Bioconversion of water treatment sludge and food waste with soldier fly larvae to produce fat with energy

Bioconversión de lodos de tratamiento de aguas residuales y residuos alimentarios con larvas de mosca soldado para producir grasa con energía

Bioconversão de lodo de tratamento de esgoto e resíduos alimentares com larvas de mosca-soldado para produzir gordura com energia

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Abstract

Introduction: This article presents the results of research conducted in 2025 at the Universidad de América in collaboration with the Universidad Distrital, entitled "Evaluation of the biotransformation process of food waste for the production of biodiesel and organic fertilizers using black soldier fly larvae (*Hermetia illucens*)." The study evaluates the bioconversion of wastewater treatment sludge and food waste using *Hermetia illucens* larvae to obtain lipid-rich biomass for energy production within a circular economy approach.

Problem: The increasing generation of sludge and food waste generates environmental impacts and limits resource recovery, thereby requiring sustainable strategies that convert waste into value-added bioenergy products.

Objective: To assess the feasibility of producing energy-rich lipids through larval bioconversion of sludge–food waste mixtures, optimizing substrate ratios and downstream processes.

Methodology: Four treatments with different sludge/food waste proportions were evaluated. Indicators included survival, growth, waste reduction, and bioconversion efficiency. Batch and countercurrent washing were compared; drying was evaluated at 60 °C, 75 °C, and 100 °C; and oil extraction was performed using Soxhlet (hexane), countercurrent ethanol extraction, and mechanical pressing. Data were analyzed using one-way ANOVA ($\alpha = 0.05$).

Results: A higher proportion of food waste improved survival (>95%), growth, and lipid yield. Countercurrent ethanol extraction achieved the highest recovery (33.27%), exceeding Soxhlet extraction (27.70%) and mechanical pressing (0.055%). Drying at 100 °C was the most energy-efficient, while countercurrent washing reduced water consumption by 80%.

Conclusion: Optimized feeding conditions and resource-efficient operations enable the sustainable conversion of sludge–food waste mixtures into lipid-rich biomass for bioenergy applications.

Originality: The study integrates substrate design, water-efficient washing, energy-efficient drying, and green extraction techniques within a scalable framework.

Limitations: The study was conducted at laboratory scale.

Keywords: Bioconversion, waste, lipids, sustainability, *Hermetia illucens*.

Resumen

Introducción: Este artículo presenta los resultados de la investigación titulada "Evaluación del proceso de biotransformación de residuos alimentarios para la producción de biodiésel y fertilizantes orgánicos utilizando larvas de mosca soldado negra (*Hermetia illucens*)", desarrollada en 2025 en la Universidad de América en colaboración con la Universidad Distrital. El estudio evalúa la bioconversión de lodos de tratamiento de aguas residuales y residuos alimentarios utilizando larvas de *Hermetia illucens* para obtener biomasa rica en lípidos destinada a la producción de energía dentro de un enfoque de economía circular.

Problema: La creciente generación de lodos y residuos alimentarios genera impactos ambientales y limita la recuperación de recursos, lo que exige estrategias sostenibles que conviertan estos residuos en productos bioenergéticos de valor agregado.

Objetivo: Evaluar la viabilidad de producir lípidos ricos en energía mediante la bioconversión larvaria de mezclas de lodos y residuos alimentarios, optimizando las proporciones de sustrato y los procesos posteriores.

Metodología: Se evaluaron cuatro tratamientos con diferentes proporciones de lodos y residuos alimentarios. Los indicadores incluyeron supervivencia, crecimiento, reducción de residuos y eficiencia de bioconversión. Se compararon el lavado por lotes y el lavado en contracorriente; se evaluó el secado a 60 °C, 75 °C y 100 °C; y la

extracción de aceite se realizó mediante Soxhlet (hexano), extracción en contracorriente con etanol y prensado mecánico. Los datos se analizaron mediante ANOVA de un factor (α = 0,05).

Resultados: Un mayor contenido de residuos alimentarios mejoró la supervivencia (>95%), el crecimiento y el rendimiento de lípidos. La extracción con etanol en contracorriente presentó la mayor recuperación (33,27%), superando a Soxhlet (27,70%) y al prensado (0,055%). El secado a 100 °C fue el más eficiente desde el punto de vista energético, y el lavado en contracorriente redujo el consumo de agua en un 80%.

Conclusión: La optimización de la alimentación y de las operaciones eficientes en el uso de recursos permite la conversión sostenible de mezclas de lodos y residuos alimentarios en biomasa rica en lípidos para aplicaciones bioenergéticas.

Originalidad: Integra el diseño del sustrato, el lavado con ahorro de agua, el secado eficiente y la extracción ecológica dentro de un marco escalable.

Limitaciones: Estudio desarrollado a escala de laboratorio.

Palabras clave: Bioconversión, residuos, lípidos, sostenibilidad, *Hermetia illucens*.

Resumo

Introdução: Este artigo apresenta os resultados do projeto de pesquisa intitulado "Avaliação do Processo de Biotransformação de Resíduos Alimentares para a Produção de Biodiesel e Fertilizantes Orgânicos Utilizando Larvas da Mosca Soldado Negra (Hermetia illucens)", realizado em 2025 na Universidade da América em colaboração com a Universidade do Distrito de Columbia. O estudo avalia a bioconversão de lodo de tratamento de águas residuais e resíduos alimentares utilizando larvas de Hermetia illucens para obtenção de biomassa rica em lipídios para produção de energia dentro de uma estrutura de economia circular.

Problema: A crescente geração de lodo e resíduos alimentares causa impactos ambientais e limita a recuperação de recursos, exigindo estratégias sustentáveis que convertam esses resíduos em produtos bioenergéticos de valor agregado.

Objetivo: Avaliar a viabilidade da produção de lipídios ricos em energia por meio da bioconversão larval de misturas de lodo e resíduos alimentares, otimizando as proporções de substrato e os processos subsequentes.

Metodologia: Quatro tratamentos com diferentes proporções de lodo e resíduos alimentares foram avaliados. Os indicadores incluíram sobrevivência, crescimento, redução de resíduos e eficiência de bioconversão. A lavagem em lote e a lavagem em contracorrente foram comparadas; a secagem a 60 °C, 75 °C e 100 °C foi avaliada; e a extração de óleo foi realizada utilizando extração Soxhlet (hexano), extração com etanol em contracorrente e prensagem mecânica. Os dados foram analisados utilizando ANOVA de uma via (α = 0,05).

Resultados: Um maior teor de resíduos alimentares melhorou a sobrevivência (>95%), o crescimento e o rendimento de lipídios. A extração com etanol em contracorrente apresentou a maior recuperação (33,27%), superando a extração Soxhlet (27,70%) e a prensagem (0,055%). A secagem a 100 °C foi a mais eficiente em termos energéticos, e a lavagem em contracorrente reduziu o consumo de água em 80%.

