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Review

Hermetia illucens as an innovative feedstock for biodiesel: properties, production techniques, and regulatory compliance

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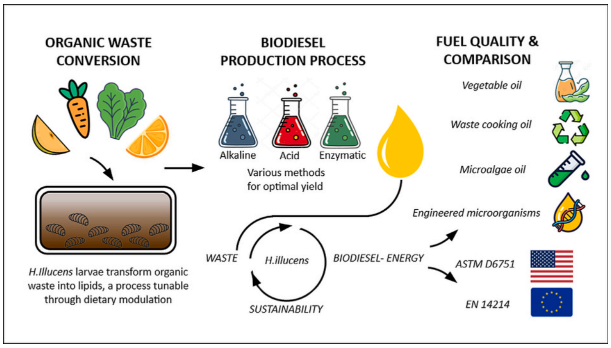
HIGHLIGHTS

- *H. illucens* is a sustainable feedstock for biodiesel.
- *H. illucens* converts organic waste into lipid-rich biomass for biodiesel.
- Biodiesel from BSFL meets EN 14214 and ASTM D6751 regulatory standards.
- Larval diet influences lipid profile and final biodiesel quality.
- BSFL biodiesel supports the circular economy and reduces fossil fuel dependency.

GRAPHICAL ABSTRACT



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ABSTRACT

The urgent need for sustainable energy is intensifying research on biodiesel feedstocks that cut environmental impact and fossil dependence. Among these, *Hermetia illucens* upcycles organic waste into lipid-rich biomass, enabling a circular bioeconomy. Compared with first- to fourth-generation feedstocks (edible oils, waste cooking oils/residues, microalgae, engineered microorganisms), it avoids food competition and dedicated cropland, supports diet-tunable lipid profiles, requires no water-intensive cultivation, and has a short production cycle; indicative carbon footprint values (~0.8 kg CO_2 eq/kg lipid) suggest advantages over conventional oils. This review critically examines the fuel properties of *H. illucens* biodiesel and benchmarks them across feedstocks. Under optimized conditions, literature-reported yields reach ~94–98 % (defined here as FAME/biodiesel mass yield on a lipid-feedstock basis). The fuel shows cetane ~50–58, kinematic viscosity 4.0–5.2 mm^2/s (generally compliant, near the EN upper bound), and flash point >120 °C; water and total glycerol meet international standards. Oxidative stability reaches 7.7 h with antioxidants (meets ASTM $\geq$3h, typically below EN $\geq$8h without optimization). In *H. illucens* biodiesel, the acid value is usually >0.50 mg KOH/g; however, standard treatments bring it within limits. In *H. illucens*, diet can be leveraged to tailor both the fatty-acid profile and the lipid yield for target applications. Preliminary evidence on process energy and costs across extraction and transesterification routes indicates that economics remain feedstock-driven and depend on scale, heat/solvent

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recovery, and enzyme reuse. In sum, *H. illucens* is a scalable platform for waste-derived biodiesel; this review distills evidence and practical levers to close remaining gaps and accelerate adoption.

1. Introduction

The increasing global energy demand, depletion of fossil fuel reserves, and rising greenhouse gas emissions necessitate the development of renewable energy sources (Golomb, 2020; Perera, 2018). According to the International Energy Agency (IEA, 2022), in 2021, renewable energy accounted for 29 % of global electricity production, with projections estimating a rise to 50 % by 2050. However, fossil fuels still meet around 81 % of the global energy demand (IEA, 2021), resulting in CO_2 emissions of 36.3 billion metric tons in 2021 (Global Carbon Project, 2021). This continued dependence on fossil fuels poses environmental and economic risks, as fluctuations in oil prices affect market stability and the viability of renewable alternatives. Moreover, climate change introduces physical risks that affect how reliably renewable energy sources can deliver energy; in particular, solar photovoltaics are sensitive to temperature and irradiance, wind resources vary by region and season, hydropower depends on precipitation, snowmelt and runoff with stronger seasonality, and geothermal is relatively robust but constrained by cooling water and infrastructure exposure (IPCC, 2022a; IPCC, 2022b; IEA, 2023; World Meteorological Organization, 2022). In this context, waste-based bioenergy pathways reduce dependence on crop yields and irrigation. Controlled rearing of *Hermetia illucens* converts heterogeneous organic residues into lipid-rich biomass within days, with low water needs, providing a more predictable and climate-resilient feedstock supply (Surendra et al., 2020; van Huis, 2013; Oonincx and de Boer, 2012).

1.1. The role of biodiesel in the era of energy transition

Biodiesel has emerged as a promising solution to mitigate the environmental impact of the energy sector, as it is primarily derived from vegetable oils and animal fats through transesterification and is highly biodegradable (Demirbas, 2002). Its use can reduce CO_2 emissions by up to 78 % (U.S. Environmental Protection Agency, 2010) and PM2.5 fine particulate matter by 47 % (U.S. Department of Energy, 2021), improving air quality and reducing urban pollution. However, producing biodiesel from oilseed crops raises sustainability issues, as it requires large agricultural areas that may compete with food production (FAO, 2021). To address these limitations, research has increasingly focused on alternative feedstocks such as organic waste and insect biomass, particularly *H. illucens* (Surendra et al., 2016). Globally, biodiesel production has grown rapidly, rising from 3.9 billion liters in 2005 to 18.1 billion liters in 2010, and is projected to reach 41.4 billion liters by 2025 (Rouhany and Montgomery, 2019). The leading biodiesel-producing countries include the United States, Brazil, Argentina, and Indonesia, with biodiesel primarily used in the transportation sector and, to a lesser extent, for electricity generation (Kim and Shandilya, 2019; Rouhany and Montgomery, 2019). The economic impact of biodiesel varies depending on the availability of feedstock. In countries such as Malaysia, Papua New Guinea, and Indonesia, biodiesel production contributes between 5.57 % and 10.36 % of Gross Domestic Product growth (Ng et al., 2017), while in Malaysia, the national biodiesel program has significantly boosted economic activity, with a production multiplier of 2.84 (Jaafar et al., 2010). In Europe, biodiesel has supported economic growth, although its role in the transport sector remains limited (Streimikiene et al., 2019).

The financial viability of biodiesel is closely linked to crude oil prices: during periods of high oil prices, up to 74 % of global biodiesel production is economically feasible, contributing 135.45 GW to the renewable energy mix (Ng et al., 2017). Nevertheless, feedstock availability and sustainable production practices remain critical to the sector long-term profitability. From an environmental perspective, biodiesel reduces greenhouse gas emissions by 20 % to 80 % compared to fossil diesel (Rouhany and Montgomery, 2019) and contributes to lowering air pollution due to its physicochemical properties. Biodiesel-diesel blends lower emissions of particulate matter, carbon monoxide, and hydrocarbons, contributing to improved air quality (McCormick, 2007). The B20 blend (20 % biodiesel, 80 % fossil diesel) lowers emissions by at least 15 % (Pala-en et al., 2013), while life-cycle CO_2 emissions are significantly lower, as the carbon released during combustion is largely reabsorbed by feedstock plants (McCormick, 2007). However, NO_X emissions vary depending on engine type and fuel composition (Balakrishnan et al., 2016). The role of biodiesel in the energy transition is strengthened by government policies such as tax exemptions and blending mandates (Europeran Commision, 2021a), along with technological improvements like enzymatic transesterification, which enhance production efficiency (Brennan and Owende, 2010). Despite challenges related to crude oil price fluctuations and feedstock availability, biodiesel remains one of the most promising solutions for reducing fossil fuel dependence and advancing toward a more sustainable energy system. Viewed through this climate change lens, waste-derived lipid pathways, including *H. illucens*, are attractive because they decouple feedstock supply from crop yield variability and irrigation constraints (Surendra et al., 2020; van Huis, 2013; Oonincx and de Boer, 2012).

Interest in *H. illucens* as a biodiesel feedstock has grown substantially in recent years. This insect species, known for its ability to bioconvert organic waste into lipid-rich biomass, represents an innovative and sustainable solution for biodiesel production, reducing environmental impact and avoiding competition with food production (Franco et al., 2021). Recent studies have demonstrated the efficiency of *H. illucens* in bioconverting a wide range of organic substrates, including fruit processing byproducts (Scieuzo et al., 2023), former foodstuffs of animal origin (Franco et al., 2025). These findings highlight the insect's adaptability to different waste streams and suggest that the resulting larval biomass could be further valorized for bioenergy purposes, including biodiesel production. Compared to traditional feedstocks, *H. illucens* offers multiple advantages, including fast growth, a high lipid conversion rate, and the ability to thrive on organic waste. Nonetheless, challenges related to lipid composition, extraction techniques, and biodiesel yield persist—topics that this review will address by analyzing current literature and outlining future directions for the valorization of *H. illucens* in the biofuel sector.

1.2. Fossil fuel pollution and waste management: potential of Hermetia illucens for a circular bioeconomy

The growing environmental burden caused by fossil fuel pollution and inadequate waste management constitutes a dual crisis demanding urgent solutions. Fossil fuel extraction and combustion are leading contributors to climate change, with global CO_2 emissions reaching 36.3 billion metric tons in 2021 (Global Carbon Project, 2021). Coal alone is responsible for 40 % of these emissions, while the transportation sector, heavily reliant on petroleum, accounts for 24 % of total greenhouse gas emissions (IEA, 2022). Additionally, the release of nitrogen oxides (NO_X) and sulfur oxides (SO_X) from fossil fuel combustion is linked to severe public health risks, including respiratory and cardiovascular diseases (Perera, 2018). Simultaneously, municipal solid waste (MSW) generation is escalating at an unprecedented rate. In 2020, global MSW was estimated between 2.3 and 3.1 billion metric tons, with projections pointing to 4.54 billion metric tons by 2050 (Maalouf and Mavropoulos, 2023). Currently, 42 % of global waste is either dumped in unregulated landfills or openly burned, leading to major environmental and health hazards (Kaza et al., 2018). Landfills contribute significantly to methane (CH_4) emissions, a greenhouse gas 25 times more potent than CO_2 (IPCC, 2019). Waste management costs also vary drastically by region, exceeding $100 per ton in high-income countries while averaging $35 per ton in low-income countries, where effective waste treatment strategies are often lacking (World Bank, 2018). To address these issues, zero-waste strategies and circular economy models have been proposed. The zero-waste approach aims to minimize waste generation at its source, reducing landfill dependency and encouraging material reuse (Zero Waste International Alliance, 2018). Meanwhile, the circular economy framework promotes resource regeneration, reduced raw material extraction, and a lower overall environmental footprint (Geissdoerfer et al., 2017). However, implementing these models requires scalable, integrated solutions that link waste recovery with renewable energy generation, ensuring environmental and economic sustainability.

