39	*
Ni	mg/kg	14.5 ± 3.0	17.7 ± 2.7	1.41	ns
Cu	mg/kg	14.9 ± 0.9	20.5 ± 2.8	3.37	*
Zn	mg/kg	66.2 ± 3.7	87.0 ± 6.2	5.01	**
As	mg/kg	4.3 ± 0.8	3.2 ± 1.3	1.26	ns
Cd	mg/kg	0.1 ± 0.0	0.2 ± 0.0	9.96	**
Pb	mg/kg	9.0 ± 1.6	11.1 ± 3.0	1.12	ns

[a] U.M., unit of measurement. [b] GD = Gainsville diet, FVW = fruit and vegetable waste. [c] ns = not significant, * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$ after Student's *t*-test.

In general, no significant differences were observed among the treatments. Compost seems to have a slightly depressive effect on plant development (Table 9) but the chlorophyll content of unfertilized plants was lower than in the other three treatments. The same trend was observed for the fruit set in the second truss, while, in the first truss, there was a positive effect of both chemical fertilization and FVW compost.

Table 9. Main characteristics of tomato plants (mean ± SD).

Treatment [a]	Plant Height (cm)	Chlorophyll Content (SPAD Unit)	Fruit Set	
			1st Truss	2nd Truss
Unfertilized	70.7 ± 5.1	44.7 ± 2.1	63.7 ± 22.1	60.1 ± 10.9
Fertilized	70.7 ± 3.2	47.3 ± 4.9	74.2 ± 4.7	66.1 ± 19.3
GD_{comp}	67.9 ± 6.1	46.5 ± 3.6	66.2 ± 14.8	67.2 ± 4.8
FVW_{comp}	66.7 ± 5.6	46.8 ± 3.0	71 ± 7.1	67.6 ± 8.1

[a] GD_{comp} = compost from Gainsville diet, FVW_{comp} = compost from fruit and vegetable waste.

From a production point of view, there were also no significant differences between treatments (Figure 7), although a definite trend can be appreciated. In terms of the production of marketable fruits, the average fruit weight ranged from 70 (unfertilized control) to 74 g (GD_{comp}). However, the most noticeable differences were in the number of fruits produced per plant, which ranged from 24 (fertilized) to 15 (GD_{comp}). The GD_{comp} figure was confirmed by the high incidence (by weight) of unripened and damaged fruits, which reduced the percentage of marketable fruits to 76%. The final figure indicates decreasing productivity according to the order Fertilized > FVW_{comp} > Unfertilized > GD_{comp}.

Figure 7. Productive traits observed for the studied treatment. Where present, bars represent the standard deviation. GD_{comp} = compost from Gainsville diet, FVW_{comp} = compost from fruit and vegetable waste.

The characteristics of the fruit did not show significant differences either (Table 10). The fruit length of the unfertilized plants was slightly lower than the other treatments, while the fruits treated with FVW_{comp} had the lowest diameter. The pH value was around 4.6 for all treatments, while there was a slight difference in soluble solids between the plants treated with compost and the fertilized or unfertilized plants.

Table 10. Mean (±SD) of marketable fruit weight, pH, and °Brix.

Treatment [a]	Fruit Traits			pH	Soluble Solids
	Lenght (cm)	Diameter (cm)	FSI [b]		°Brix
Unfertilized	6.6 ± 0.2	4.8 ± 0.2	1.4 ± 0.0	4.6 ± 0.2	5.2 ± 0.7
Fertilized	6.8 ± 0.2	5.0 ± 0.3	1.4 ± 0.0	4.7 ± 0.2	5.2 ± 0.6
GD_{comp}	6.8 ± 0.2	5.1 ± 0.2	1.3 ± 0.0	4.6 ± 0.3	5.0 ± 0.3
FVW_{comp}	6.6 ± 0.3	4.8 ± 0.3	1.4 ± 0.0	4.6 ± 0.2	5.0 ± 0.7

[a] GD_{comp} = compost from Gainsville diet, FVW_{comp} = compost from fruit and vegetable waste. [b] FSI = fruit shape index.

