USING BLACK SOLDIER FLIES (HERMETIA ILLUCENS) TO BIOCONVERT WASTE FROM THE LIVESTOCK PRODUCTION CHAIN: A LIFE CYCLE ASSESSMENT CASE STUDY

ROSANGELA SPINELLI[1], PAOLO NERI[1], MARTINA PINI[1], SILVIA BARBI[2], MONIA MONTORSI[1] & ANNA MARIA FERRARI[1]
[1]Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, Italy
[2]Interdepartmental Centre for Applied Research and Services in Advanced Mechanics and Motoring, Italy

ABSTRACT
The aim of this study was to enhance waste from the livestock production chain using insects to produce biomaterials that can fall within the agricultural production cycle (e.g. plastic mulch), in order to achieve sustainability throughout the technological process. After stabilization by drying, mature larvae of Hermetia illucens reared on substrate composed of poultry manure, zeolite and water were chemically separated in the laboratory to extract the proteic, lipidic and chitinic fractions. Proteins were then isolated and added to other components in order to obtain bioplastics. The environmental impacts of the bioplastic production process developed at a laboratory scale was evaluated through the Life Cycle Assessment (LCA) methodology.
Keywords: life cycle assessment, Hermetia illucens, proteins, waste bioconversion.

1 INTRODUCTION

The disposal of livestock manure/waste from the zootechnical value chain and the organic fraction of municipal waste is subject to strict regulations in terms of traceability which involves high costs and prevents alternative uses. This kind of waste is ultimately used to produce poor quality compost.

In nature, these organic materials are the optimal substrate for the growth of various insect species that are able to adapt and thrive in environments with high amounts of microorganisms. Insects can play an important role in recycling waste or other nutrients accumulated in the environment [1]. According to these authors, Hermetia illucens Linneaus, 1758 (Diptera, Stratiomyidae), one of the most studied species for its potential use as a food product, seems to be able to consume manure from poultry and pig farms and to convert it into biomass with a high protein and lipid content.

Several studies have investigated the nutritive composition of insects and their potential use as a source of protein, both for human consumption and animal feed [2]–[6], or as a source of fats for biodiesel production [7]–[11]. This study investigates the potential of proteins extracted from the prepupae of Hermetia illucens grown on poultry manure substrate, as the basis for a new type of bioplastics. The creation of a system that uses insects to transform waste into usable materials for the development of biomaterials is part of a sustainable design approach that is the undisputed driver for the change and development of new production technologies.

In order to evaluate the environmental profile of insect-based products, the environmental impacts associated with the whole life cycle of these processes should be quantified. Life cycle assessment (LCA) is an essential tool for analysing and evaluating the environmental impact of production systems and insect-based products. However, the scientific literature on this specific field of research is still limited. Using the LCA approach, Oonincx and De Boer [12] published a detailed environmental impact assessment in terms of

WIT Transactions on Ecology and the Environment, Vol 231, © 2019 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/WM180051

global warming, agricultural land use and energy consumption for the mass farming of two species of mealworms (Tenebrio molitor and Zophobas morio) in comparison with traditional protein sources for human consumption (milk, chicken, pork, beef). Their results highlighted greater GHG emissions and land use associated with milk, chicken, pork and beef systems, whereas similar amounts of energy are required in conventional and mealworm protein production. However, Oonincx and De Boer [12] reported that the high energy consumption observed for the production of insects is due in large part to the need to air-condition the breeding environment.

De Boer et al. [13] used LCA to compare different protein production scenarios in order to investigate whether soybean products from South America could be replaced by protein sources produced in Europe, without negatively affecting the carbon footprint of the feed (CFP). They found that mealworms seem to have little potential for inclusion in compound feed, without increasing the CFP, because replacing 12% soybean products from South America with 6.1% insects (mealworms) increased the CFP from 595 to at least 717 g CO_2 eq. per kg of compound feed. This is partly caused by the large energy requirement for heating during the production phase and a drying step thereafter. However, they also concluded that the use of other insect species with a low energy requirement during rearing and higher nutrition values, reared on waste products instead of feed ingredients, could increase the replacement potential of insects.