One such strategy is the bioconversion of organic waste into biodiesel feedstock using *H. illucens*. This insect efficiently degrades agricultural and organic waste, reducing landfill inputs and methane emissions (Gold et al., 2020). Several studies indicate that *H. illucens* larvae can reduce organic waste mass by 50–60 % in just a few days, converting it into readily available biomass. This biomass is characterized by a lipid content ranging from 15 to 49 %, which represents a valuable renewable feedstock for biodiesel production (Diener et al., 2011; Surendra et al., 2020). Unlike first-generation biofuels derived from edible crops, biodiesel from *H. illucens* does not compete with food production or arable land use, making it a highly sustainable alternative. Framed within climate change scenarios, pairing waste management with *H. illucens* rearing provides a dual mitigation and adaptation benefit: it reduces methane emissions from landfilling while creating a feedstock pathway less exposed to heat and drought than crop-based bioenergy, thereby supporting climate-resilient liquid fuel supply chains (IPCC, 2022a; IPCC, 2022b; IEA, 2023; Surendra et al., 2020). Incorporating *H. illucens* into waste management systems can reduce environmental pollution and generate renewable fuel, in line with circular bioeconomy principles (Kim et al., 2025). The following section critically examines current biodiesel feedstocks, with a focus on *H. illucens* as an emerging sustainable alternative. Key aspects include its lipid extraction, biodiesel conversion processes, comparative evaluations against conventional sources, and regulatory standards.

2. Challenges in biodiesel production from traditional sources

Biodiesel feedstocks are commonly categorized into four generations, each with distinct technical and sustainability profiles. First-generation feedstocks, such as soybean, rapeseed, and palm oil, offer high oil yields and compatibility with conventional transesterification. However, their large-scale use raises significant ethical and environmental concerns, as they require intensive inputs of land, water, and fertilizers, and compete directly with food crops (Mat Aron et al., 2020; Souza et al., 2018). In particular, the expansion of palm oil and soybean cultivation for biofuel production has been linked to deforestation in tropical regions, biodiversity loss, and substantial greenhouse gas emissions (Pinzi, 2012; Highina et al., 2014; Ramos et al., 2019). Moreover, diverting edible crops to biodiesel production can exacerbate food insecurity, especially in developing countries, due to competition for arable land and agricultural resources (FAO, 2021). This competition also contributes to rising food prices and intensifies the environmental burden associated with intensive monoculture farming systems, which often rely heavily on chemical fertilizers and pesticides, leading to soil degradation and water pollution (Souza et al., 2018). These issues highlight the environmental and socio-economic trade-offs of using food-based feedstocks for biodiesel.

Second-generation feedstocks, such as waste cooking oils, animal fats, and non-edible plant oils like *Jatropha*, utilize agro-industrial by-products and waste materials, thereby reducing pressure on arable land (Ariyanti et al., 2024). Nonetheless, their adoption is still constrained by several limitations, including inconsistent feedstock quality, logistical challenges related to collection, and the complexity of processing (Prananta and Kubiszewski, 2021). A key technical issue is the typically high content of free fatty acids (FFAs), which react with alkaline catalysts such as sodium hydroxide (NaOH) or potassium hydroxide (KOH) during base-catalyzed transesterification. This reaction leads to soap formation rather than biodiesel, decreasing overall yield and complicating phase separation (Ariyanti et al., 2024; Prananta and Kubiszewski, 2021). To address this, pretreatment steps are often required to reduce or neutralize FFAs before transesterification. Despite these challenges, the use of second-generation feedstocks has the potential to reduce greenhouse gas emissions by up to 70 % compared to fossil fuels, making them a promising component of low-carbon energy strategies (Prananta and Kubiszewski, 2021). In response to the environmental and economic limitations of traditional biodiesel sources, research has shifted toward third- and fourth-generation feedstocks that promise higher sustainability and resource efficiency.

Third-generation feedstocks, such as microalgae and microbial oils, have attracted growing interest due to their high lipid content and ability to grow on non-arable land using saline water or wastewater (Hempel et al., 2012; Yang et al., 2023; Nazloo et al., 2022). Microalgal species such as *Chlorella sp.*, *Scenedesmus obliquus*, and *Tetraselmis sp.* have been widely studied for their lipid accumulation capacity (Hempel et al., 2012; Yang et al., 2023; Ekin, 2020). Cultivation in open raceway ponds and photobioreactors allows for optimized growth conditions, while emerging extraction technologies, including ultrasound and microwave-assisted methods, can enhance lipid recovery (Wahlen et al., 2011; Gaurav et al., 2023). Nevertheless, high energy demands, and significant production costs limit the commercial scalability of third-generation biodiesel (Nazloo et al., 2022).

Fourth-generation feedstocks represent a major advancement in biodiesel technology, combining synthetic biology and carbon capture and storage to achieve carbon–neutral or even carbon-negative fuel production. These systems employ genetically engineered microbes, such as *Escherichia coli*, *Yarrowia lipolytica* and *Rhodosporidium toruloides,* that are capable of synthesizing fatty acid methyl esters (FAMEs) through artificial metabolic pathways using CO_2 or organic waste as carbon source (Peralta-Yahya and Keasling, 2010; Nawabi et al., 2011). Despite their substantial environmental potential, these biotechnological strategies remain constrained by high costs, issues of scalability, and technical challenges related to bioreactor design, process efficiency, and genetic stability (Kumar et al., 2009; Ouellet and Abdel-Mawgoud, 2024).

A complementary and increasingly sustainable solution is the use of oil extracted from *H. illucens* larvae. Although insect oil can be chemically classified among animal fats traditionally considered first-generation feedstocks, its production system is fundamentally different, as larvae are reared on organic residues and agro-industrial by-products. This approach supports a circular economy and waste valorization model, making it conceptually closer to second-generation feedstocks (Surendra et al., 2020; Li et al., 2011). Relative to first-generation oilseeds, *H. illucens* is reared on residues rather than arable land, avoiding food competition and indirect land use change related constraints while delivering schedulable lipid supply (van Huis, 2013; Oonincx and de Boer, 2012). When compared to microalgae, which, despite their high theoretical lipid productivity, require intensive cultivation and harvesting steps and often generate biomass with highly variable lipid content depending on growth conditions, resulting in net energy balances close to unity at scale depending on pathway and assumptions (Bennion et al., 2015), *H. illucens* larvae can be produced with minimal energy input and without energy intensive dewatering and drying steps (Oonincx and de Boer, 2012; van Huis, 2013; Surendra et al., 2020). Microalgal biodiesel systems also exhibit high costs and CO_2 emissions associated with dewatering and drying, whereas insect production requires far less water and infrastructure (Nazloo et al., 2022). Compared to waste cooking oils (WCOs), which represent an important second-generation feedstock but are intrinsically heterogeneous and frequently present free fatty acid levels that necessitate an acid-esterification pretreatment before base-catalyzed transesterification (Leung et al., 2010; Canakci and Van Gerpen, 2001), *H. illucens* oil demonstrates greater consistency in fatty acid composition, particularly in saturated and monounsaturated lipids (mainly

Table 1

Comparative physicochemical properties of biodiesel from various feedstocks, including *H. illucens*, with ASTM and EN standards. The table compares key fuel parameters of biodiesel produced from first- to fourth-generation feedstocks with those of *H. illucens* biodiesel and ASTM D6751/EN 14214 specifications. Values are expressed as ranges or mean values from the literature. n.d.: not determined (property not reported in the referenced study). Report: indicates parameters that are required to be reported by the relevant standard but have no fixed pass/fail limit. Not specified: indicates parameters that are not regulated (i.e., no requirement/limit is provided) by the relevant standard.

Property	I Generation			II Generation		III Generation	IV Generation	Emerging feedstock	International standards	
	Soybean oil (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007)	Rapeseed oil (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007)	Palm oil (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007)	*Jatropha* oil (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007)	Waste Cooking oil (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007)	Microalgae *Nannochloropsis gaditana* (Sanjurjo et al. 2024)	Engineered Microorganisms *Escherichia coli* (Nawabi et al., 2011) *Yeast* (Ouellet and Abdel-Mawgoud, 2024)	*Hermetia illucens* (Nguyen et al., 2018a; Park et al., 2022)	ASTM D6751	EN 14,214
Density (kg/m^3)	882	879	873	876	877	885	n.d.	875	Not specified	860–900
Kinematic Viscosity (mm^2/s)	4.3	4.5	4.6	4.75	5.23	6.54	3.86, 4.77* (Yeasts)	5.2	1.9–6.0	3.5–5.0
Cetane Number	51.3	53.7	61.9	55.7	n.d.	n.d.	51.36, 63.70* (Yeasts)	50	≥47	≥51
Oxidative Stability (h)	2.1	7.6	10.3	2.3	n.d.	n.d.	n.d.	n.d.	≥3	≥8
Flash Point (°C)	159	169	163	152	157	140–150	152.32, 168.08* (Yeasts)121 (*E. coli*)	121	≥93	≥101
Water Content (mg/kg)	<0.0001	0.0003	n.d.	n.d.	n.d.	n.d.	0.03 (*E. coli*)	0.03	Not specified	≤500
Total Glycerin (% mass)	0.15	n.d.	0.01	0.1	n.d.	n.d.	n.d.	n.d.	≤0.24	≤0.25
Acid Value (mg KOH/g)	0.27	n.d.	0.24	0.4	0.21	0.42	0.8 (*E. coli*)	<0.8	≤0.50	≤0.50
Cloud point (°C)	0	−3	14	5	n.d.	n.d.	1.11, 21.63 (Yeasts)	n.d.	Report	Not specified
Pour point (°C)	−4	−10	13	0	n.d.	−7.2, −8.7	−4.01, 16.10 (Yeasts)	n.d.	Not specified	Not specified
CFPP (°C)	−4	−12	9	n.d.	n.d.	n.d.	−3.39, 17.13 (Yeasts)	0	Not specified	Climate-dependent**
Iodine Value g I_2/100 g	125.5	116.1	54	109.5	n.d.	n.d.	n.d.	n.d.	Not specified	≤120
Ester contents (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	99.5	Not specified	≥96.5
Sulfur content (%)	0.0002	0.0004	0.0002	0.0005	n.d.	n.d.	n.d.	n.d.	<0.05	0.001
Carbon residue wt%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	≤0.05	Not specified
Ash, wt%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01	≤0.020	≤0.02
Sediment, mg/kg	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<1	Not specified	≤24
Monoglyceride wt%	0.473	n.d.	n.d.	0.291	n.d.	n.d.	n.d.	<0.01	Not specified	≤0.70
Diglyceride wt %	0.088	n.d.	n.d.	0.104	n.d.	n.d.	n.d.	<0.01	Not specified	≤0.20
Triglyceride wt %	0.019	n.d.	n.d.	0.022	n.d.	n.d.	n.d.	<0.01	Not specified	≤0.20
Glycerol wt%	0.012	n.d.	0.01	0.006	n.d.	n.d.	n.d.	0.02	≤0.02	≤0.02
Metals Na + K	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<1 mg/kg	≤5 ppm	≤5 mg/kg
Metals Ca + Mg	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<1 mg/kg	≤5 ppm	≤5 mg/kg
Phosphorus	<0.1 mg/kg	n.d.	<0.001 mg/kg	<0.1 mg/kg	n.d.	n.d.	n.d.	<1 mg/kg	≤0.001 % mass	≤4 mg/kg

* The values reported were predicted in the study by Ouellet and Abdel-Mawgoud 2024 using *in silico* models based on the FAMEs composition. The study evaluated various oleaginous yeasts, including genetically engineered strains of *Yarrowia lipolytica*, providing a reliable estimation of the biodiesel physicochemical properties.