Conclusão: A otimização da alimentação e das operações com uso eficiente de recursos permite a conversão sustentável de misturas de lodo e resíduos alimentares em biomassa rica em lipídios para aplicações em bioenergia.

Originalidade: Integra o design do substrato, lavagem com economia de água, secagem eficiente e extração ecologicamente correta em uma estrutura escalável.

Limitações: Estudo conduzido em escala laboratorial.

Palavras-chave: Bioconversão, resíduos, lipídios, sustentabilidade, *Hermetia illucens*.

1. INTRODUCTION

The increasing generation of organic waste, together with the search for alternative sources of energy and sustainable materials, has driven the development of efficient bioconversion technologies. In this context, black soldier fly (*Hermetia illucens*) larvae have emerged as a promising biotechnological solution for the treatment of organic waste, enabling the production of value-added products such as lipids and proteins [1].

Strategies based on the formulation of food waste diets with specific nutrient compositions, as reported in [2], significantly improve larval growth performance and consistency. This approach, analogous to that used in livestock production, involves adjusting feeding conditions to maximize larval growth and, consequently, obtain an improved final fatty acid composition [3]. The use of organic waste as a nutrient source [4] in larval diets promotes the efficient utilization of different types of waste, enhances the sustainability of operations, and increases the bioconversion rate associated with this method, with potential applications at a global scale [5].

Previous studies [6] have analyzed the role of ventilation in the biotransformation of high-moisture waste, demonstrating that aeration directly affects the dry separation of larvae from the substrate. Moisture content significantly influences both the bioconversion process and larval survival rates; therefore, achieving efficient dry separation is beneficial for biomass recovery.

Additionally, alternative oil extraction methods from black soldier fly (*Hermetia illucens*) larvae have been explored [7], confirming the feasibility of obtaining lipids through sustainable techniques such as decoction, microwave-assisted extraction, solvent maceration, and ultrasound. These methods yield fatty acid profiles consistent with those reported in the literature, characterized by a high concentration of lauric acid. Furthermore, [8] highlight that lipid extraction from biomass is a critical step in biofuel production. Conventional methods such as Soxhlet extraction with solvents (e.g., petroleum ether) enable high yields without degrading lipid compounds. The lipid profile of *Hermetia illucens* is dominated by saturated fatty acids, particularly lauric acid (53.43%), followed by palmitic and stearic acids, while unsaturated fatty acids such as oleic and linoleic acids are present in lower proportions, influencing oxidative stability and thermal behavior (Almeida et al., 2022).

2. MATERIALS AND METHODS

The optimization process was structured into sequential stages, beginning with the rearing of black soldier fly (*Hermetia illucens*) larvae sourced from Neiva, Colombia.

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The process included larval hatching, growth, and substrate biotransformation. The substrate was characterized, and the diets used during the experimental phase were selected accordingly. Subsequently, unit operations including separation, washing, drying, and oil extraction were evaluated through different methods in order to identify the conditions and mechanisms that enable improved resource utilization.

Characterization of the substrate

The substrate consisted of wastewater treatment sludge from the Salitre Wastewater Treatment Plant and organic solid waste [9] collected from the cafeteria of the Universidad de América in Bogotá, Colombia. The organic waste included pineapple peel, potato peel, coriander, onion, tomato, mango peel, papaya, melon, and lettuce in different proportions. Samples were collected in March 2025 and evaluated for physicochemical characteristics to determine the influence of dietary composition on larval growth, digestion, and lipid yield. Measurements were conducted five days after collection.

Moisture determination.

The technical standard ISO 11465 [10] was followed, modifying the drying temperature to 60°C in order not to degrade the substrate in an oven (BINDER E28, BINDER®, Germany, Resolution: 1°C).

Ash determination.

The technical standard ISO 18122 [11] was followed, in a muffle furnace (Barnstead Thermolyne® 48000, Thermo Fisher Scientific, USA, Resolution: 1°C).

pH determination.

The technical standard ISO 10390 [12] was followed, without using saline solution, measuring with a potentiometer (APERA PH700, APERA®, China, Resolution: 0.01).

Lignin determination

The amount of lignin present in the substrates was determined following the ISO 21436 standard [13].

Preparation of the aqueous extract.

150 g of sludge and RSO samples were weighed in wet mass (WM), and added 200 ml of water and homogenised, transferred to a 250 ml beaker and left to settle for 45 minutes. [14].

Determination of Total Nitrogen.

Total nitrogen was determined using the Spectroquant® Total Nitrogen Test - ISO 11905-1 method [15].

Determination of Nitrites.

Total nitrite was determined using the Spectroquant® Nitrite Test Kit - EPA method 354.1 method [16].

Determination of Nitrates.

Nitrate determination was performed using the Spectroquant® Nitrate Test Kit - DIN 38405-9 method [17].

Determination of ammonium.

Ammonium was determined using the Spectroquant® Ammonium Test Tube - DIN 38406-5 method [18].

Total phosphorus determination.

The determination of total phosphorus was carried out using the Spectroquant® Total Phosphorus Test - method ISO 6878 [19].
Spectroquant® Total Phosphorus Test) - ISO 6878 method [19].

Determination of phosphates.

Phosphate determination was performed using the Spectroquant® Phosphate Test in Cuvettes - ISO 6878 method [19].A photometer (Spectroquant® NOVA 60- A, Merck KGaA, Germany, resolution: 0.01A) was used for all measurements with the cuvette test.

Larval Growth and Biotransformation.

Setup.

The growth of larvae fed four different diets/treatments was evaluated. The compositions are presented in Table 1, in dry mass. (MS). The larvae were fed on days 0, 5 and 10. Figure 1 shows the setups used for each treatment. Replicates (n=3) were performed for each treatment. Table 2 shows the experimental parameters of the setup, which were similar for all four treatments, especially the feeding rate, which is the amount of substrate (food) supplied to each larva per day [20].

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Larval density, which refers to the concentration of larvae in the rearing system, generally expressed as larvae per unit area (larva/cm²), influences competition for food and the microclimate generated [21].

Table 1. Composition of total feed supplied per diet.

Treatment	Sludge (%)	RSO (%)	Sludge (g)(DM)	RSO (g)(DM)
T1	100	0	144	0
T2	75	25	108	36
T3	25	75	36	108
T4	0	100	0	144

Source: own work

Table 2. Experimental parameters for larval rearing.