The analysis provided by PCA (Figure 8) confirmed how the different treatments do not clearly differ from each other. The main productive traits (such as number and weight of marketable fruits) were associated with the fertilized treatment or FVW_{comp} application. On the other hand, fruit characteristics and the number of immature fruits were shifted more toward GD_{comp}. Overall, the two main components explain 61.9% of the total variability.

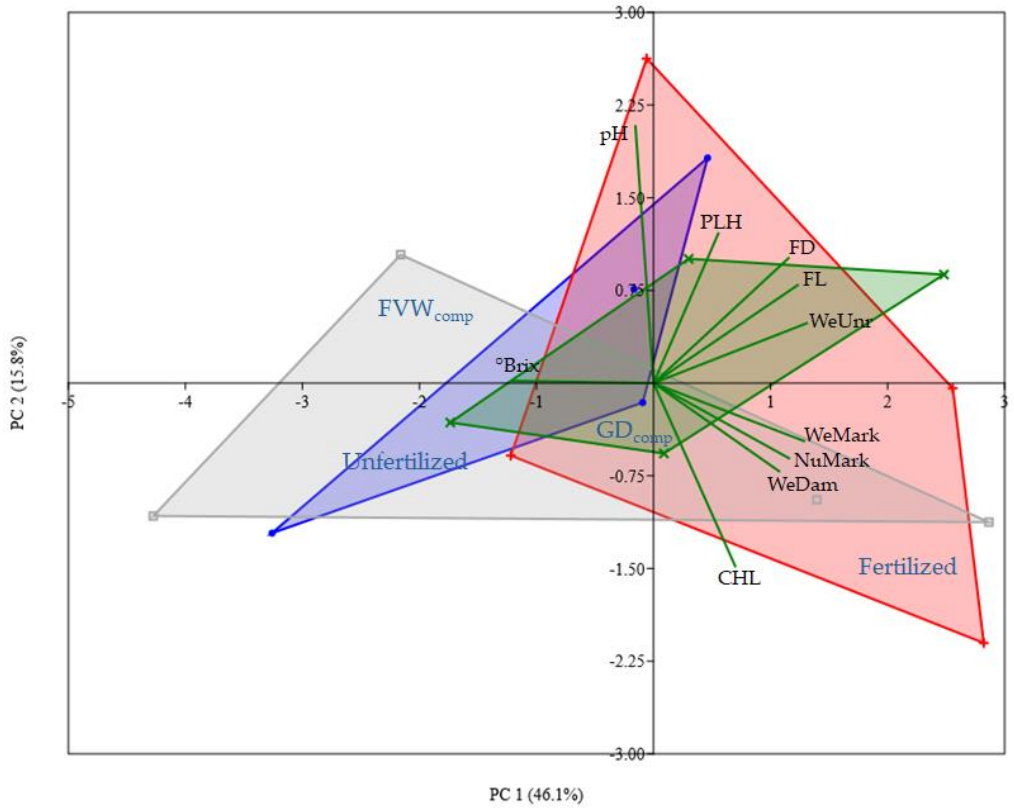

Figure 8. Biplot showing the PCA results of treatment separation based on the observed traits. °Brix (soluble solids); CHL, chlorophyll content; FD, fruit diameter; FL, fruit length; NuMark, number of marketable fruits per plant; PLH, plant height; WeMark, weight of marketable fruits per plant; WeUnr, weight of unripened fruits per plant; WeDam, weight of damaged fruits per plant. Polygon colors: purple, Unfertilized; pink, Fertilized; green, GD_{comp}, compost from Gainsville diet; grey, FVW_{comp}, compost from fruit and vegetable waste.

4. Discussion

This aimed to analyze the influence of rearing conditions (feeding regimes and rearing scales) on bioconversion efficiency parameters of BSFL in view of system scale-up, comprising the whole supply chain till the field evaluation of the composted frass. Fruit- and vegetable-based diets represent the largest amount of useable organic waste from large-scale retail trade and from local markets, together with a wide range of by-products from the agrifood industry.