Van Zanten et al. [14] focused on the use of housefly larvae grown on poultry manure and food waste as livestock feed. They concluded that the energy use is the main contributor to the direct environmental impact of larvae meal production. Indirect environmental consequences entail the inclusion of the environmental impact of producing the energy needed to replace the original bioenergy function of FW used for feeding housefly, which is "situation specific" depending on the use of FW in the system under investigation (aerobic digestion, anaerobic digestion, etc.).

Salomone et al. [15] used LCA methodology to assess the potential environmental impacts of food-waste bioconversion into compost and dried larvae through the action of Hermetia illucens. Results related to the functional unit of 1 tonne of food waste treated show a value of 30.2 kg CO_2 eq. in terms of global warming potential, 215.3 MJ in terms of energy use, and 0.661 m2a in terms of land use. When compared with alternative sources of raw material for feed or biodiesel, these results show that the most significant benefits of insect production are connected to Land Use, while Energy Use is the main burden.

In this study, we applied LCA approach to evaluate the environmental performance of the production process of innovative bioplastics created from biopolymers obtained from proteins extracted from the black soldier fly (BSF), grown on poultry manure. The aim was to develop a detailed picture of the environmental profile of the bioconversion process and of the availability and quality of the data.

2 MATERIALS AND METHODS

This section characterises the insect species under investigation, and then details the LCA method and data.

2.1 The insect species under investigation: Hermetia illucens (Diptera: Stratiomyidae)

The black soldier fly (BSF) is a Dipteran fly of the Stratiomyidae family and Hermetia illucens species. The BSF is native to the warm and temperate zone of America, and was then introduced to the tropical and subtropical regions worldwide [16]. The larvae are extremely voracious (they consume a quantity of food substrate equal to twice their weight on a daily

basis) and can grow on different types of organic waste, including waste from the agri-food industry and agricultural processes, zootechnical waste, and urban wet waste, thereby reducing its mass significantly [17]–[21]. Adult BSFs are large and can reach a maximum length of 20 mm. Under ideal conditions (food supply, temperature, and humidity), BSF larvae can develop into prepupae within two weeks, however the developmental timing depends heavily on farming conditions. The BSF life cycle can be divided into five main phases: egg, larva, prepupa, pupa and adult. A special feature of the adult stage is that they do not have mouthparts, thus they do not feed. However, they survive thanks to the fat stored from their larvae stage [22], [23] and therefore adequate nutrition is important in this stage. The BSF does not come into contact with any degrading or fresh organic material including foodstuffs, and can therefore not be regarded as unsanitary or a vector of diseases [24]. Adults only live for 5–8 days, during which time they need be able to mate and lay eggs. When the moment of mating arrives, the males come together to "court" and "conquer" the females of the same species. Eggs are laid inside cracks and crevices, in dry environments near to the larval substrate, where the offspring can easily flourish. Once mated, the female lays an egg clutch of 200 to 700 eggs which hatch within 4 days [25]. Once born, the larvae fall into the substratum, the source of food where they will feed. The larval stage, in fact, seems to be the most vital in terms of waste management as it represents the stage where the larvae feed and thus contribute to the bioconversion. The larvae feed for about two weeks, before moving to the prepupae stage, in which they accumulate sufficient fat which will be an energy source for the adult stage. Newton [26] states that the larval stage can be extended for up to four months if food is limited [19]. The prepupae stage, which is the last BSF larvae stage, is important in terms of waste transformation that is still happening [27] and in conversion of biomass. The prepupae are characterized by a change in colour: they turn from white to dark brown. They also tend to migrate from the larval habitat (food source) to another place which ideally must be dry and dark [16], [22]. The pupal stage represents the last stage before the appearance of adult flies and takes about two weeks together with the prepupae phase. The prepupe of Hermetia illucens, grown on animal excrement, have a high protein and lipid content, of 43.2% and 28%, respectively, and an ash content of 16.6%. The values are expressed on dry matter. The composition of the BSF is strongly influenced by the stage of development and, above all, by the type of substrate on which it is grown.

2.2 The implemented method: life cycle assessment (LCA)

The present study was carried out adopting the LCA methodology [28], [29]. An LCA entails identifying and assessing the potential environmental impact associated with a material, product, service or process throughout its entire life cycle, from the raw material extraction and processing, through manufacturing, transport, use and final disposal.