Parameter	Value
Number of larvae	200
Larvae mass (g)	0.7473±0.0142
Days	17
Feeding rate (mg (DM)/larva/day)	42.3
Container area (cm^2)	100
Larval density (larva/cm^2)	2

Source: own work

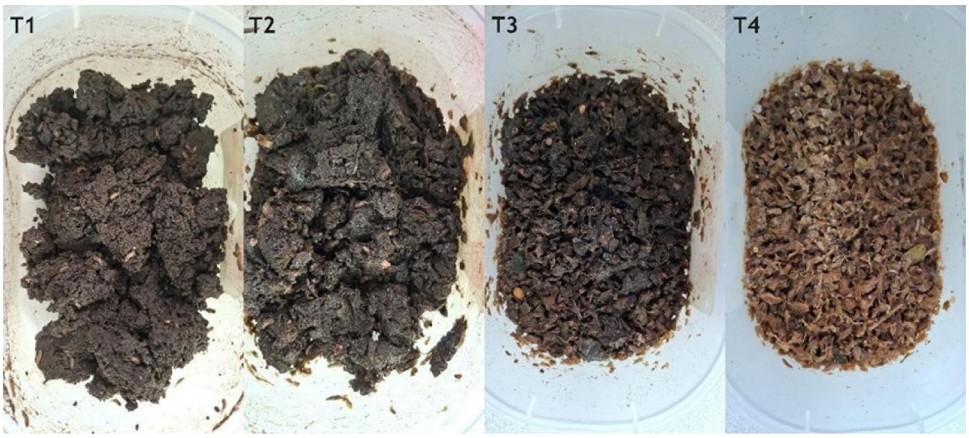

Figure 1. Assemblies by diet.
Source: own work

Bioconversion monitoring.

Table 3. Bioconversion monitoring equations.

Indicator	Equation	Citation
Survival Rate	$\text{Rate of } (\%) = \frac{larvas\ final}{larvae\ initial} * 100\ (1)$	[22]
Waste Reduction	$\text{of } Residues\ (\%MS) = \frac{\text{Feed}_{residual}\ (g)}{(1 - \text{Feed}_{Initial}\ (g))} * 100\ (2)$	[23]
Bioconversion Rate	$\text{Rate of } Bioconversion\ (\%MS) = \frac{\text{Mass Gain}\ (g)}{\text{Feed}_{Initial}\ (g)} * 100\ (3)$	[2], [24]
Bioconversion efficiency	$\text{Efficiency}\ (\%MS) = \frac{\text{Mass Gain}\ (g)}{\text{Feed}_{Consumed}\ (g)} * 100\ (4)$	[24]

Source: own work

Larvae Growth.

The initial and final weights of the larvae are compared on a dry basis, and the length and width of the larvae are measured using graph paper on a sample of six larvae per treatment.

Substrate evolution.

In order to observe possible changes in the substrate throughout the biotransformation process, 3 grams of sample are taken every 3 days from each of the treatments and the moisture, ash and pH measurements described above are carried out.

Washing.

Batch Washing.

This washing strategy involves washing the same batch of larvae at a ratio of 1:3 between larval mass and millilitres of water per wash, in 5 washes with fresh water in each, in which constant agitation is applied for 20 minutes, seeking to separate the dirt from the larvae with the water and agitation, and then separate the larvae with the help of a sieve. As shown in Figure 2.

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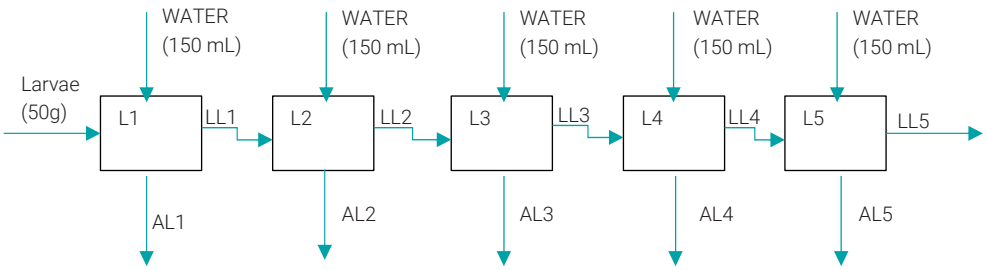

Figure 2. Batch washing system.
Source: own work

Countercurrent washing.

With which they were fed. For countercurrent washing, a proportion is used a countercurrent larval washing scheme is proposed in order to remove the dirt present on them after slaughter, dirt belonging to the substrate.1:3 between the mass of larvae and the millilitres of water used in washing (50 grams of larvae and 150 ml of water respectively), following the diagram in Figure 3.

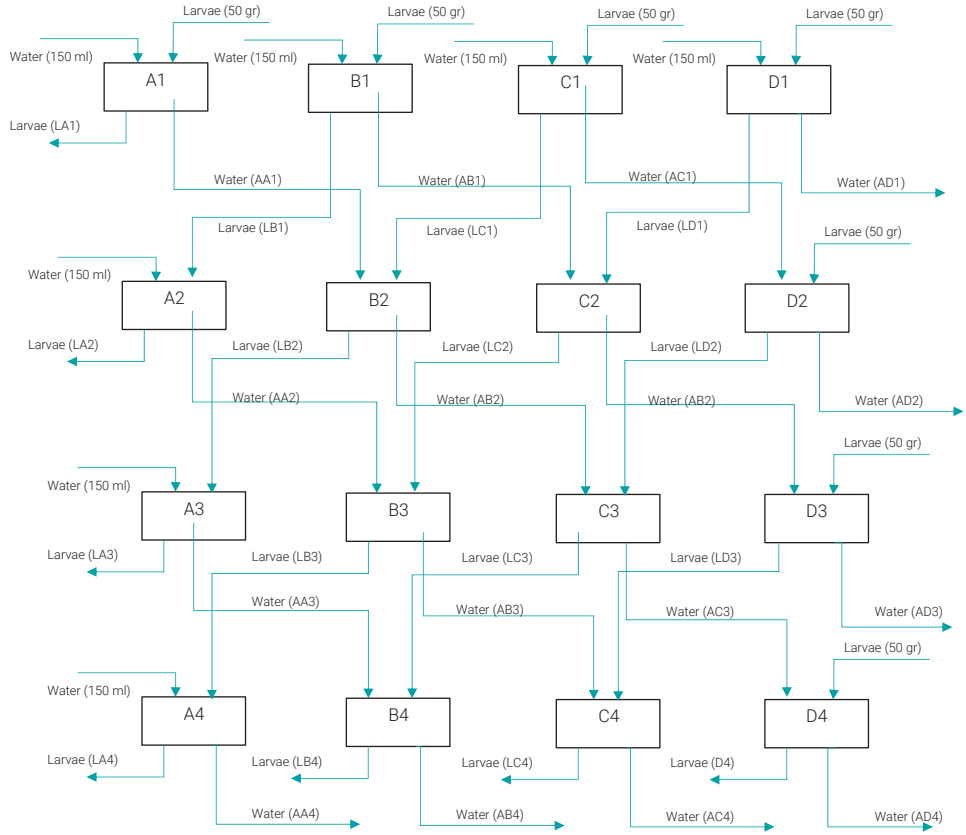

Figure 3. Countercurrent washing system.
Source: own work

Determination of Total Solids.

(BINDER E28, BINDER®, Germany, Resolution: 1°C) with the previously determination of total solids (TS) in the washing water was carried out using the SM 2540 B gravimetric method (APHA, AWWA, & WEF, 2023) using a balance (RADWAG AS 220/C/2, RADWAG®, Poland, Resolution: 0.1 mg). For this, 10 ml of water sample was taken from each batch and countercurrent wash.