** Under EN 14214, no single universal limits are specified for cloud point and CFPP, as low-temperature operability requirements depend on climate grades defined in the National Annex.

lauric and palmitic acids), which are ideal as a raw material for biodiesel (Meneguz et al., 2018; Caligiani et al., 2018). Therefore, *H. illucens* larvae provide a consistent feedstock quality and predictable yields, avoiding the high energy demands and variability issues associated with microalgae and waste oils. Operationally, larvae reduce waste mass by approximately 50 to 80 % within 10 to 14 days, achieving substrate to biomass bioconversion of about 10 to 20 % and oil contents of about 25 to 40 % of dry matter (Diener et al., 2011; Mertenat et al., 2019; Surendra et al., 2020; Li et al., 2011; Spranghers et al., 2017). Collectively, this fast and resource-efficient upcycling, together with minimal competition for arable land, underpins the distinct feedstock advantage of *H. illucens* as a second-generation biodiesel feedstock. In addition, *H. illucens* oil yields biodiesel properties broadly consistent with conventional fuel quality targets following standard transesterification and, where appropriate, antioxidants or blending (Li et al., 2011; Ewald et al., 2020). Compared with fourth-generation engineered microbes, *H. illucens* relies on commercially deployed rearing and conventional transesterification, whereas synthetic biology platforms still face nontrivial cost, scale, and genetic-stability constraints for the manufacture of commodity fuels (Peralta-Yahya and Keasling, 2010; Nawabi et al., 2011). Taken together, the unique innovative value of *H. illucens* as a feedstock lies in the combination of controlled waste to lipid bioconversion, tighter upstream quality windows than WCOs, the avoidance of the algal dewatering bottleneck, and an MCFA-rich (medium-chain fatty acid–rich) oil that supports standard-compliant biodiesel after conventional processing (Li et al., 2011; Ewald et al., 2020). However, standardization of oil quality and optimization of extraction protocols remain essential for large-scale biodiesel production (Caligiani et al., 2018; Meneguz et al., 2018).

2.1. *The potential of Hermetia illucens-derived biodiesel compared to other generations of biofuels*

A comparative evaluation of physicochemical properties reveals that biodiesel from *H. illucens* offers performance parameters broadly aligned with those of first-, second-, third-, and fourth-generation biofuels, while complying with most ASTM D6751 (ASTM International, 2021) and EN 14214 requirements (European Committee for Standardization, 2019) (Table 1). Its cetane number, reported at 50 (Nguyen et al., 2018a), ensures acceptable ignition quality and engine performance, positioning it close to soybean oil (51.3, Hoekman et al., 2012) and *Jatropha* oil (55.7), and within the range for engineered yeast biodiesel (51.36–63.70, Ouellet and Abdel-Mawgoud, 2024), albeit slightly lower than palm (61.9) and rapeseed oil (53.7). The kinematic viscosity meets ASTM specifications and only marginally exceeds the EN upper limit, situating it between waste cooking oil and yeast-derived biodiesel, and suggesting that minor process adjustments or blending could ensure full compliance with EU standards. Flash point values comply with both ASTM and EN criteria, confirming safe handling, although they are moderately lower than those of several plant- and algae-derived biodiesel. Density is within the international range and comparable to *Jatropha* and waste cooking oil biodiesel, indicating compatibility with conventional engines. Water content is well below international thresholds, reflecting excellent moisture control and reduced risks of microbial contamination or corrosion. However, the acid value (<0.8 mg KOH/g) slightly exceeds the EN limit (≤ 0.5 mg KOH/g), indicating that additional refining could improve stability and compliance. First-generation feedstocks such as soybean and palm generally require less post-treatment, while some microbial biodiesel present even higher acid values. Overall, Table 1 further supports a satisfactory conversion/purification quality for *H. illucens* biodiesel, as indicated by high ester content and low residual contaminants (free glycerol and mono-/di-/triglyceride fractions), together with favorable levels of alkali metals (Na + K) and phosphorus. However, relevant data gaps persist for several key descriptors that are still sparsely reported or listed as “not determined” (n.d.), particularly oxidative stability, total glycerin (when not explicitly reported), iodine value, cold-flow properties (cloud and pour points, and Cold Filter Plugging Point (CFPP)), and, where unavailable, sulfur content. Addressing these gaps through standardized measurements would enable a more robust assessment of overall performance and a clearer evaluation of full compliance with international fuel specifications, especially under low-temperature operation and storage conditions.

From a sustainability standpoint, *H. illucens* biodiesel offers advantages over first-generation oils (Hoekman et al., 2012; Karmakar et al., 2010) by avoiding food–fuel competition and enabling waste bioconversion. Environmental Footprint (EF 3.1) benchmark ingredient datasets for vegetable oils reported by Francis et al. (2025) reach values up to 3.75 kg CO_2 eq/kg for palm kernel oil (Table 2). In the same source, the climate-change impact for 1 kg of extracted *H. illucens* fat at the production gate is 0.8 ± 0.1 kg CO_2 eq/kg (EF 3.1), based on an industrial facility using wind electricity and applying mass–economic allocation in a multi-product system (fat and defatted protein meal). Importantly, the cradle-to-production gate (cradle-to-gate) boundary explicitly includes substrate pre-treatment and feedstock handling/preparation (collection, mixing, and storage), climate-controlled rearing, larval separation from frass, dewatering/drying, and lipid extraction (yielding fat and defatted protein meal), without downstream refining. Accordingly, the observed gap between *H. illucens* fat and vegetable oils is consistent with differences in feed supply chains (including the use of food-industry side streams/waste), site-specific electricity mixes, process energy demand (notably dewatering/drying and extraction), and allocation choices. Therefore, the values reported in Table 2 are comparable only within the stated assumptions (EF 3.1; cradle-to-gate) and should be interpreted as a quantitative indication under a harmonised methodological framework. Consistent with Francis et al. (2025), the footprint is particularly influenced by the electricity mix, the energy required for dewatering/drying and extraction, and the co-product allocation basis. Finally, Table 2 covers lipid/oil production up to the production gate and does not include subsequent conversion of these lipids to biodiesel, nor downstream lipid refining.

Moreover, in the rearing of *H. illucens*, dietary modifications, such as substrate acidification with acetic acid, have been shown to reduce NH_3 and attenuate CH_4/N_2O. The direct global warming potential (100-year horizon; $CH_4 + N_2O$ only; biogenic CO_2 excluded), expressed per kilogram of dry larvae, can decrease to 2.67 ± 0.12 g CO_2 eq/kg with 2 %

Table 2
Comparative climate-change footprint of *H. illucens* lipids and conventional vegetable oils (EF 3.1; cradle-to-production gate; values from Francis et al., 2025). Values are expressed as kilograms of CO_2 equivalent per kilogram of product (kg CO_2 eq/kg oil/lipid). The *H. illucens* value refers to an industrial-scale production system and to 1 kg of extracted *H. illucens* lipids at the production gate. The underlying system boundary is cradle-to-production gate and includes feedstock handling and preparation (collection, mixing, and storage), climate-controlled rearing, larval separation from frass, drying, and lipid extraction yielding fat and defatted protein meal; impacts are calculated using mass–economic allocation between co-products as reported by Francis et al. (2025). Rapeseed, soy, and palm kernel oil values correspond to EF benchmark ingredient datasets (market averages) reported in the same source; standard deviations are not provided for these benchmark entries.

Feedstock (product)	Climate-change footprint (EF v3.1) kg CO_2 eq/kg oil/lipid	Dataset/allocation note
H. illucens lipids (extracted; production gate)	0.80 ± 0.10	Mass economic allocation between defatted protein meal and lipids; an industrial facility with wind electricity.
Rapeseed oil	1.30	EF benchmark dataset (market-average).
Soybean oil	2.12	EF benchmark dataset (market-average).
Palm kernel oil	3.75	EF benchmark dataset (market-average).

acetic acid. This indicator pertains exclusively to the rearing phase and is not directly comparable with cradle-to-gate life cycle assessment (LCA) per kg oil. (Elsayed et al., 2024).

Compared to second-generation sources such as waste cooking oil (Yamane et al., 2007), it provides a more uniform lipid profile, facilitating transesterification and consistent fuel quality. Third-generation options like microalgae (Sanjurjo et al., 2024) demand costly infrastructure and high energy inputs, whereas *H. illucens* farming relies on different, potentially less resource-intensive systems. Similarly, relative to fourth-generation microbial biofuels (Ouellet and Abdel-Mawgoud, 2024), it represents a more immediately scalable solution, avoiding industrial fermentation complexities. The regulatory context further shapes potential applications. Within the EU, the distinction between feed and non-food technical uses determines how insect-derived lipids can be produced and placed on the market. For feed, insects must be reared only on approved substrates, the 2017 reform and the 2021 update (EU Regulations 2017/893 and 2021/1372) European Commission, 2017, European Commission, 2021a,b) maintained explicit bans on using catering waste, manure, and unprocessed animal by-products as rearing substrates, in line with the risk profile outlined by EFSA (2015) and subsequent reviews (van der Fels-Klerx et al., 2018). By contrast, for non-food technical uses (e.g., biodiesel, oleochemicals, cosmetics), insect lipids fall under the Animal By-Products (ABP) framework (Reg. (EC) 1069/2009; Reg. (EU) 142/2011, European Commission, 2011; European Parliament and Council, 2009), where biodiesel is an “end point”: once produced via authorised routes in ABP-approved installations, the product exits ABP controls and is governed by the sectoral legislation for fuels. In this context, catering waste is generally Category 3 ABP (while international catering waste is Category 1), and manure is Category 2; insects reared on these substrates cannot enter the feed chain, but their lipids may be processed to biodiesel under the ABP rules in authorised installations with full authorisation and traceability (Reg. (EC) 1069/2009; Reg. (EU) 142/2011). This creates a compliant pathway to valorize otherwise excluded waste streams for energy uses without breaching legal limits (EFSA, 2015; van der Fels-Klerx et al., 2018). From a technical standpoint, peer-reviewed studies show that *H. illucens* lipids are convertible to biodiesel and that optimizing rearing conditions, lipid extraction, and transesterification parameters improves both yields and fuel properties (Leong et al., 2016; Franco et al., 2021; Surendra et al., 2020). Scaling production will require optimization of farming practices, lipid conversion processes, and dietary/rearing strategies to enhance biodiesel-suitable lipid profiles (Leong et al., 2016; Surendra et al., 2020). Presently, farming focuses on protein meal, with limited emphasis on lipid extraction for energy (van Huis, 2013). Increasing competitiveness will depend on automation, efficient extraction systems, and cost reduction (Surendra et al., 2020). Overall, *H. illucens* biodiesel emerges as a sustainable and technically viable alternative that satisfies many international standards, with targeted improvements in processing and stabilization likely to enhance its large-scale applicability (Leong et al., 2016; Franco et al., 2021; Surendra et al., 2020).