2.2.1 Goal and scope

We assessed the environmental impact associated with the laboratory-scale production of innovative bioplastics produced from biopolymers obtained from proteins extracted from BSFs. These bioplastics, mainly used in the agricultural sector (e.g. sheet mulching), aside from carrying out their primary function, act as a slow-release fertilizer, releasing nitrogen during their decomposition. The residue, which is not assimilated by the larvae, is also useful for agronomic purposes, as it is a high quality compost.

2.2.2 Functional unit

The functional unit is represented by the amount of bioplastic produced (0.403 gr).

2.2.3 System boundaries

The system boundaries range from the BSFs reared on organic substrates to the bioplastic production, by passing through the phase of isolation, characterization and extraction of the biomolecules. The energies, materials, water, main equipment with their end of life, transport, waste and their treatment, emissions into the air at the continental level, the aspiration system and purification plant as well as the recovery and reuse of certain solvents were also considered.

2.2.4 Life cycle inventory (LCI)

Regarding the quality of data used for the life cycle inventory (LCI), lab-scale data were directly collected from the experimental procedure. In particular, laboratory data on breeding, biomolecule extraction and bioplastic production were the primary data collected during experimental tests. Where the data were not available, the study was completed on the basis of secondary data obtained from the Ecoinvent databases v3 [30] which were used to model the background processes (land use, material production, fuel and electricity production, and transport). The emissions were calculated assuming that the laboratories were fully ventilated after the conclusion of each working day (8 hr). For these emissions, the indoor concentrations were calculated considering a total laboratory volume of 480 m^3 and assuming that 1% of the emissions come from the aspiration filters with 99% efficiency. The extracted lipidic and chitinic fractions are not used for the production of bioplastic, and thus were considered as co-products. A mass allocation was used. Data relating to the following phases were considered: (a) BSF breeding; (b) BSF biomolecule fractionation; (c) bioplastic production.

2.2.4.1 (a) BSF breeding

The pilot plant consists of two main parts (physically separated):

1. *Nursery*: for the adult mating, oviposition and hatch of eggs;
2. *Bioconverter*: for the conversion of the organic waste into BSF adult larvae.

In addition, a substrate preparation system and a sorting system are installed in the demonstration plant (see Fig. 1).

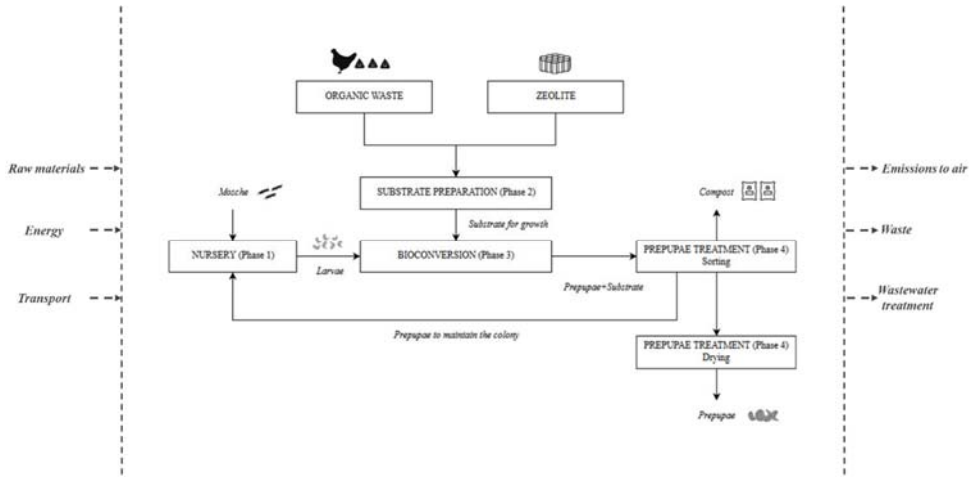

Figure 1: Black soldier flies breeding.