Drying.

Three temperatures were evaluated for drying the larvae, a necessary preliminary procedure for oil extraction [25]. The gravimetric method was used to dry the larvae, seeking a constant weight for each temperature. A mass of 50 grams of previously washed larvae is placed in a drying oven programmed temperature (60°C, 75°C and 100°C), and in turn the consumption of the equipment will be measured using an electricity consumption meter (WM03-US, MECHEER US, China, Resolution: 0.1 W).

The mass of the larvae is measured every hour during the first 5 hours of drying, then every 2 hours, as is the electricity consumption data. The aim is to construct a drying curve.

Oil extraction.

Soxhlet extraction.

The technical standard ISO 659 [26] is followed.

Countercurrent extraction.

Countercurrent extraction is performed using a green solvent such as ethanol, following the diagram shown in Figure 4.

The process conditions for this extraction were a ratio of 1:5 between larval mass and millilitres of solvent. The operating conditions were a temperature of 50°C and 1 hour for each extraction stage. These conditions were chosen with the aim of promoting the solubilisation of lipids in ethanol. The excess solvent promotes extraction efficiency by maintaining a favourable concentration gradient, the temperature promotes viscosity reduction and contact between the phases, while the time allows equilibrium to be reached without unnecessarily prolonging the operation, reducing energy consumption and preventing oil degradation.

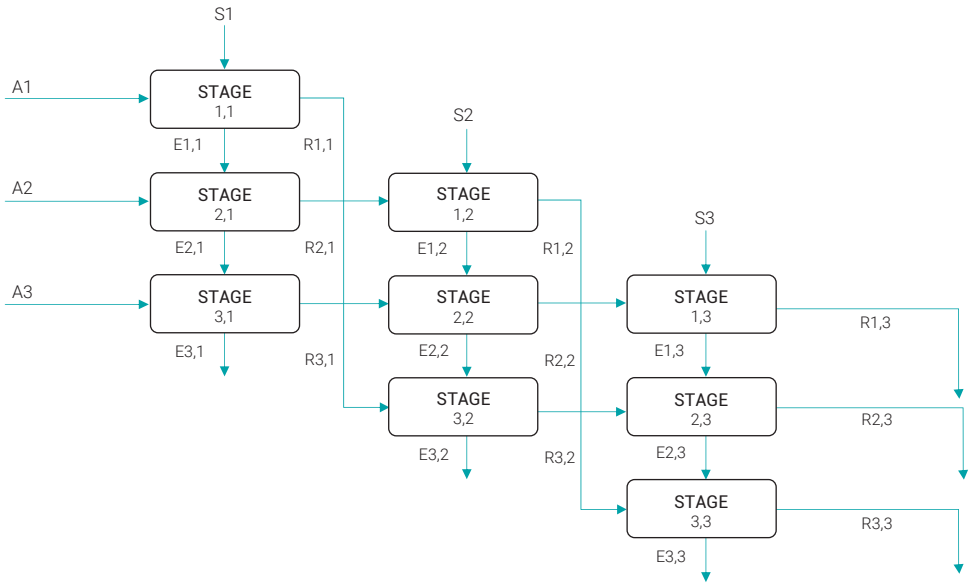

Figure 4. Countercurrent extraction system. Where A represents the mass input of dried larvae, S represents the input of new solvent, R represents the refined solid, and E represents the extract.

Source : own work

The quantification of oil at each extraction stage was performed by taking 1 mL of sample at each stage and drying it at 80°C to remove the ethanol, then calculating the total oil using Equation 1.

$$SampleOil\ \% = \frac{Mass\ (g)}{SampleMass\ (g)} * 100 \quad (1)$$

The composition of the extraction at each stage is calculated using Equation 2.

$$Oil_{Stage}\ (g) = Mass_{Extract}\ (g) * Oil_{Sample}\ (\%) \quad (2)$$

The total oil extracted from food 1 (A1) is calculated using Equation 3.

$$OilExtracted\ (A1) = Oil_{E1,1} + Oil_{E1,2} + Oil_{E1,3} \quad (3)$$

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The total oil extracted from food 2 (A2) is calculated using Equation 4.

$$OilExtracted\ (A2) = (Oil_{E2,1} - Oil_{E1,1}) + (Oil_{E2,2} - Oil_{E1,2}) + (Oil_{E2,3} - Oil_{E1,3}) \quad (4)$$

The total oil extracted from food 3 (A3) is calculated using Equation 5.

$$OilExtracted\ (A3) = (Oil_{E3,1} - Oil_{E2,1}) + (Oil_{E3,2} - Oil_{E2,2}) + (Oil_{E3,3} - Oil_{E2,3}) \quad (5)$$

The hexane extract is separated from the aqueous phase using a rotary evaporator (Hei-VAP Core, Heidolph Instruments GmbH & Co. KG, Germany, resolution: 0.1°C). It is dried at 70°C until a constant weight is achieved and the oil obtained is quantified.

Extraction yield.

The extraction yield was calculated in the same way for the three methods studied, using Equation 6 [27]

Extraction by mechanical pressing.

For extraction by pressing, a mass of larvae with 50% moisture content is used, which is continuously fed into the equipment through the hopper. The compression exerted by the screw (Oil Extractor, GaRcan, China, Resolution: 1°C) and its temperature (100°C) [28]forces the release of the liquid contained, which is collected in a collection jar [29]. This process is repeated on the same mass three times in order to obtain as much extract as possible. The weight data of the extract and the resulting solid are recorded. Subsequently, hot vacuum filtration is performed on the extract obtained. From the filtered and heated extract, a 10 mL sample is taken and subjected to simple liquid-liquid extraction with hexane to separate the oil from the aqueous phase. This is done for each of the three extracts obtained.

$$Performance\ (\%) = \frac{Mass\ (g)^{Oil} * 100}{MassSolidDry\ (g)} \quad (6)$$

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Statistical Analysis.

For the statistical analysis of the data obtained, a one-way analysis of variance (ANOVA) was applied with a significance level of 5% ($\alpha = 0.05$) in order to determine whether there were statistically significant differences between the treatments evaluated.

This analysis was performed using the ANOVA: one-way tool from the Microsoft Excel Data Analysis Tools add-in. Prior to this, the assumptions of normality and homogeneity of variances of the data were verified. In cases where significant differences were detected ($p < 0.05$), a comparison of means was performed using Tukey's test or subsequent graphical analysis.

3. RESULTS

Characterisation of Substrates (Waste and Sludge).

Table 4. Physicochemical properties of the substrate.