FVW supply from the supermarket was heterogeneous. The heterogeneity was not related to the seasonality because of the continuous presence of greenhouse-grown products provided off-season. A wide range of fruits and vegetables (domestic and exotic) were provided by the supermarket and used in the various tests in the different compositions and proportions as they arrived. Similarly, the supply of BW was very variable in quantity (sometimes huge, sometimes few, since it was as a priority targeted to ethical food banking) and quality (pizza and sweets contain fats and carbohydrates). For this reason and to respect a real scenario of a continuous supply by several supermarkets to a CORS-based bioconversion plant mediated by BSFL, the qualitative and quantitative compositions of each supply were not considered as a variable.

The addition of bakery waste (BW) or bran (BR) was effective in lowering the initial FVW moisture content, absorbing a part of the excess leachate, and facilitating the bioconversion process. In contrast, the addition of brewer's spent grain (BSG) was not sufficient to lower the moisture content to the needs of BSFL. A combination of the three types of waste (FVW, BW, and BSG) could represent an improvement in the composition of the substrate and thus the efficiency of the bioconversion process. In addition to the moisture content,

the feeding rate (amount of substrate/larva/day) is an important factor that influences the bioconversion process. Diener et al. [14] evaluated the effects of different feeding rates on waste reduction and final biomass production, demonstrating that the optimal value was 0.1 g/larva/day if a balanced feed from the nutritional point of view was ensured. Parra Paz et al. [21] estimated as the ideal condition of BSFL feeding rate a range between 0.095 and 0.163 mg/larva/day. The present study demonstrated that, with the combination FVW:BW, valuable yields of larval biomass and added-value products can be obtained at a low feeding rate (0.066–0.089 g/larva/day), no matter the production scale. Higher yields are attainable (as observed also in our work) with high feeding rate but, when applied to a firm scale, it means higher operating cost. Thus, a trade-off between valuable production and wise economic management must be considered.

BSFL can be reared on a wide range of organic substrates [34] but the bioconversion process is influenced by feedstock characteristics [10]. It was shown that dietary protein and nonstructural carbohydrate contents are primary determinants of bioconversion efficiency [17,22]. Nevertheless, high dietary protein levels do not necessarily lead to higher larval performance [12] since the amount, quality, and ratio of protein, carbohydrates, and lipid should be considered as well [52]. Compared to other studies [53] and considering BSFL nutritional needs [22], the substrates used in this work were poor in protein and lipid content, with a high percentage of carbohydrates. This led to a P:C ratio higher than the optimal rates of 1:2–1:3 [11,54]. Nevertheless, mature larval weights did not differ from those observed by other authors using FVW as the main substrate for BSFL rearing [20,55,56]. At a low feeding rate, the maximum larval weight (MLW) was around 250 mg/BSFL for FVW:BW and remained around 200 mg/BSFL with FVW:BSG and FVW:BR mixtures. The best results in terms of MLW were achieved by using a substrate with a P:C ratio of 1:5.5–1:6.5, which means an unbalanced diet, with a high percentage of carbohydrates. Such data suggest that BSFL are able to exploit poor nutritional diets to reach a body mass comparable to supplying a richer diet, such as kitchen waste with a high level of protein and fat [57].