3. Lipid profile of *Hermetia illucens*

Hermetia illucens, commonly known as the Black Soldier Fly (BSF), is a species of fly belonging to the family *Stratiomyidae* (Diptera order) (Qi et al., 2017). Originally native to the Neotropics, it has spread globally due to human activities and is now found in every zoogeographic region (Marshall et al., 2015). *H. illucens* larvae are saprophagous, inhabiting various organic remains of plants and animals (Gladun, 2019). This adaptability has led to numerous applications in industrial and agricultural areas, including organic waste management, composting, and animal feed production (Kaczor et al., 2022). The species has also shown potential in entomoremediation, biodiesel production, and as a source of chitin and chitosan (Kaczor et al., 2022). Its widespread distribution and versatility have made *H. illucens* a subject of significant scientific and industrial interest in recent years (Kaczor et al., 2022). Key data on the biology, applications, and industrial relevance of *H. illucens* are summarized in Table 3.

Importantly, the table provides quantitative indicators that help interpret *H. illucens* larvae-based bioconversion across operational scales, from organism-level performance metrics to pilot/field evidence, as well as an operating bioconversion facility case study. These case studies collectively highlight that process outcomes are not fixed values, but are influenced by both substrate characteristics and operational settings, including feeding load and moisture management (e.g., drainage), thereby offering a clearer basis to contextualize “real-world scale” in terms of waste processing and mass-flow partitioning (Surendra

Table 3

Key features and data on *H. illucens* relevant to biodiesel production. This table summarizes the main biological traits, industrial applications, and quantitative data related to *H. illucens*, highlighting its relevance as a sustainable feedstock for biodiesel. Values refer to specific case studies and operational settings.

Aspect	Features and data	References
Distribution and ecology	Native to Neotropics, now cosmopolitan; saprophagous larvae feeding on organic residues.	Qi et al., 2017; Marshall et al., 2015; Gladun, 2019
Life cycle	38–55 days; five stages (egg, larva, prepupa, pupa, adult). Lipid accumulation peaks in larval–prepupal stages.	Padmanabha et al., 2020
Applications	Waste management, composting, animal feed, entomoremediation, biodiesel, chitin/chitosan, cosmetics, pharmaceuticals.	Kaczor et al., 2022; Li et al., 2011; Surendra et al., 2016; Almeida et al., 2022
Waste treatment performance	Per-larva intake: 25–500 mg organic matter·larva^{-1}·day^{-1}. Waste reduction examples: ~39 % (pig manure), ~50 % (chicken manure), ~68 % (municipal organic waste)	Surendra et al., 2016
Pilot/field treatment metrics	In a medium-scale field experiment on mixed municipal organic waste, reported waste reduction 65.5–78.9 % (depending on feeding rate and drainage), with prepupae (late larval stage) harvested at the end of the larval phase reported as 252 g·m^{-2}·day^{-1} (wet weight) under favourable conditions.	Diener et al., 2011
Real facility scale	15 t food waste·day^{-1} (wet weight) in an operating BSFL bioconversion plant assessed via material flow analysis (MFA) and life cycle assessment (LCA). Reported dry-basis mass balance: 6 % converted to harvested insects (pre-pupae), 51 % to matured compost, 43 % emitted to air	Guo et al., 2021
Lipid content and diet effect	General content: 35–40 % of dry weight. Strongly diet-dependent: 20.8–84.5 %. Fat-rich food waste → >80 %; plant residues (e.g., avocado) → ~20 %; agri-by-products → 24–30 %.	Li et al., 2011; Surendra et al., 2016; Abduh et al., 2020; Jung et al., 2022; Liew et al., 2022; Pang et al., 2020
FA composition and diet effect	Lauric acid (C12:0) is dominant (16–70 %). Oleic (C18:1) and linoleic (C18:2) peak early and then decline. Diet modifies FA profile: coconut waste → lauric > 60 %; food waste → stable oleic, variable linoleic.	Liu et al., 2017; Zhu et al., 2019; Wong et al., 2020; Liu et al., 2023
Implications for biodiesel	High SFAs (lauric, myristic) → oxidative stability and higher cetane number. Higher UFAs → better cold-flow but lower stability. Diet modulation optimizes biodiesel quality.	Surendra et al., 2016; Jung et al., 2022

et al., 2016; Diener et al., 2011; Guo et al., 2021).

Beyond these quantitative indicators, the European sector is growing significantly due to increasing awareness of the potential applications of *H. illucens* larvae and their potentially lower environmental impacts compared to traditional livestock farming (Thrastardottir et al., 2021; Tomberlin et al., 2015). The industry success is driven by substantial investments in technology and automation, with venture capital firms playing a significant role (Thrastardottir et al., 2021). In Italy, a case study assessed the feasibility of implementing *H. illucens* production facilities based on regionally sourced agri-food by-products, evaluating substrate availability within a 100 km radius and reporting advantages relative to composting in terms of waste reduction and economic output value, supporting local circular-economy models (Cattaneo et al., 2024). As the sector expands, researchers emphasize the importance of addressing *H. illucens* welfare in commercial rearing facilities, focusing on disease prevention, abiotic conditions, and nutritional requirements (Barrett et al., 2022).

H. illucens undergoes a life cycle that spans approximately 38 to 55 days, consisting of five distinct stages: egg, larval stage, prepupal stage, pupa, and adult (Padmanabha et al., 2020). Particular attention is given to the larval stage, which is the most metabolically active and represents the critical window for lipid accumulation. During this phase, BSF larvae are particularly efficient at storing lipids, which can account for up to 35 %–40 % of their dry weight, depending on the rearing substrate (Li et al., 2011; Surendra et al., 2016). These lipids are predominantly composed of medium-chain saturated fatty acids (SFAs), particularly lauric acid (C12:0), which confer oxidative stability and favorable combustion properties, making them especially suitable for biodiesel production (Spranghers et al., 2017; Almeida et al., 2022). This biochemical profile not only supports biofuel applications but also adds value in sectors such as animal feed, cosmetics, and pharmaceuticals (Li et al., 2011; Surendra et al., 2016; Almeida et al., 2022).

3.1. Lipid content variation across the life cycle of Hermetia illucens

The lipid content of *H. illucens* undergoes significant fluctuations throughout its life cycle, reaching its peak during the prepupal stage before decreasing sharply upon pupation and adulthood. Lipids serve as a crucial energy reserve, supporting larval growth and later sustaining the metabolism of adults, which do not feed and rely entirely on stored fat from the larval phase (Banks, 2014).

Analyses of larvae reared on chicken feed indicate an initial decline in lipid content immediately after hatching, from 15.8 % to 4.8 %, as energy is expended for larval development (Liu et al., 2017). During the feeding phase, lipid accumulation resumes, increasing rapidly to 28.4 % and peaking at the early prepupal stage. As larvae transition into the pupal stage, lipid levels decline progressively, reaching their lowest levels in adults, where a slight increase is observed, likely to meet the energy demands of reproduction (Liu et al., 2017).

Similar trends have been reported in studies analyzing lipid dynamics across eight experimental timepoints spanning the entire life cycle from egg to adult (Zhu et al., 2019). Lipid content remains low in early larval stages (5.3–7.5 %) but rises sharply to 17.8 % by day 8. The highest lipid accumulation (26.1 %) is recorded in the late prepupal stage, followed by a marked decline during pupation (8.9 % in early pupae and 7.7 % in late pupae). While Liu et al. (2017) identified the lipid peak in the early prepupal phase, Zhu et al. (2019) observed it later, in the late prepupal stage, highlighting minor differences in the exact timing of peak lipid accumulation. This slight discrepancy in timing likely reflects differences in experimental setups or developmental stage classification, rather than variation in larval diet. Sexual dimorphism in lipid content has also been observed in adults, with females exhibiting higher lipid levels than males. Additionally, females have a greater proportion of SFAs, whereas males display higher levels of unsaturated fatty acids (UFAs) (Liu et al., 2017).

3.2. Fatty acid composition across the life cycle of Hermetia illucens

The fatty acid (FA) composition of *H. illucens* undergoes significant variations throughout its life cycle, with a fluctuating distribution of SFAs and UFAs depending on the developmental stage. Lauric acid (C12:0) is a predominant component of the lipid fraction, accounting for 16 % to 70 % of total lipids at different stages (Liu et al., 2017). Lauric acid synthesis remains stable throughout the life cycle, except in 4-day-old larvae, where lower levels are observed.

During the early larval stages, UFAs, particularly oleic acid (C18:1) and linoleic acid (C18:2, ω-6), peak between days 4 and 6, reaching concentrations of 36.4 % and 31.4 %, respectively. After this period, UFA levels progressively decrease, whereas SFAs follow the opposite trend, reaching their lowest value on day 4 and steadily increasing until the prepupal stage (Liu et al., 2017). This trend was also observed by Zhu et al. (2019), who observed similar FA profiles in larvae reared on chicken feed. The influence of diet on FA composition in *H. illucens* larvae was also highlighted by Liu et al. (2023), who analyzed larvae fed on food waste. Palmitic acid (C16:0) remained stable in larvae fed on food waste, whereas it gradually decreased in those reared on chicken feed (Liu et al., 2017; Zhu et al., 2019). Differences were also observed in UFA levels: in larvae fed on chicken feed, oleic acid (C18:1) and linoleic acid (C18:2) progressively decreased, whereas in larvae fed on food waste, the former remained stable while the latter increased until day 11 before declining (Liu et al., 2023). These findings confirm that the FA composition of *H. illucens* is influenced by both the developmental stage and diet. Chicken feed promotes a continuous increase in C12:0 through the acetyl-CoA pathway (Liu et al., 2017; Zhu et al., 2019), while fluctuations in C12:0 levels in larvae fed on food waste are linked to substrate variability (Liu et al., 2023). Additionally, the higher UFA content in larvae reared on food waste reflects the dietary composition, as BSF can only accumulate MUFAs and PUFAs from their diet (Hadj Saadoun et al., 2020). Ultimately, lipid composition and FA profiles in insects are influenced by multiple factors, including habitat, temperature, humidity, metamorphic stage, and diet. By modulating these parameters, it is possible to optimize lipid quantity and quality for specific industrial or nutritional applications (Hadj Saadoun et al., 2020).

3.3. Lipid yield and fatty acid composition variability in Hermetia illucens

The ability of *H. illucens* larvae to efficiently convert organic waste into lipid-rich biomass makes them a promising feedstock for biodiesel production (Bertinetti et al., 2019). In this section, 'lipid yield' denotes larval lipid content expressed as wt% of dry biomass; because this metric is reported on different analytical bases (dry vs wet biomass) and may be derived using different extraction and quantification procedures, comparisons across studies should be considered indicative unless methodologies are harmonized. However, the quantity and composition of the lipids accumulated in the larvae are strongly influenced by their diet, which directly affects the quality and properties of the extracted biodiesel (Mohd-Noor et al., 2017). Previous studies have demonstrated that lipid yield can vary significantly depending on the type of organic substrate provided during larval rearing, with recorded values ranging from 20.8 % to 84.5 % (Abduh et al., 2020; Jung et al., 2022). Generally, larvae fed on fat-rich food waste exhibit the highest lipid content, with yields exceeding 80 %, whereas those raised on low-lipid plant-based residues, such as decayed avocado, tend to accumulate lower lipid levels, around 20 % (Abduh et al., 2020). Agricultural by-products, including rice straw and palm kernel expeller, support intermediate lipid yields, typically between 24 % and 30 % (Liew et al., 2022; Pang et al., 2020). These findings highlight the critical role of diet composition in shaping the lipid biosynthesis process within *H. illucens* larvae.