Property	Sludge	RSO
Moisture (%) (MH)	74.7634±0.0040	75.9992±0.0215
Ash (%) (DM)	1.3871±0.0017	1.2920±0.0006
pH (MH)	6.89±0.09	6.12±0.05
Lignin (%) (MS)	22.6167±0.0291	9.0200±0.0246
Total nitrogen (mg/kg) (MH)	17.07±1.09	17.56±0.27
Nitrates (mg/kg) (MH)	3.93±0.20	4.91±0.26
Nitrites (mg/kg) (MH)	12.36±0.33	12.89±0.27
Total phosphorus (mg/kg) (MH)	40.71±1.76	28.00±0.87
Phosphates (mg/kg) (MH)	124.36±0.13	2.42±0.10
Ammonium (mg/kg) (MH)	0.90±0.15	6.89±0.13

Source: own work

Monitoring of biotransformation.

Table 5. Monitoring of biotransformation by treatment.

Treatment	Survival Rate (%)	Waste Reduction (%)	Bioconversion Rate (%)	Bioconversion Efficiency (%)
T1	86.50±0.08	18.2300±0.0711	0.8162±0.0024	5.1896±0.0280
T2	97.83±0.02	37.0358±0.0489	4.8295±0.0020	13.1569±0.0140
T3	96.00±0.01	51.9171±0.0624	8.5179±0.0038	16.6356±0.0286
T4	99.50±0.01	50.7940±0.0283	9.3217±0.0084	18.3715±0.0169

Note. Prepared by the authors.

Larvae growth.

Table 6. Final Larvae Dimensions by Treatment

Treatment	Growth (%)	Length (cm)	Width (cm)
T1	483.4105±1.5401	0.95±0.01	0.20±0.01
T2	2975.1474±1.3594	1.75±0.02	0.45±0.02
T3	5052.6609±0.9001	2.20±0.02	0.50±0.01
T4	5631.9098±6.5473	2.15±0.02	0.50±0.01

Source: own work

Substrate Evolution Over Time.

Humidity.

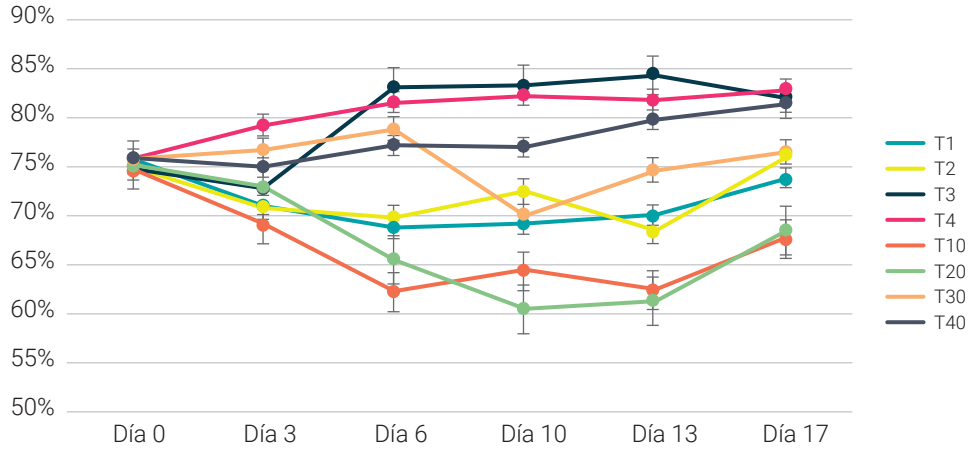

Figure 5. Evolution of humidity in the treatments.
Source: own work

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Ash.

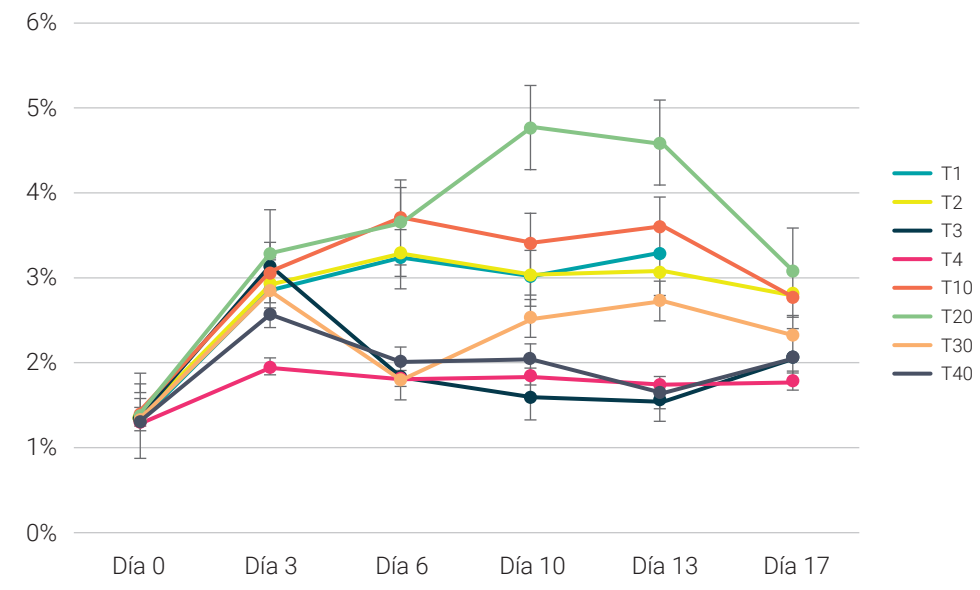

Figure 6. Evolution of ash content in the treatments.
Source: own work

pH.

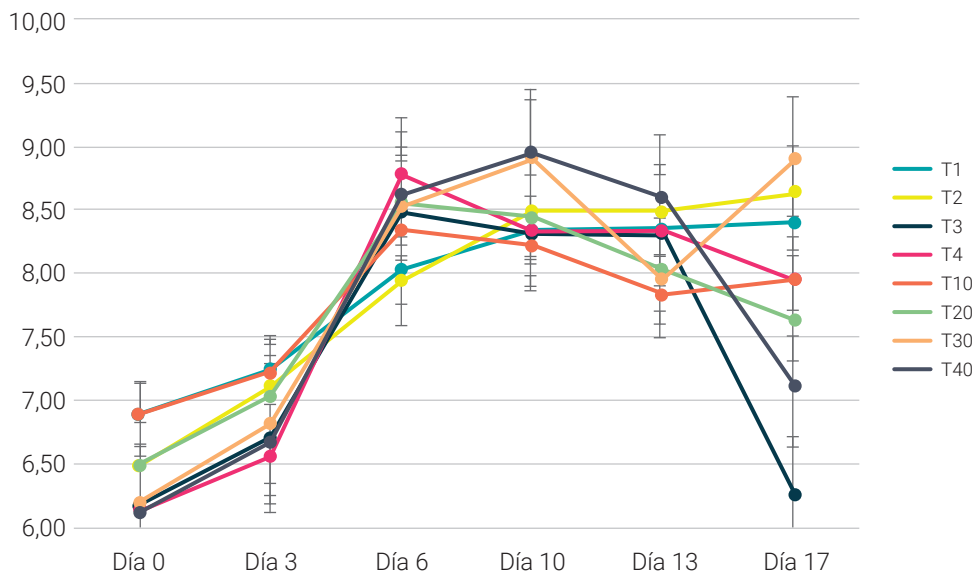

Figure 7. Evolution of pH in the treatments.
Source: own work

Obtaining of Oils According to the Treatment.