Once the reference unit was defined represented by the production in terms of 26 trays, the data relating to the following four phases were collected:

1. *Nursery (Phase 1)* This covers the production of eggs and larvae of Hermetia Illucens at the first stage of life. These larvae are used to transform waste and to maintain the colony in order to continue the larvae production cycle. After the adults emerge from their pupal cases, their primary focus is to mate and lay eggs. They do not feed, and only drink water and sugar. The adults are kept in a nylon cage (60 cm X 60 cm X 120 cm) inside an isothermal box (80 cm X 100 cm X 200 cm) which is made up of a rigid PE shell insulated with expanded polyurethane foam. LED lamps with three specific wavelengths (515 nm, 450 nm, 365 nm), which have the best effect on the mating and oviposition of Hermetia illucens, illuminate the cage [31]. The temperature of the nursery is fixed at 26°C and 70% of Relative Humidity [31]. A miniaturized single-board computer mounted directly on the box monitors the temperature and humidity. Attached to the single-board computer is an integrated camera in order to extrapolate with dedicated algorithms the number of flies, number of matings, and number of dead flies. Just a single initial input of pupae is needed to start up the process, thereafter the production chain constantly supports itself through limited withdrawals from the larvae colony.
2. *Substrate preparation system (Phase 2)* The ideal substrate for the growth of larvae is prepared. The experimental design includes three components for the blend of the BSF growth substrate. The percentage (by weight) of each substrate component was optimized based on the experiments conducted in the laboratory in order to obtain the maximum number of prepupae (Table 1).

   The chicken manure is supplied by the Farm Sant'Andrea S.r.l which belongs to the Amadori group, who are key player in the livestock sector. Zeolite is added to overcome the problem of odorous emissions from the organic waste, and particularly animal manure. Zeolite is like a kind of sponge, it absorbs ammonia by reducing the unpleasant emissions and then releases it into the soil to the plants that need it. Once the zeolite is in the ground, it acts as a fertilizer.
3. *Bioconvertor (Phase 3)* The substrate is distributed in trays, which are inserted into a steel structure by an electric forklift, and larvae are positioned on the substrate and are kept at a temperature of 30-35 °C, humidity >65%. The amount of larvae introduced varies depending on the type and amount of organic waste. The bioconversion process lasts about 12 days in which the larvae go through the various life cycle phases. Under ideal conditions, it takes about two weeks for the larvae to reach maturity. BSF larvae pass through five larval stages; on reaching the pre-pupal stage, the larva detaches from the substrate. Larvae need to leave the manure to successfully pupate into an adult, so the separation of larvae from compost is easy.
4. *Prepupae treatment (Phase 4)* After the bioconversion, larvae and compost products are separated by a vibrating screen. Part of the colony is kept to evolve into pupae and maintain the production chain; the rest are dried at 45°C (using a dryer of 3.3 kWh) to remove water by evaporation (weight reduction of about 40%).

Table 1: Components for the blend of the BSF growth substrate.

Component	**Max prepupae**
Poultry manure	34.5%
Zeolite	7.20%
Water	58.30%

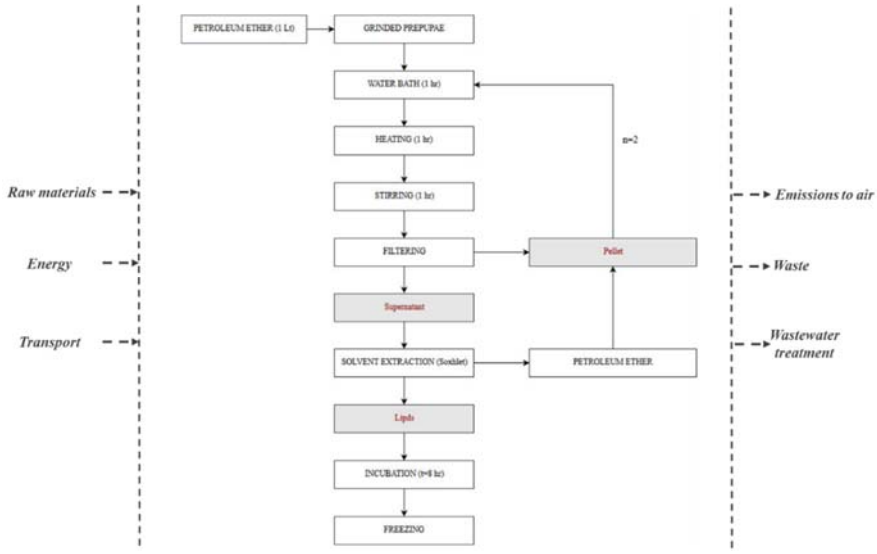

Figure 2: Flowchart of lipid extraction protocol, showing the system boundaries considered in the LCA study.