Beyond lipid yield, fatty acid composition is also highly dependent on the substrate. *H. illucens*-derived lipids are generally rich in SFA, with lauric acid (C12:0) being the dominant component, particularly in larvae fed with coconut endosperm waste or food scraps, where it can exceed 60 % of total fatty acids (Wong et al., 2020; Jung et al., 2022). Other relevant fatty acids include palmitic (C16:0), oleic (C18:1), and linoleic (C18:2), with varying proportions depending on the diet (Surendra et al., 2016). The ability to modulate fatty acid profiles by adjusting larval diet presents a strategic advantage for biodiesel optimization. For instance, increasing UFAs content through lipid-rich feedstocks can enhance fuel fluidity and cold-flow properties, which are essential for compliance with international biodiesel standards in colder climates. However, this must be balanced with the need for oxidative stability, typically provided by SFAs. Conversely, a higher saturation level, particularly with lauric and myristic acids, enhances oxidative stability and increases the cetane number, making the biodiesel more suitable for diesel engines (Jung et al., 2022). Because larval diet modulates the lipidome of *H. illucens*, typically yielding a lauric-rich, SFA-dominated profile (Liland et al., 2017; Ewald et al., 2020; Jung et al., 2022), the melting point and apparent viscosity increase, with adverse consequences for mixing and mass transfer during alcoholysis (Hoekman et al., 2012). Accordingly, transesterification conditions should be calibrated to the feedstock free fatty acid content and moisture level (Meher et al., 2006). Thus, optimizing *H. illucens* feeding strategies provides a viable approach to enhancing both lipid yield and fuel quality, ensuring greater efficiency and sustainability in biodiesel production. Additionally, utilizing organic waste substrates not only reduces production costs but also supports a circular bioeconomy model, transforming waste into a renewable energy resource.

4. Technologies and processes for biodiesel production from *Hermetia illucens*

4.1. Lipid extraction: methods and innovations

The extraction of lipids from *H. illucens* larvae is a critical step in biodiesel production, as the chosen method can significantly affect both the quantity of oil recovered (yield) and the relative abundance of specific fatty acids (lipid composition), key parameters for biodiesel quality. Various extraction techniques have been developed, each with specific advantages and limitations regarding efficiency, sustainability, and cost. These methods can be broadly classified into solvent-based extraction, enzymatic extraction, and mechanical extraction.

4.1.1. Solvent-based extraction techniques

Solvent-based techniques are among the most widely used at the laboratory scale, due to their high efficiency in lipid yield extraction. However, in BSF production environments, solvent-based methods are less commonly applied, mainly due to cost, safety, and regulatory concerns. The choice of solvent is crucial, as different solvents target specific lipid fractions. Non-polar solvents, such as *n*-hexane, petroleum ether, chloroform, and benzene, are commonly used to extract neutral lipids like triacylglycerols, while polar solvents, such as methanol (MeOH) and ethanol, are preferred for extracting membrane lipids by disrupting protein-lipid interactions (Kates, 1986; Lee, 2003; Nelson and Cox, 2018).

- Soxhlet Extraction: recognized as the gold standard for lipid extraction, this method employs continuous solvent circulation, ensuring thorough extraction. Dried and ground BSF samples are placed in a Soxhlet apparatus, where a heated solvent repeatedly washes the biomass, dissolving lipids. Although effective, it requires long processing times and careful solvent handling (Surendra et al., 2016).
- Folch Method: this technique utilizes a chloroform–MeOH (2:1) solvent system to disrupt cellular structures and separate lipids. The process involves homogenization, phase separation, and centrifugation, yielding high lipid recovery. However, the use of toxic solvents raises environmental and safety concerns (Folch et al., 1957; Eggers and Schwudke, 2016).
- Ultrasound-Assisted and Microwave-Assisted Extraction: these emerging methods improve lipid recovery while reducing solvent consumption. Ultrasound treatment enhances mass transfer by creating cavitation bubbles in the solvent, while microwave irradiation accelerates lipid release through rapid heating (Almeida et al., 2022).
- Maceration and Decoction: in maceration, BSFL are soaked in *n*-hexane, acetone, or a solvent mixture for several hours under agitation, followed by filtration and vacuum evaporation to recover lipids. Decoction, on the other hand, involves boiling the biomass in water and centrifuging the mixture to isolate lipid fractions (Lopez-Bascón and Luque De Castro, 2020).

4.1.2. Enzymatic extraction of Hermetia illucens lipids

Enzymatic methods offer an eco-friendly alternative by using biological catalysts to break down cellular components and facilitate lipid release. These processes are typically carried out under controlled conditions of temperature and pH, optimizing enzyme activity while preserving lipid integrity. Such strategies, which have been successfully applied to insects and in particular to *H. illucens*, are today encompassed under the broader concept of enzyme-assisted aqueous extraction (EAAE), which defines all approaches that combine aqueous media with enzymatic hydrolysis. Compared with solvent-based techniques, EAAE avoids toxic residues, preserves thermosensitive bioactive compounds (e.g., tocopherols and carotenoids), and generates defatted protein-rich flours that can be further valorized in food and feed applications (Cruz et al., 2025).

Different enzymatic methodologies have been explored for insects, particularly for *H. illucens*:

- Protease-Assisted Extraction: enzymes such as proteases (from *Bacillus licheniformis*) hydrolyze proteins and free lipids, increasing extraction efficiency. Studies have reported lipid recovery rates of up to 81 % under optimized enzymatic hydrolysis (Woods et al., 2020).
- Sequential Enzymatic Fractionation: this method, detailed by Smets et al. (2020), involves an initial enzymatic lipid extraction followed by protein solubilization at high pH (~11) and subsequent precipitation at low pH (~4). This approach allows for the simultaneous recovery of lipids, proteins, and chitin, optimizing biomass utilization.
- Biological Decomposer-Assisted Extraction: Rabani et al. (2019) proposed a mild enzymatic extraction at 39.5 °C and pH 6, yielding a three-phase solution with separate lipid, protein, and chitin fractions. This process reduces solvent use while maintaining the fatty acid composition of the extracted lipids.

At the industrial scale, the concept of EAAE has been implemented in the patent WO2013191548A1 (World Intellectual Property Organization, 2013), which includes applications to *H. illucens* and describes a “wet fractionation” process combining mechanical disruption (e.g., grinding and decanter centrifugation) with enzymatic hydrolysis. This integrated approach enables the simultaneous separation of oils, soluble proteins, and chitin without the use of organic solvents. Beyond improving lipid recovery, it provides distinct nutrient streams that can be directed to food, feed, cosmetic, or pharmaceutical applications, supporting the full valorization of BSFL biomass.

Despite these advantages, enzymatic extraction still faces challenges related to the relatively high cost of enzymes and the need for precise optimization to achieve competitive lipid yields compared with conventional solvent-based methods (Caligiani et al., 2018).

4.1.3. Mechanical extraction of Hermetia illucens lipids

Mechanical lipid extraction represents an efficient, solvent-free approach that can be broadly divided into dry mechanical pressing and wet mechanical extraction.

Dry pressing, primarily performed through screw pressing or hydraulic pressing, applies high mechanical pressure to dried *H. illucens* larvae, physically expelling lipids while leaving a residual protein-rich press cake. Studies suggest that a screw rotation speed of 20 ± 5 rpm, an outlet diameter of 7–10 mm, and a moisture content of 8 ± 2 % maximize lipid recovery (Maltseva et al., 2024; Leong et al., 2022). Higher pressing temperatures, typically ranging from 140 to 150 °C, can enhance lipid yield; however, they may also degrade the protein content in the press cake (Lewandoski et al., 2021). Although mechanical pressing yields slightly lower lipid recovery than solvent-based methods, it offers significant advantages, including lower environmental impact, cost-effectiveness, and preservation of bioactive compounds, making it particularly suitable for industrial applications (Kim et al., 2022; Bhuiya et al., 2020).

Wet mechanical extraction, also referred to as enzyme-free wet extraction, is based on the processing of fresh or minimally treated biomass and relies on centrifugal separation technologies, such as decanter centrifuges, to fractionate lipids, proteins, and aqueous components. This approach avoids the use of solvents and enzymatic hydrolysis, thereby ensuring low environmental impact while enabling efficient fractionation. Laboratory-scale investigations, such as the wet mode fractionation scheme described by Ravi et al. (2021), have demonstrated the feasibility of this strategy for obtaining distinct lipid, protein, and chitin fractions (Ravi et al., 2021). At the industrial scale, this methodology has been implemented and protected under patent WO2014123420A1 (World Intellectual Property Organization, 2014), which provides a detailed description of its application for converting insects or worms into nutrient streams, and it currently represents the reference processing route adopted by European large-scale producers.

Few comparative studies have systematically evaluated lipid extraction techniques in *H. illucens*. Caligiani et al. (2018) compared Soxhlet extraction, maceration, and enzymatic hydrolysis, identifying significant differences in lipid yield and environmental impact. Soxhlet extraction achieved the highest lipid recovery (37.1 %), but its reliance on hazardous solvents limits its sustainability. Maceration with petroleum ether provided a slightly lower yield (32.5 %) while requiring longer extraction times, making it less efficient for large-scale applications. Enzymatic hydrolysis, though environmentally friendly, resulted in a substantially lower lipid yield (10 %), demonstrating the need for further optimization to improve efficiency. Similarly, Almeida et al. (2022) compared different extraction techniques, including decoction, maceration, ultrasound-assisted, and microwave-assisted methods. Their results indicated that maceration in *n*-hexane achieved the highest lipid yield (39 %), followed by ultrasound-assisted extraction (23 %), which offered a balance between efficiency and sustainability. These comparative analyses highlight the trade-offs between yield, environmental impact, and scalability, emphasizing the necessity of refining extraction methodologies to enhance the feasibility of lipid recovery from *H. illucens* for biodiesel production.

4.1.4. *Emerging trends in sustainable lipid extraction*

Growing environmental concerns have prompted significant interest in green solvents as alternatives to conventional petrochemical-based extraction methods for insect-derived lipids. Among these, 2-Methyloxolane (2-MeO), a bio-based solvent derived from renewable lignocellulosic biomass, has shown remarkable promise. In a study by Ravi et al. (2019), 2-MeO extracted 35.8 % of lipids from dried BSFL, outperforming *n*-hexane (32.5 %) while maintaining a similar fatty acid profile (Ravi et al., 2019). In addition to enabling efficient lipid extraction, this solvent did not compromise the quality of the remaining proteins, highlighting its suitability for integrated biorefinery processes targeting the simultaneous recovery of lipids and high-value proteins. Further evidence of 2-MeO effectiveness comes from Smets et al. (2021), who evaluated 2-methyltetrahydrofuran (2-MeTHF), the IUPAC equivalent of 2-MeO, for lipid extraction from fresh BSFL. By applying response surface methodology, they identified optimal extraction conditions (15 % fresh larvae, 40 % 2-MeTHF, 45 % water at 45 °C), under which the process achieved a lipid recovery of 94.9 % ± 1.8 %. Additionally, the extracted lipid fraction was rich in lauric acid, oleic acid, and phospholipids, suggesting high applicability for biodiesel production and nutraceutical uses (Smets et al., 2021). Wang et al. (2020) demonstrated that maceration in a 90:10 ethyl acetate-water solution achieved a degreasing efficiency of 29.04 %, while avoiding the toxicity associated with petroleum-derived solvents. Although the extraction was partial, the use of this green solvent highlights its potential as a safer and more environmentally friendly alternative for lipid recovery from BSFL. Future research should focus on optimizing green solvents for industrial scalability, developing hybrid extraction methods (e.g., enzymatic pre-treatment combined with solvent systems), and integrating mechanical pressing techniques. Moreover, the implementation of solvent recovery processes and LCA will be essential to fully realize the environmental and economic potential of these approaches for circular bioeconomy applications. A thorough comparison of the efficiency of new solvent-based and mechanical methods, especially those relying on wet extraction, should be performed to understand their benefits.