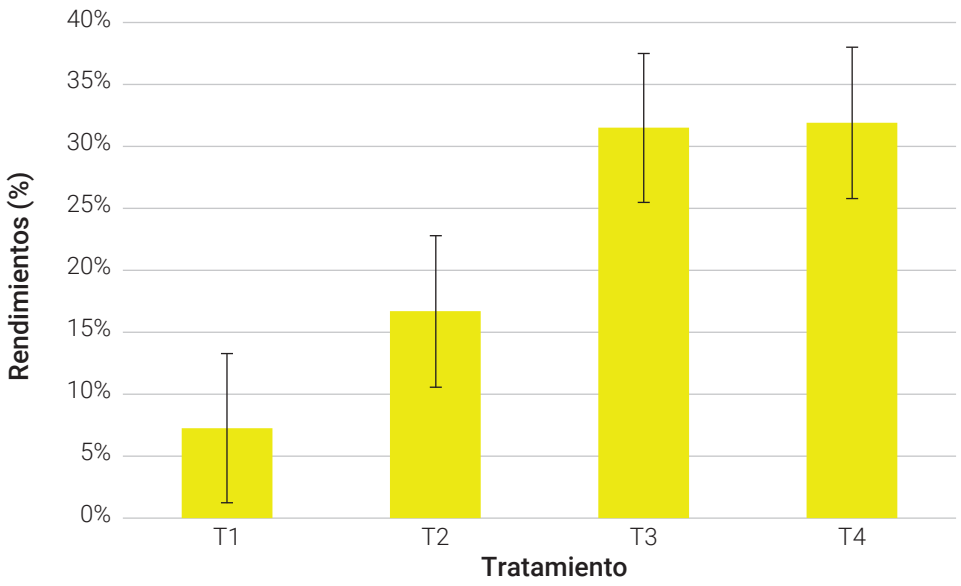

Figure 8. Oil Yield by Treatment.
Source: own work

Washing.

Batch washing.

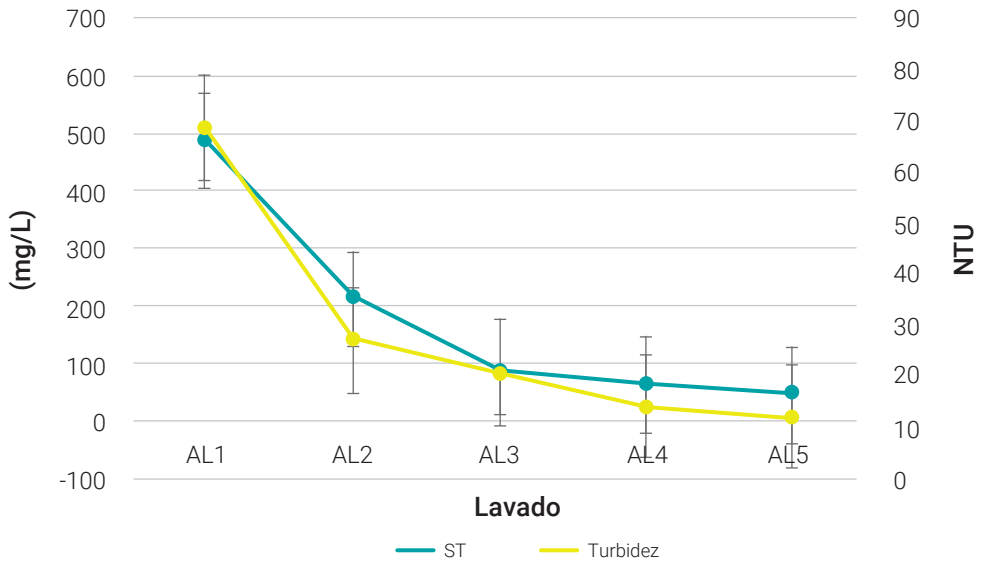

Figure 10. Turbidity and ST behaviour in batch washing.
Source: own work

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Countercurrent washing.

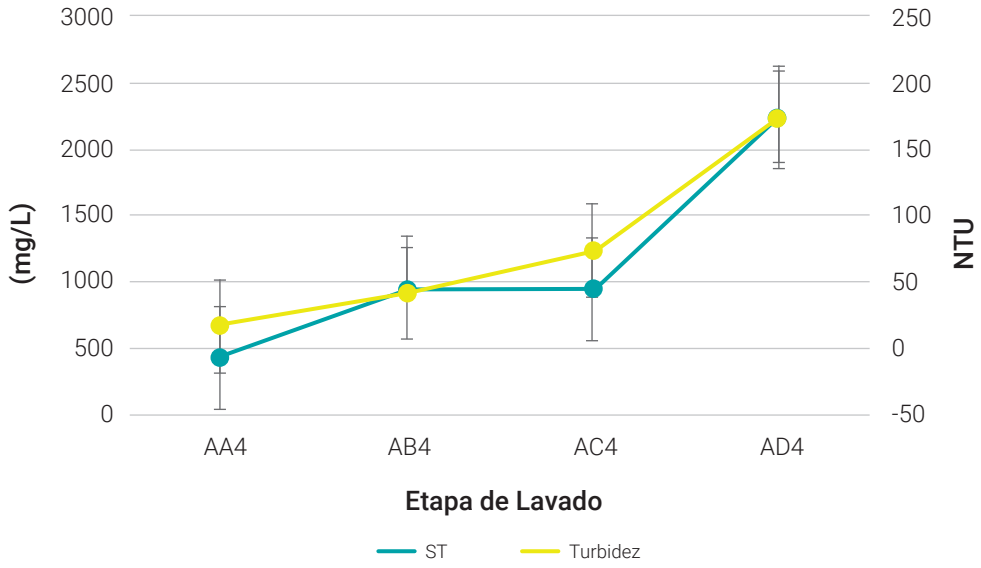

Figure 11. Turbidity and ST behaviour in countercurrent washing.
Source: own work

Drying.