Shorter development time is especially important, as it leads to less production of greenhouse gases such as CO_2 and NH_3 during the waste bioconversion process by BSFL [58]. In this work, the larval lifetime until the mature stage (i.e., at highest larval weight) was influenced by the scale of the trials, confirming how the transition from laboratory to mass rearing of insects could affect this parameter [59,60]. In the MS trial, BSFL had a development time not exceeding 14 days, while, in LS condition, the lifetime lasted up to 19 days. The weight decrease following the MLW is typical of the prepupae, the last larval stage before pupation. In this phase, the larvae empty their digestive tube, do not feed anymore, and move away from the food source towards a place to pupate [10]. Similarly, Caruso et al. [61] obtained mature larvae under mass production conditions in a time (14 days) longer than under laboratory conditions (9 days). Arabzadeh et al. [62] evaluated that the required time to attain 40% prepupae was 15 days. In their study, they suggested that the faster development time could be due to higher levels of proteins in the diet rather than the presence of BSG and bread in addition to FVW. Other studies reported that BSFL fed with vegetable-based diets completed their larval cycle in 36–52 days [34,55]. Meneguz et al. [63] found a significant difference in the development time comparing BSG diet (8 days) with FV diets (20–22 days), where BSG has a considerably higher protein content. However, protein level was not the key factor in our study, because, despite having a low protein content compared to the carbohydrates, the percentage of larval crude protein was in agreement with those of Arabzadeh et al. [62].

Although the addition of BW and BR did not show linearity between MS and LS trials, probably due to the better conditions in terms of moisture content, the percentage of waste reduction was the highest, ranging from 59.8 to 79.3%. These results are in line with Arabzadeh et al. [62] and Candian et al. [20], who showed that, with the addition of bread to FVW, the substrate reduction reached values of 66.8 and 76.9%, respectively.

In the current study, BSFL reared on FVW with added BW, in both the scales, had the highest crude lipid content. The role of carbohydrates-to-lipids conversion has been well documented [53,64]. Again, the results are nonlinear between MS and LS trials; only in LS does the lipid content of BSFL appear to be affected by the carbohydrate content of the substrates, with the lowest values in BSFL fed with FVW:BSG or FVW:DS. These results, ranging from 24.8 ± 0.3 to 45.7 ± 1.0%, are similar to values found in other studies for FVW-fed larvae [62,65]. Larval crude protein content, ranging from 23.7 ± 0.5 to 37.1 ± 1.3, with the lowest values in FVW:BW, appeared to mirror the protein content of the substrate. This result is in contrast with the findings of Spranghers et al. [53] and Tschirner & Simon [66], who reported that crude protein was higher in larvae fed on a diet with the lowest crude protein content. Nevertheless, the percentages of BSFL crude protein are in agreement with other authors [9,63].

A significantly lower percentage of crude protein content of substrate than BSFL confirmed the efficient capacity of larvae to convert dietary proteins into larval protein biomass [22,34]. The trend of PrCR values was not perfectly comparable between the medium and large scale. In MS trials, the highest values were obtained for FVW:BW and FVW:BSG, while, in LS trials, for FVW:BSG and FVW:DS. In addition, in LS condition, the PrCR showed higher values than in MS. In any case, the PrCR values were in line with the results of Lalander et al. [34] and Arabzadeh et al. [62], who tested FVW diets for BSFL feeding. Lower efficiency in PrCR values in the MS trial and, in general, the disagreement between the scales could be explained by the influence of the scale. At the laboratory scale, with small amounts of larvae, BSFL performance could be more affected by rearing conditions (substrate, temperature, and relative humidity), with a reduction in bioconversion efficiency [65]. These results show how relevant the scale factor is for BSFL rearing and, as for key performance variables (e.g., waste reduction, lipid content, and PrCR), the transition from a benchtop to a large/industrial scale may not be linear [30,67]. In the setting up of the experiments, it was not possible to maintain the same proportion between the quantity of feed provided to BSFL and the size of the container. In LS trials, the fresh food column (about 12–13 cm) was reduced to 4–5 cm during the bioconversion process because of larval activity and water evaporation. Considering the number of starting larvae and the surface area of the container, the final density was about 3.5 BSFL per cm^2. In the MS trials, the larvae were more affected by the weight of the food column weighing on them. In a preliminary test we observed that the same rearing conditions produced much higher amounts of leachate than in LS. Under these conditions, the larvae were unable to co-operate in bioconversion easily because of the lack of available oxygen. Therefore, the food column was halved to about 6–7 cm, halving the number of larvae and the total amount of food. This way, we had about 1.7 BSFL per cm^2, which is still within the optimal range of larval density obtained by Parra Paz et al. [21] between 1 and 5 BSFL/cm^2. Therefore, the conditions for the best performance of the bioconversion process and the parameters to be set (whether they are expressed as feeding regime, larval density, or feeding rate) cannot necessarily be the same on a small, medium, and large scale, thus causing the nonlinearity of the scaling up. Results on BSFL rearing may provide the basis to compare findings from previous small-scale studies. Moreover, they are a paradigm to help in optimizing the mass-rearing conditions of BSFL fed with FVW and agro-industrial by-products.