4.2. *Biodiesel production from Hermetia illucens*

In lipid matrices dominated by lauric and other saturated fatty acids, as is often the case with *H. illucens* oils, base-catalyzed transesterification is suitable only when FFAs are low and water is minimized; under these conditions, preheating to approximately 50–65 °C improves interfacial contact and mass transfer and helps manage viscosity (Meher et al., 2006; Ishak et al., 2018). When FFAs are high, acid esterification or a two-stage acid-then-base configuration is indicated (Zheng et al., 2012; Nguyen et al., 2018a; Khizar et al., 2024). For crude feeds or matrices with non-negligible FFAs or moisture, an enzymatic route is appropriate, using controlled MeOH dosing or methyl acetate as the acyl acceptor to limit enzyme inhibition (Nguyen et al., 2018b; He et al., 2022). Where broad tolerance to FFAs and water is required, supercritical methanolysis can be employed, accepting the higher thermal duty (Jung et al., 2022). Because the fatty-acid composition of *H. illucens* is diet-responsive, temperature, mixing intensity, alcohol-to-oil ratio, and catalyst choice should be calibrated to the actual profile; in general, more oleic-rich formulations tend to require less preheating and favor base catalysis, whereas more saturated profiles may demand more vigorous mixing or alternative routes (Spranghers et al., 2017; Liland et al., 2017; Park et al., 2022). Within this context, biodiesel production from *H. illucens* lipids involves different transesterification techniques, each employing specific catalysts, solvents, and reaction conditions to optimize conversion efficiency (Table 4).

4.2.1. *Alkaline-catalyzed transesterification*

This is the most commonly used method due to its high efficiency and relatively mild reaction conditions. It employs alkaline catalysts such as NaOH and KOH, typically dissolved in MeOH as a solvent (Ishak et al., 2018; Kathumbi et al., 2022). The process is generally conducted at temperatures ranging from 50 °C to 65 °C, with a MeOH-to-lipid molar ratio of approximately 6:1 to 9:1. The reaction is usually performed under constant stirring for 60–90 min to achieve complete conversion of lipids into FAMEs. As a result of the transesterification process, glycerol is formed as a by-product from the cleavage of the triglyceride backbone. After the reaction, the mixture undergoes phase separation: the denser glycerol layer is removed, and the biodiesel phase is subsequently purified through washing with distilled water and drying. Ishak et al. (2018) performed the process after acid pretreatment to reduce FFAs, using NaOH in MeOH (molar ratio 9:1) at 60 °C for 68 min, achieving a biodiesel yield of 96 %. The initial oil, derived from larvae reared on kitchen waste, had low FFA content, thus avoiding saponification and producing biodiesel that met EN 14214 standards. Kathumbi et al. (2022) applied base-catalyzed transesterification after acid pretreatment, comparing NaOH prepared using citric acid (NaOH/CA), calcium oxide (CaO) prepared with citric acid (CaO/CA), and commercial CaO.

Table 4
Comparison of biodiesel production yields and techniques from *H. illucens* lipids. Transesterification methods, biodiesel yields, and reaction conditions applied to *H. illucens* lipid extracts. The molar ratio refers to the solvent-to-lipid ratio.

Study	Production Technique	*Biodiesel Yield (%)	Optimal Conditions
Ishak et al., 2018	Alkaline transesterification (after acid pretreatment)	96 %	Catalyst: NaOH; Solvent: MeOH; Reaction Time: 68 min; Temperature: 60 °C; Molar Ratio: 9:1
Kathumbi et al., 2022	Alkaline transesterification (after acid pretreatment)	>98 %	Catalyst: NaOH/CA and CaO/CA (citric acid synthesized), commercial CaO; Solvent: MeOH; Reaction Time: 60 min; Temperature: 90 °C; Molar Ratio: Not specified
Leong et al., 2016	Acid transesterification	48.46 %	Catalyst: H_2SO_4; Solvent: MeOH; Reaction Time: 60 min; Temperature: 55 °C; Molar Ratio: 8:1—12:1
Nguyen et al., 2018a	Acid transesterification	94.14 %	Catalyst: H_2SO_4; Solvent: MeOH; Reaction Time: 90 min; Temperature: 120 °C; No Molar Ratio – Direct transesterification
Zheng et al., 2012	Two-step acid-base transesterification	93.1 %	Catalyst: H_2SO_4 (acid phase) + NaOH (base phase); Solvent: MeOH; Reaction Time: 90 min (60 min acid + 30 min base); Temperature: 75 °C acid, 65 °C base; Molar Ratio: 8:1 acid, 6:1 base
Khizar et al., 2024	Two-step acid-base transesterification	96.7 %	Catalyst: H_2SO_4 (acid phase) + NaOH (base phase); Solvent: MeOH; Reaction Time: 150 min (60 min acid + 90 min base); Temperature: 75 °C acid, 60 °C base; Molar Ratio: 8:1 acid, 6:1 base
Jung et al., 2022	Supercritical (non-catalytic) transesterification	94.1 %	Catalyst: None (porous SiO_2 support used); Solvent: MeOH; Reaction Time:1 min; Temperature: 390 °C; Molar Ratio: Not specified
Nguyen et al., 2018b	Enzymatic transesterification	96.97 %	Catalyst: Novozym® 435 (immobilized, *Candida antarctica*); Acyl acceptor: Methyl acetate; Reaction Time: 12 h; Temperature: 39.5 °C; Molar Ratio: 14.64:1
He et al., 2022	Enzymatic transesterification	98.45 %	Catalyst: Eversa® Transform 2.0 (free, T. lanuginosus) + SMG1 (free, microbial); Solvent: MeOH, Reaction Time: 8 h; Temperature: 25 °C; Molar Ratio: 3:1

[*] Biodiesel yield basis: lipid basis (Ishak 2018; Zheng 2012; Khizar 2024; Jung 2022; Nguyen 2018a,b; He 2022); whole-larvae basis (Leong 2016). Note: Kathumbi 2022 reports ester content (conversion), not a mass yield.

The reactions were conducted at 90 °C for 60 min with 1.8 % catalyst loading (w/w relative to oil), achieving methyl ester content exceeding 98 %. NaOH-based catalysis provided higher yields and better biodiesel properties than CaO-based systems, particularly in terms of residual FFA content and cold-flow performance. Despite its simplicity, this method requires strict control of FFA levels (<1%) to prevent soap formation. Since FFA content in BSFL-derived oils can vary depending on the larval substrate, acid pretreatment or refining may be necessary prior to transesterification.

4.2.2. *Acid-catalyzed transesterification*

Acid-catalyzed transesterification is particularly advantageous when processing lipid feedstocks with high FFA content, as it prevents soap formation, a common issue in base-catalyzed reactions (Meher et al., 2006). This is especially relevant for oils extracted from *H. illucens* larvae reared on protein-rich or decaying substrates, which tend to accumulate elevated FFA levels (>5%) (Nguyen et al., 2018a). Researchers have applied acid-catalyzed transesterification to *H. illucens* biomass to produce biodiesel. Leong et al. (2016) investigated an ultrasonic-assisted *in-situ* acid transesterification using MeOH and sulfuric acid (5 % v/v) on dried whole larvae. The reaction was conducted at 55 °C for up to 60 min with MeOH-to-larvae mass ratios of 8:1 and 12:1, achieving a maximum FAME yield of 48.46 % when larvae were fed with fruit waste. Nguyen et al. (2018a) developed two approaches: (i) a direct *in-situ* acid transesterification that integrated lipid extraction and conversion into a single step, employing sulfuric acid as a catalyst, MeOH as solvent, and *n*-hexane as co-solvent. Under optimal conditions (120 °C, 90 min, 1:2 n-hexane-to-MeOH volume ratio, and 12 mL of solvent per 2 g of biomass), this method achieved a FAME yield of 94.14 %; (ii) a conventional acid-catalyzed transesterification of extracted BSFL oil using sulfuric acid (H_2SO_4) as a homogeneous catalyst, with a 30:1 MeOH-to-lipid molar ratio at 60 °C for 90 min, which resulted in a 93.7 % yield. Together, these studies confirm that acid transesterification is an effective and practical approach for converting insect biomass into biodiesel, especially when processing raw or minimally pretreated materials (Leong et al., 2016; Nguyen et al., 2018a). In such cases, alkaline catalysis would lead to significant soap formation, reduced biodiesel yield, and emulsification problems during purification. The most commonly used acid catalyst is H_2SO_4, typically dissolved in MeOH (Nguyen et al., 2018a; Leong et al., 2016). Compared to base catalysis, acid-catalyzed reactions are slower and require more extreme conditions, including higher MeOH-to-lipid molar ratios (typically 15:1 to 30:1), longer reaction times (up to 4 h), and higher catalyst concentrations (1–5 % v/v). After the reaction, the mixture is neutralized, commonly with sodium bicarbonate, then washed and dried to remove residual MeOH, glycerol, and acid traces. Although the corrosive nature of sulfuric acid and the longer reaction times require more robust reactor materials and increased safety precautions, this method enables both esterification of FFAs and transesterification of triglycerides to occur in a single step. It is particularly suitable for unrefined or crude insect oils, where pre-esterification is impractical.

4.2.3. *Two-step acid-base transesterification*

The two-step acid–base transesterification process has been successfully applied to *H. illucens* lipids to produce biodiesel from feedstocks with high FFA content. This method combines an initial acid-catalyzed esterification, which reduces the FFA content and minimizes soap formation, with a subsequent base-catalyzed transesterification that converts the remaining triglycerides into FAMEs. Zheng et al. (2012) applied this approach using 1 % v/v H_2SO_4 in MeOH at 75 °C for 60 min, with a MeOH-to-fat molar ratio of 8:1 in the esterification step. This was followed by base-catalyzed transesterification with 0.8 % w/w NaOH in MeOH at 65 °C for 30 min, with a 6:1 M ratio, achieving a final FAME yield of 93.1 %. Similarly, Khizar et al. (2024) used 0.5 % v/v H_2SO_4 at 75 °C for 60 min with an 8:1 MeOH-to-lipid molar ratio, followed by transesterification with 1 % w/w NaOH in MeOH at 60 °C for 90 min, using a 6:1 M ratio. This process yielded 96.7 % FAME. The two-step acid–base transesterification process is a well-established strategy for converting high-FFA oils into high-quality biodiesel and has been specifically adapted for *H. illucens* lipids, which often exhibit variable FFA levels depending on the rearing substrate (Khizar et al., 2024). This sequential approach allows for the minimization of soap formation, improved conversion efficiency, and enhanced phase separation. In the first step, acid-catalyzed esterification is employed to convert FFAs into esters, thereby reducing FFA concentrations below the critical threshold (<1%) that would otherwise interfere with base-catalyzed transesterification. Sulfuric acid is commonly used as a homogeneous catalyst, typically at 0.5–1 % v/v in MeOH, with high MeOH-to-lipid molar ratios (often 8:1 to 20:1) and reaction temperatures between 50 °C and 75 °C. Once esterification is complete, the mixture may require neutralization (e.g., with sodium bicarbonate) and removal of excess acid before proceeding to the second stage. In the subsequent step, standard alkaline transesterification is performed, often using NaOH or KOH in MeOH under conditions similar to those described in Section 4.2.1. Both Zheng et al. (2012) and Khizar et al. (2024) confirmed that this method is particularly valuable for semi-industrial processing of insect-derived biodiesel, where lipid feedstocks frequently exceed 5 % FFA due to organic-rich substrates. However, its limitations include increased processing time, higher MeOH and energy consumption, and the need for multiple purification steps.