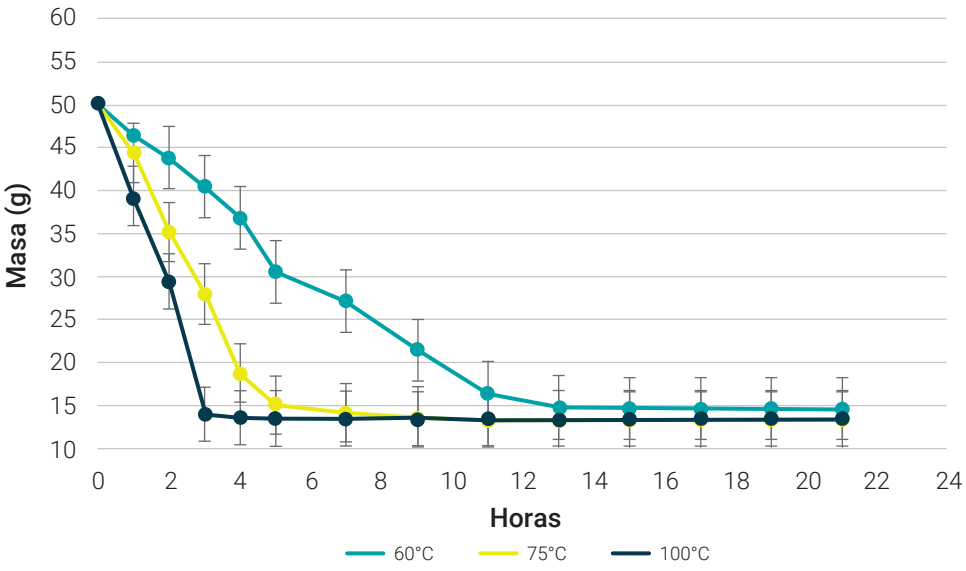

Figure 12. Drying curves.
Source: own work

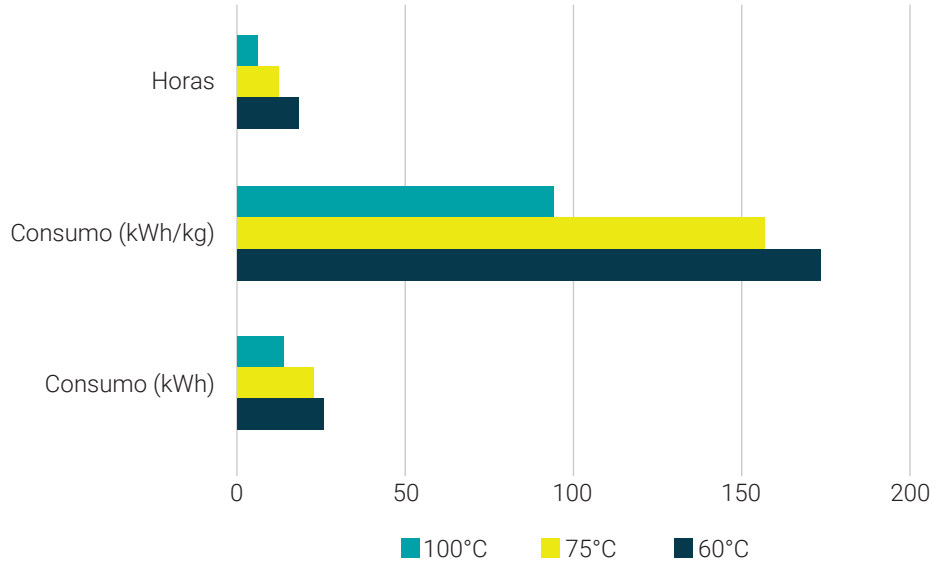

Figure 13. Time and energy consumption by temperature.
Source: own work

Oil extraction.

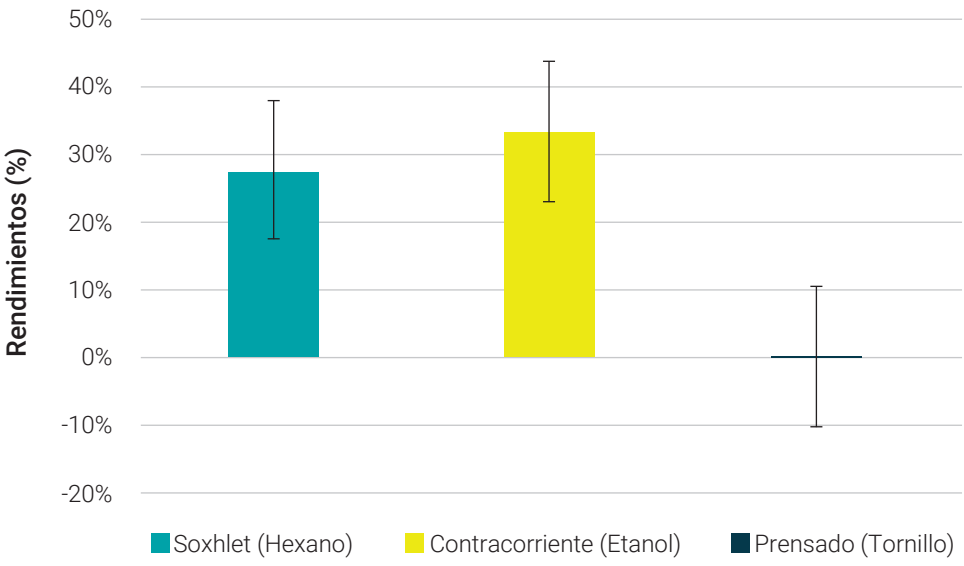

Figure 14. Oil extraction yield by method.
Source: own work

4. DISCUSSION

As shown in Table 3, both substrates exhibit similar moisture and ash contents. The observed moisture levels indicate that the substrates are suitable for biological processes, while the low ash content reflects a limited inorganic fraction. These parameters are consistent with values reported in the literature, although some differences are noted. For instance, [30] report a sludge pH of 7.12, while [4] describe sludge from the same treatment plant with a moisture content of 87.57%, highlighting variations with respect to the characterization obtained in this study.

The lignin content is higher in sludge, indicating greater resistance to biological degradation [31]. In contrast, the organic solid waste (RSO) facilitates larval bioconversion due to its lower lignin content and higher biodegradability.

The total nitrogen content in both substrates is comparable, as are nitrite and nitrate levels, suggesting similar nitrogen availability for larval metabolism. However, ammonium concentrations are higher in sludge, which can be attributed to its origin as domestic wastewater sludge.

Regarding phosphorus, sludge presents a higher total concentration; however, phosphorus in RSO exhibits greater bioavailability due to its higher phosphate content, whereas in sludge it is often bound to less soluble compounds.

Treatment T1 showed a survival rate below 95% compared to the other treatments. This result is associated with the limited accessibility of nutrients when sludge is used as the sole substrate, due to its resistance to biological decomposition, as previously discussed.

In contrast, treatments T2 and T3, which included mixtures with RSO, as well as T4, composed exclusively of this substrate, exhibited survival rates above 95%. This suggests that the inclusion of RSO enhances nutrient availability. Additionally, the acidic pH of this waste favors enzymatic activity, reduces lignin content, and provides a physical structure more suitable for ingestion and digestion by the larvae.

Treatment T1 also showed significantly lower growth compared to the other treatments. In contrast, the remaining treatments demonstrated that RSO has a positive effect on larval development. The limited growth observed in T1 is reflected in larval size, which was 41.8% smaller than that observed in T4, consistent with its lower mass gain. Conversely, the dimensions observed in treatments T2, T3, and T4 indicate a more complete and favorable development.