The most obvious finding from the results of the field trial is the lack of significance between different treatments. There is a trend that indicates a decreasing productivity according to the order Fertilised > FVW_{comp} > Unfertilized > GD_{comp}. Nevertheless, based on the data collected, it is not possible to state if this is due to an intrinsic difference among the fertilization strategies applied. A possible cause may be associated with the high experimental variability responsible for masking the real differences. However, the magnitude of the observed differences does not appear particularly consistent, and it can therefore be an indication of a real equivalence of effects, in particular, between chemical fertilization and compost administration. Although the tested compost was not able to completely

equate the results obtained with the synthetic fertilizer, the yields were comparable. This observation is supported by the work of Anyega et al. [26], who, by comparing composted BSF frass fertilizer (BSFFF), composted brewer's spent grain, commercial organic fertilizer (Evergrow), mineral fertilizer, and some combinations, observed a substantial equivalence between BSFFF and chemical fertilization in terms of tomato yield in both greenhouse and in open field conditions. The two treatments were only overcome by the combination of BSFFF with synthetic fertilizer. The reader must be aware that only the repeated medium- and long-term administration of compost at agronomic or organic doses triggers improvement trajectories affecting both the physical and hydraulic properties of the soil [68] and the bulk soil chemical and biological parameters, thus contributing to decreasing the amount of conventional fertilizer [69].

The results obtained using FVW_{comp} were slightly higher than with GD_{comp}. This could be explained by a better composition in terms of micro- and macro-nutrients, which favored a higher production of marketable fruits. Based on the C/N ratio value, GD_{comp} appeared more mature and, therefore, more stable than FVW_{comp}. From a nutritional point of view, this means a slower nutrient release than FVW_{comp}. Conversely, FVW_{comp}, still not fully mature, could promote a faster release and, therefore, a prompt and greater availability of nutrients. The fact that the production of plants fertilized with GD_{comp} included the highest proportion of unripened fruits would confirm the effect.

5. Conclusions

The primary objective of project Hermes was to create an operating model to valorize the waste of a large supermarket and by-products of the agro- or food industry within the territory of the Lazio Region, carrying out experiments at a large-scale level. The heterogeneity of fruit and vegetable waste coming from a large distribution did not appear a limiting factor about the possibility to standardize the production in an insect farm as well as to organize the bioconversion process at an industrial scale. The study demonstrates that the addition of co-substrate such as bakery waste or bran to fruits and vegetable waste lowers the final moisture content and improves larval growth. At a large scale, an increase in the ratios of larvae to feed, even if it resulted in inducing individual lower larval weight, produced high larval biomass as well as substantial total production of crude proteins and lipids. The transition from medium scale to large scale in this study resulted in being partially linear. So, it is possible to expect that, by modifying the feeding regime (and we noticed that there is room for a reduction) and by studying the proportions between the basic substrate (FVW) and the co-substrates, we can identify a model for the development of an insect farm based on the waste supplies of one or more supermarket chains.

The fertilizer trials found no significant difference between fertilized and unfertilized plants regardless of the type of fertilizer used. However, the data from the field trial provided indications about the possibility of using the compost obtained from the residues of BSF rearing as a soil conditioner even in partial replacement of the amount of synthetic fertilizer. Such a result increases the degree of integration of the bioconversion system within a circular economy context.

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