4.2.4. *Supercritical transesterification*

Non-catalytic transesterification has been successfully applied to *H. illucens* lipids as an innovative method for biodiesel production. Jung et al. (2022) investigated both lipid extracts and whole dried larvae as feedstocks, employing MeOH at high temperature (390 °C) in the presence of porous silica (SiO_2) to enhance the reaction by increasing the contact area between the MeOH vapor and the lipid phase. For lipid extracts, the optimal conditions included a MeOH-to-lipid ratio of 1/20 (w/v) and a reaction time of only 1 min, achieving a FAME yield of 94.1 wt%. When whole dried larvae were directly used without prior lipid extraction, the process was optimized at the same temperature and reaction time, with a MeOH-to-larvae ratio of 1/10 (w/v), leading to a FAME yield of 94.7 wt%. The process avoids the use of acid or base catalysts and benefits from the enhanced mass transfer provided by the SiO_2 support.

This technique offers several advantages, including simplified post-reaction separation, elimination of soap formation, and the potential to process unrefined or minimally pre-treated insect biomass. These yields were markedly higher than those obtained with conventional acid- or base-catalyzed methods previously applied to BSFL (27.9–36.3 wt%), demonstrating the efficiency of this thermally induced transesterification process. The method relies on MeOH in the vapor phase reacting directly with triglycerides without chemical catalysts or post-reaction neutralization, allowing the direct conversion of unrefined biomass into biodiesel. This approach represents a promising and environmentally friendly alternative to traditional catalytic methods for insect-derived lipids, although its high operating temperature requires careful consideration of energy efficiency and process design.

4.2.5. *Enzymatic transesterification*

Enzymatic transesterification of *H. illucens* lipids has emerged as an effective strategy for biodiesel production, particularly suitable for substrates with a high content of FFAs. Unlike chemical catalysis, the use of lipases enables the reaction to proceed under milder conditions, thereby avoiding soap formation and reducing the need for downstream purification steps. Nguyen et al. (2018b) employed Novozym® 435, an immobilized lipase derived from *Candida antarctica* (lipase B), known for its high operational stability in non-aqueous media, using methyl acetate as the acyl acceptor and optimizing the process via response surface methodology to achieve a biodiesel yield of 96.97 % at 39.5 °C, a 14.64:1 M ratio, and 12 h reaction time. In a subsequent study, He et al. (2022) applied a dual-enzyme system consisting of Eversa® Transform 2.0, a free liquid lipase from *Thermomyces lanuginosus*, and SMG1, a free microbial lipase produced via fermentation. Under optimal conditions (25 °C, 1:3 M ratio, 8 h), this combination achieved a FAME yield of 98.45 % and significantly reduced the FFA content to 0.17 %. The combined use of these biocatalysts resulted in a synergistic effect, enhancing reaction efficiency by enabling the conversion of triacylglycerols, diacylglycerols, monoacylglycerols, and FFAs in a single step. Among the key advantages of enzymatic transesterification are the high selectivity of the catalysts, tolerance to water and FFAs, the potential for enzyme recovery and reuse (particularly when immobilized), and compatibility with heterogeneous and continuous systems. These characteristics make the enzymatic approach particularly attractive for integration into sustainable biorefinery chains based on non-conventional feedstocks such as insects (Nguyen et al., 2018b; He et al., 2022).

When compared with other transesterification methods, each technique presents distinct advantages and limitations. Basic transesterification remains the most widely applied method due to its simplicity, low cost, and high efficiency under mild conditions, although it requires prior reduction of FFA content to avoid soap formation. Acid-catalyzed approaches are better suited for high-FFA feedstocks, enabling simultaneous esterification and transesterification, but they demand longer reaction times, higher alcohol-to-lipid ratios, and more corrosive conditions. The two-step acid–base process effectively combines the benefits of both, reducing FFAs in the first stage and achieving high conversion efficiency in the second, making it particularly suitable for feedstocks derived from organic-rich substrates. Supercritical transesterification eliminates the need for chemical catalysts and allows direct processing of unrefined biomass, simplifying downstream separation and avoiding soap formation. However, the high energy input required for operation at elevated temperatures can limit its industrial feasibility. Enzymatic transesterification offers notable sustainability advantages, including mild operating conditions, high selectivity, and tolerance to water and FFAs, while enabling integration into biorefinery systems. Its current limitations lie in higher enzyme costs and longer reaction times compared to chemical processes. Overall, the choice of transesterification method for *H. illucens* lipids depends on the characteristics of the feedstock, desired product quality, process economics, and environmental considerations. Future developments should aim at optimizing energy efficiency, reducing processing costs, and integrating these methods into scalable, sustainable biodiesel production chains.

4.3. *Process energy and cost considerations*

The production cost of biodiesel varies markedly by feedstock: consolidated data report WCO/UCO at ~0.55 USD L^{-1} (USD L^{-1} = U.S. dollars per liter; 2023) and 0.1318 USD L^{-1} (2017; best-case), rapeseed ~0.75 USD L^{-1} (2020), palm ~0.68 USD L^{-1} (2020), sunflower ~0.71 USD L^{-1} (2017), and microalgae ~0.275 USD L^{-1} (2020, best-case); for *H. illucens*, costs are reported in £ t^{-1} (U.K. pounds per metric ton) at ~800–819 £ t^{-1} for live/dried biomass and are not directly comparable to USD L^{-1} without additional assumptions (He et al., 2025). On transesterification routes, supercritical MeOH is flowsheet- simple but operates at high temperature and pressure. Economic feasibility depends on scale, heat recovery, and MeOH recycle, and ~0.17–0.52 USD L^{-1} has been reported for WCO systems (van Kasteren & Nisworo, 2007). Under homogeneous catalysis, acid pretreatment followed by alkaline transesterification is customary for high-FFA WCO, whereas alkaline catalysis is preferred for low-FFA virgin oils; overall process energy is moderate, and overall economics remain feedstock-driven (Zhang et al., 2003a, 2003b). Enzymatic routes operate at low temperature and under mild conditions, but operating costs are dominated by the immobilized biocatalyst (e.g., Novozym® 435), making competitiveness sensitive to purchase price and effective reuse/regeneration (Fjerbaek et al., 2009). For lipid extraction, pre-press plus closed-loop *n*-hexane remains a low unit-cost option at comparable scale due to high oil recovery and high solvent-recovery efficiency; the energy duty (and thus a large share of energy Operating Expenditure (OPEX)) concentrates in meal desolventizing/drying and miscella distillation/stripping, while the main capital items are the extractor, solvent-recovery trains (evaporators/strippers/condensers), and the desolventizer-toaster (Carré, 2024; Cheng & Rosentrater, 2019). Bio-based solvents (2-MeTHF, CPME, MeOAc) reduce petroleum-solvent use but, at equal yield, tend to shift costs to distillation/heat-exchange duties, and may require adapted solvent-recovery trains (e.g., decanters or additional columns) depending on solvent–water phase behavior, potentially affecting Capital Expenditure (CAPEX) (Chemat et al., 2020; Cravotto et al., 2022). Single-stage mechanical pressing has low CAPEX/OPEX but lower oil recovery (typical use as pre-press) (Carré, 2024; Cheng & Rosentrater, 2019). SC-CO$_2$ (supercritical carbon dioxide) yields solvent-free, high-purity oils but is more energy-intensive and requires high-pressure equipment and recycle compression, increasing capital and energy demands compared with conventional solvent extraction (Uwineza and Waśkiewicz, 2020; Khalati et al., 2023). In insect oils, initial moisture and drying are primary drivers of the thermal load and influence downstream separations (Fornari et al., 2023); pressing reduces solvent handling but entails lower lipid recovery, whereas hexane extraction achieves oilseed-like yields (Franco et al., 2021).

Industrial-scale biodiesel output from *H. illucens* can be contextualized through scenario-based estimates that combine publicly reported industrial capacity indicators with experimentally reported conversion performance. Public disclosures for the ADM–InnovaFeed Decatur project indicate a potential production of ~20,000 t y^{-1} of oils (currently intended for feed markets) from a single site (Chemical and Engineering News, 2020; World Grain, 2022). If, purely as an illustrative scenario, this oil stream was diverted to fuel, then applying literature values for FAME/biodiesel production from BSFL lipids (~0.94–0.98 on a lipid-feedstock mass basis) would correspond to roughly ~18,800–19,600 t y^{-1} of biodiesel (Jung et al., 2022; Nguyen et al., 2018b; He et al., 2022). On an energy basis, FAME has a net specific energy of ~37 MJ kg^{-1} (CIMAC, 2024), and potential GHG (greenhouse gases) implications can be benchmarked against the RED II fossil fuel comparator for transport biofuels (94 g CO$_2$eq MJ^{-1}) (European Parliament and the Council, 2018). However, net GHG savings are system-dependent and sensitive to process choices (notably dewatering/drying energy demand, heat/solvent recovery, electricity mix, and allocation in multi-product systems); accordingly, industrial LCA evidence indicates that the climate-change impact attributed to *H. illucens* fat can vary across configurations and assumptions (Francis et al., 2025).

5. Regulations and standards for biodiesel from *Hermetia illucens*

The large-scale commercialization of biodiesel from *H. illucens* depends on strict compliance with international fuel standards, such as EN 14214 in Europe and ASTM D6751 in the United States, which establish limits for key properties including ester content, oxidative stability, and cetane number (Table 5). One of the main challenges for *H. illucens*-derived biodiesel lies in its distinctive lipid profile, dominated by SFAs, particularly lauric acid (C12:0), which can influence viscosity and oxidative stability. To ensure conformity with specifications, strategies such as blending with other lipid sources, antioxidant supplementation, or targeted refining may be required. The regulatory framework for insect-derived oils in fuel applications is still evolving, highlighting the need for additional studies to fully assess their safety, sustainability, and market integration. Data from Zheng et al. (2012), Nguyen et al. (2018a), Jung et al. (2022), and Park et al. (2022) suggest that, for the properties reported, *H. illucens* biodiesel can meet ASTM D6751 and EN 14214 specifications, with some exceptions. Cetane numbers reported range between 50 and 58, satisfying ASTM requirements (≥47); however, only the higher value (58) reported by Zheng et al. (2012) also meets the stricter EN limit (≥51), underscoring the importance of feedstock and process conditions for ignition quality. Kinematic viscosity falls within ASTM limits (1.9–6.0 mm^2/s) across all studies, but only the values reported by Jung et al. (2022) and Park et al. (2022) are

Table 5

Comparative analysis of biodiesel properties from *H. illucens* and international standards. Biodiesel samples were obtained from larvae reared on different organic substrates and processed with distinct lipid extraction or direct transesterification methods. Properties are compared against ASTM **D6751** (US) and EN 14214 (EU) specifications.