Regarding substrate consumption, treatment T1 exhibited low intake, consistent with its reduced growth and survival rates. In contrast, treatments T2 and T3, which included RSO, showed improved consumption patterns, particularly T3, which

achieved the highest percentage of substrate consumption. In this case, larvae were able to partially hydrolyze the sludge, improving its ingestion, as illustrated in Figure 15.

Figure 15. Hydrolysed sludge in treatment T3.
Source> own work

Treatment T1 exhibited the lowest bioconversion rate and efficiency, as shown in Table 8. This confirms that, although substrate consumption occurred, the larvae's capacity to convert it into biomass was limited.

In contrast, treatments T2, T3, and T4 showed an increasing trend in both bioconversion rate and efficiency, indicating greater assimilation of the supplied feed and a higher capacity of the larvae to convert it into larval biomass.

As previously noted, treatment T4, despite presenting a feed consumption similar to that of treatment T3, achieved a bioconversion rate 0.8 percentage points higher and an efficiency 1.73 percentage points greater. This indicates that the larvae converted a higher proportion of the ingested feed into useful biomass, demonstrating superior performance when operating with 100% organic solid waste (RSO).

It is noteworthy that treatments T3 and T4, although exhibiting very similar consumption rates, showed slight differences in growth. This suggests that feed conversion efficiency does not depend solely on the amount of substrate consumed, but also on how effectively it is metabolized by the larvae.

Additionally, in treatments T2 and T3, only a small proportion of larvae reached the prepupal stage [32], whereas in treatment T4 more than 50% of the larvae reached this stage. This indicates that diets composed exclusively of organic solid waste favor larval development toward the prepupal stage, which is considered undesirable for oil production processes [33].

Figure 8 illustrates the oil yield obtained through Soxhlet extraction of the larval biomass produced in each treatment. Treatment T1 exhibited the lowest yield,

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consistent with its reduced consumption and growth, resulting in lower biomass accumulation and lipid content. This confirms that sludge alone is not a suitable substrate for lipid production through bioconversion using *Hermetia illucens* larvae.

The incorporation of organic solid waste improved oil yields. In particular, treatments T3 and T4 presented similar extraction yields, suggesting that the lipid accumulation in larvae was comparable, regardless of differences in bioconversion rate or efficiency.

Regarding the washing stage, the batch washing system, following the scheme presented in Figure 2, required a water consumption of 15 mL per gram of larvae. As shown in Figure 9, this method produces cleaner water at the end of the process, with lower turbidity and reduced total solids in the final effluent (AL5).

In contrast, the countercurrent washing system, based on the configuration shown in Figure 3, used only 3 mL of water per gram of larvae, representing a fivefold reduction compared to batch washing. However, this method resulted in higher turbidity and greater total solids content in the outlet stream (AA4), which may indicate a lower degree of cleaning and potential contamination of the larvae.

Thus, batch washing provides higher cleaning efficiency at the expense of greater water consumption, whereas countercurrent washing offers improved water-use efficiency but reduced cleaning effectiveness. The selection of the method depends on the operational scale: batch washing is more suitable for small-scale applications, while countercurrent washing is more efficient for large-scale processing.

The drying curves presented in Figure 12 reveal significant differences in mass loss rates as a function of temperature. At 100 °C, a rapid reduction in mass is observed, reaching near-constant weight within approximately 3 hours, indicating an efficient evaporation process and a short constant-rate drying phase, associated with high heat transfer. At 75 °C, intermediate behavior is observed, with equilibrium reached after approximately 5 hours. In contrast, at 60 °C, the drying process is considerably slower, requiring 12–14 hours to stabilize. This extended duration is attributed to the lower energy available to overcome water–matrix interactions within the larvae. These results highlight the direct influence of temperature on drying kinetics. Although higher temperatures enhance evaporation, they also increase the risk of thermal degradation or loss of volatile compounds if not properly controlled.

In terms of energy consumption, drying at 60 °C, despite lower instantaneous power usage, resulted in the highest total and specific energy consumption due to prolonged operation, exceeding 170 kWh/kg. At 75 °C, intermediate energy consumption values were obtained. At 100 °C, although higher instantaneous power was required,

the shorter processing time compensated for energy use, making it the most energy-efficient condition among the temperatures evaluated.

Regarding oil extraction, the method with the highest performance was countercurrent extraction with ethanol, as shown in Figure 14, followed by Soxhlet extraction with n-hexane. Mechanical pressing exhibited a negligible yield (0.055%).

Ethanol demonstrated strong affinity for the lipids present in the larvae during countercurrent extraction, achieving an extraction yield of 33.27%. It represents a more sustainable alternative due to its lower toxicity and potential for recovery. It was also observed that ethanol tends to become saturated with lipids, as illustrated in Figure 16. This method stands out due to the enhanced contact between biomass and solvent, which improves the extraction of partially polar lipids such as lauric acid, the predominant fatty acid in the oil [7].

Figure 16. Ethanol saturated with oil.
Source: own work

Soxhlet extraction achieved a high yield (27.70%), which is consistent with its classification as a conventional technique for oil extraction, primarily due to the non-polar nature of the solvent (n-hexane). However, this method presents operational risks associated with solvent volatility, toxicity, and flammability, and its scale-up may be costly due to the need for solvent recovery systems.

In contrast, screw pressing, which does not involve solvents, extracts only free or surface oil from the larvae. Additionally, the lack of sufficient moisture in the sample limits the efficiency of the pressing process, as moisture is required for proper equipment performance. The low yield obtained suggests that most of the oil is retained within the internal structure of the larvae rather than on the surface. To verify this, a subsequent Soxhlet extraction was performed on the residual solid, yielding 22.47%,

which confirms that a significant proportion of lipids remains in the solid fraction after pressing. Therefore, the pressing system primarily facilitates moisture removal rather than efficient oil extraction, making it an unsuitable method for lipid recovery in this context.

5. CONCLUSIONS

The results obtained in this study demonstrate the significant influence of substrate composition on larval growth, development, and productivity, indicating that a high proportion of organic solid waste (OSW) enhances survival rates and promotes efficient conversion into lipid-rich biomass.

The optimization of energy use during the drying process revealed that higher temperatures, particularly 100 °C, reduce oven operating time and total energy consumption without compromising the quality of the final product. Furthermore, the cleaning and separation stages identified countercurrent washing as an efficient strategy in terms of water consumption, achieving substantial savings while maintaining acceptable cleanliness levels.

The evaluation of different oil extraction methods demonstrated that countercurrent extraction with ethanol, used as a green solvent, provides the highest yield, surpassing the conventional Soxhlet method with hexane and representing a more sustainable and viable alternative.

Overall, the findings validate the feasibility of a technical process for waste valorization through bioconversion using *Hermetia illucens*, capable of generating value-added products—particularly lipids—through an efficient, optimized, and environmentally sustainable operation, with potential for industrial-scale implementation.

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