	Jung et al., 2022	Park et al., 2022	Nguyen et al., 2018a	Zheng et al., 2012	ASTM D6751 (US Standard)	EN 14,214 (EU Standard)
Density (kg/m^3)	880 ASTM✓ EN✓	875 ASTM✓ EN✓	875 ASTM✓ EN✓	860 ASTM✓ EN✓	Not specified	860–900
Kinematic Viscosity (mm^2/s)	4.0 ASTM✓ EN✓	4.0 ASTM✓ EN✓	5.2 ASTM✓ EN×	4.9 ASTM✓ EN✓	1.9–6.0	3.5–5.0
Cetane Number	–	–	50 ASTM✓ EN×	58 ASTM✓ EN✓	≥47	≥51
Oxidative Stability (h)	–	7.7 h ASTM✓ EN× (after adding antioxidant)	–	–	≥3	≥8
Flash Point (°C)	131 ASTM✓ EN✓	138 ASTM✓ EN✓	121 ASTM✓ EN✓	128 ASTM✓ EN✓	≥93	≥101
Water Content (wt. %)	0.02 ASTM✓ EN✓	<0.01 ASTM✓ EN✓	0.03 ASTM✓ EN✓	0.02 ASTM✓ EN✓	≤0.05	≤0.05
Total Glycerin (% mass)	0.03 ASTM✓ EN✓	0.02 ASTM✓ EN✓	–	–	≤0.24	≤0.25
Acid Number (mg KOH/g)	0.12 ASTM✓ EN✓	0.16 ASTM✓ EN✓	<0.8 ASTM× EN×	0.6 ASTM× EN×	≤0.50	≤0.50
Larvae feeding	Food waste	Food waste	Fermented wheat bran	Restaurant waste SRF*	–	–
Lipid extraction method	Soxhlet (*n*-hexane)	*n*-hexane (liquid–liquid)	Direct transesterification (no prior lipid extraction)**	Petroleum ether (liquid–liquid)	–	–

* SRF = Solid Residual Fraction, obtained after grease extraction from restaurant waste.

** Direct transesterification indicates that no preliminary lipid extraction was performed; the biomass was reacted directly with methanol and catalyst. Notes. ✓ = compliant; × = non-compliant;

within the narrower EN range (3.5–5.0 mm^2/s), while Nguyen et al. (2018a) slightly exceeds it (5.2 mm^2/s), suggesting that viscosity control may be necessary for full EU compliance. Oxidative stability data are scarce; Park et al. (2022) report 7.7 h after antioxidant addition, meeting ASTM criteria (≥3h) but remaining below the EN minimum (≥8h). In that study, the baseline oxidation stability was 0.8 h and increased to 7.7 h by adding the phenolic antioxidant *tert*-butylhydroquinone (TBHQ) at 200 ppm (mg/kg). The iodine value, a parameter linked to unsaturation and oxidation susceptibility, was not reported in these studies, yet EN 14214 sets a limit of ≤120 g I_2/100 g, highlighting the need for further evaluation. Flash point values (121–138 °C) are consistently above ASTM (≥93 °C) and EN (≥101 °C) requirements, ensuring safe handling and transport. Water content is minimal across all reports (≤0.03 wt%), well below the 0.05 wt% limit, confirming adequate dehydration and reduced risk of microbial growth or corrosion. Similarly, total glycerin content remains far within ASTM (≤0.24 %) and EN (≤0.25 %) specifications, supporting good combustion performance and minimal deposit formation. Acid number results are generally favorable, ranging from 0.12 to 0.16 mg KOH/g in Jung et al. (2022) and Park et al. (2022), which are well below the 0.50 mg KOH/g limit. However, Zheng et al. (2012) report 0.6 mg KOH/g, exceeding both ASTM and EN thresholds, indicating that in some cases additional refining or pretreatment to lower FFAs may be required. Representative examples include: (i) chemical refining of the oil (degumming–alkali neutralization–bleaching–deodorization), which in *H. illucens* yields AV ≈ 0.90 mg KOH/g (Mai et al., 2019); (ii) for feedstocks with elevated FFA (>5%), application of acid-catalyzed pre-esterification/transesterification to prevent soap formation and reduce FFA to levels suitable for subsequent base-catalyzed processing (Meher et al., 2006; Nguyen et al., 2018a); (iii) adsorptive polishing of the FAME with magnesium silicate or silica (dry-wash) or ion-exchange resins, which can lower the acid number to ≤ 0.17 mg KOH/g (Faccini et al., 2011). The reporting of cold-flow metrics and compositional parameters (cloud/pour point, CFPP, copper strip corrosion, total/free glycerol, mono- and diglycerides, metals, and phosphorus) remains heterogeneous and, at times, incomplete; future priorities include systematic measurements and harmonized protocols to enable reliable quantitative comparisons. Overall, biodiesel from *H. illucens* demonstrates encouraging potential to meet international fuel quality and safety requirements, already complying with several parameters specified by EN 14214 and ASTM D6751, albeit with some remaining costraints. To achieve consistent compliance and enable industrial scalability, future research should focus on optimizing transesterification conditions, refining larval diet formulations, and improving refining steps and co-product management; in parallel, bridging the oxidative-stability gap relative to EN 14214 remains a priority by optimizing antioxidant type and dose together with storage practices. These improvements, coupled with clearer regulatory guidelines for insect-based fuels, could facilitate its adoption as a sustainable alternative to fossil diesel.

6. Emerging genetic approaches to enhance lipid yield in *Hermetia illucens*

Genetic improvement of BSF larvae is an active area of research with direct implications for industrial applications. Pioneering studies have demonstrated that selective breeding programs can significantly enhance larval growth and lipid yield, confirming the feasibility of gradual improvement strategies under industrial conditions (Facchini et al. 2022). Importantly, subsequent research by Shrestha et al., 2025 showed that such selection does not compromise fecundity, thereby ensuring the long-term reproductive sustainability of selected colonies, a crucial aspect for large-scale rearing. In parallel, industrial developments have moved in this direction: the BugEra technical report (2023), an industry technical report, suggests that genetically improved lines have shown a potential increase of up to twofold in larval lipid yields under controlled production conditions, highlighting the economic and industrial potential of these innovations. Beyond selective breeding, recent advances in genome editing using CRISPR/Cas9 have shown the effectiveness of this tool in targeting genes associated with key traits in BSF, including pigmentation (Sui et al., 2024; Dong et al., 2025) and biosafety through the generation of wingless lines (Kou et al., 2024). More recently, Jiang et al., 2024 provided the first functional evidence of CRISPR/Cas9-mediated disruption of a metabolic gene directly linked to lipid homeostasis, the Lipid Storage Droplet 1 (LSD1) gene. Knockout mutants (lsd1−/−) displayed enhanced larval growth, larger lipid droplets, and increased feed conversion efficiency, without developmental defects. These findings reveal that targeted manipulation of lipid metabolism pathways can alter biomass composition and energy utilization, paving the way for rational metabolic engineering of BSF lines tailored to industrial goals (Jiang et al., 2024). However, approaches aimed directly at modulating metabolic pathways linked to lipid or protein accumulation remain largely unexplored. In this context, future research applying genome editing to modify biomass composition could represent a transformative step, enabling the development of *H. illucens* strains tailored to specific industrial applications: lipid-rich lines for biodiesel and sustainable aviation fuel production, and protein-rich lines for feed and food industries. Overall, the integration of selective breeding and genetic engineering opens new opportunities for growth, economic profitability, and reduced environmental impact in biofuel-oriented production strategies.

7. Research needs and future directions

As already demonstrated in the most recent studies on *H. illucens* biodiesel, the main bottleneck is achieving compliance with standards and industrial-scale implementation (He et al., 2025; Jung et al., 2022; Park et al., 2022). In particular, future studies should evaluate the parameters that are still often missing (especially descriptors related to oxidation and low-temperature operability), as these gaps currently prevent ‘fair’ quantitative comparisons between raw materials and conversion pathways (EN 14214; ASTM D6751; Nguyen et al., 2018a; Zheng et al., 2012; Jung et al., 2022; Park et al., 2022; He et al., 2025). Another prerequisite will be the selection of specific breeding substrates that lead to the production of lipids with physicochemical characteristics optimized for biodiesel (Meneguz et al., 2018; Ewald et al., 2020; Hadj Saadoun et al., 2020). Starting from upstream control, a key direction is an integrated, scale-relevant optimization that connects substrate/diet design with defined oil specification windows (including FFA and moisture), guides the selection of the most suitable conversion pathway (e.g., alkaline/acid–base, enzymatic, or non-catalytic routes), and informs the downstream purification strategy, while prioritizing process robustness under realistic raw-material variability (Canakci and Van Gerpen, 2001; Meher et al., 2006; Jung et al., 2022). Finally, translation requires more pilot-scale demonstrations with realistic mass/energy balances and harmonized TEA/LCA assumptions for multi-output insect systems, along with the continued development of breeding/genetic strategies to reduce variability and improve breeding performance (Guo et al., 2021; Mertenat et al., 2019; Francis et al., 2025; Facchini et al., 2022; Shrestha et al., 2025; Kou et al., 2024; Jiang et al., 2024).

8. Conclusion

Using *H. illucens* for biodiesel couples waste valorization with reduced reliance on conventional feedstocks. An indicative carbon footprint (∼0.80 ± 0.10 kg CO_2 eq/kg) shows an environmental advantage (Francis et al., 2025). In terms of performance, cetane number (≈50–58) is comparable to soybean/rapeseed/Jatropha biodiesel (Hoekman et al., 2012; Karmakar et al., 2010; Yamane et al., 2007); flash point (∼121 °C), density (∼875 kg m^{-3}), water (∼0.03 wt%), and total glycerol fall within ASTM D6751/EN 14214 (Nguyen et al., 2018a) kinematic viscosity (4.0–5.2 $mm^2\ s^{-1}$) is generally ASTM-compliant but near the EN upper bound. In crude *H. illucens* biodiesel, acid value is often >0.50 mg KOH/g; refining or acid-then-base processing restores compliance. Oxidative stability meets ASTM (≥3h) but is below EN (≥8h) without antioxidants (Park et al., 2022). Overall, *H. illucens* biodiesel is a promising, adaptable option, already viable as a waste-derived blendstock; genetic improvement and genome editing, plus clearer regulation, standardization, and stability management, can support scale-up.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the clarity and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

CRediT authorship contribution statement

Valentina Pucciarelli: Writing – review & editing, Writing – original draft. **Dolores Ianniciello:** Writing – review & editing. **Eric Schmitt:** Writing – review & editing. **Carmen Scieuzo:** Writing – review & editing. **Patrizia Falabella:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare no competing financial interest.

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Data availability

No data was used for the research described in the article.

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