2.2.4.2 (b) BSF biomolecule fractionation

Hermetia illucens prepupae are stored at -20°C in ziplock bags until use. Frozen prepupae are ground and immediately used for the various extraction analyses/treatments. As a first step, BSFs are subjected to lipid extraction (Siteia Parma Laboratory) (Fig. 2).

Lipids are extracted with petroleum ether (40–60°C boiling point fraction) in a two-step method. BSF biomass (375 gr) and petroleum ether (1 Lt) are stirred by a magnet stirrer for 1 hr. The solvent containing fats is decanted and recovered by paper filtration. The procedure is repeated. Lipids are recovered by solvent extraction with a Soxhlet extractor. Residual solvent is removed from the defatted insect pellet by evaporation overnight. The defatted insect pellet is prepared for protein extraction (Siteia Parma Laboratory) (Fig. 3). It is treated with 40 mL of 1 M NaOH in a water bath at 40°C for 1 hr. The solution is centrifuged for 15 min at 4000 rpm. The pellet is demineralized with 40 mL of 2N HCl for 24 hours at room temperature. The proteins are recovered from the supernatant by precipitation with a solution of normal HCl 6N:

1. HCl is added to the supernatant;
2. the solution is incubated overnight at -20°C (i.e. the temperature that facilitates the aggregation of proteins);
3. the solution is centrifuged at 4°C, 4000 rpm for 15 min;
4. the pellet is dried in an oven at 60°C for 8 hr, while the supernatant is disposed of as a hazardous special waste.

The final step is the chitin separation (Fig. 4). The pellet that has been demineralized with 40 mL of 2N HCl for 24 hours at room temperature is centrifuged for 15 min at 4000 rpm. The precipitate is washed twice with distilled water, while the supernatant is disposed of as hazardous special waste. The final residue is dried in an oven at 40°C overnight.

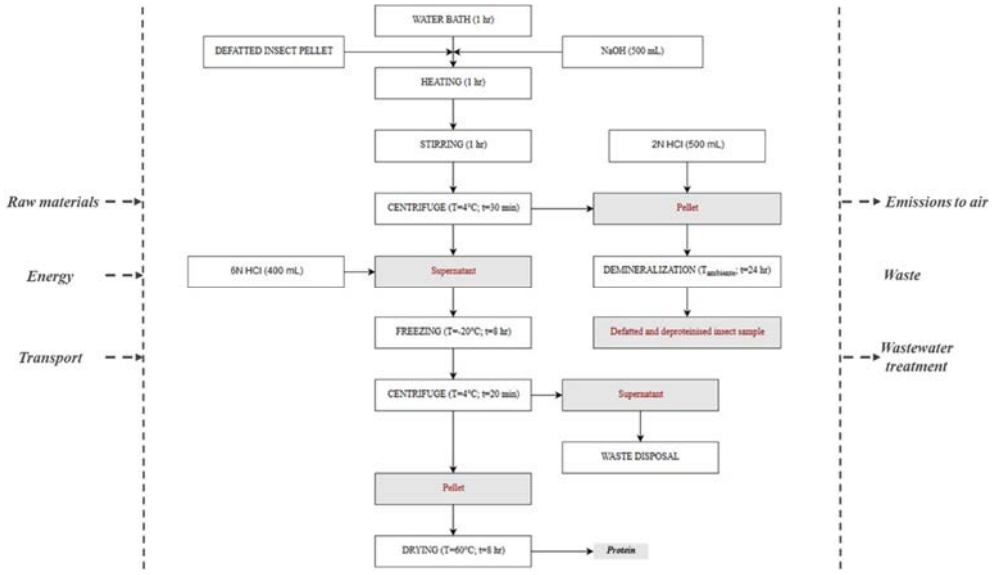

Figure 3: Flowchart of protein extraction protocol, showing the system boundaries considered in the LCA study.

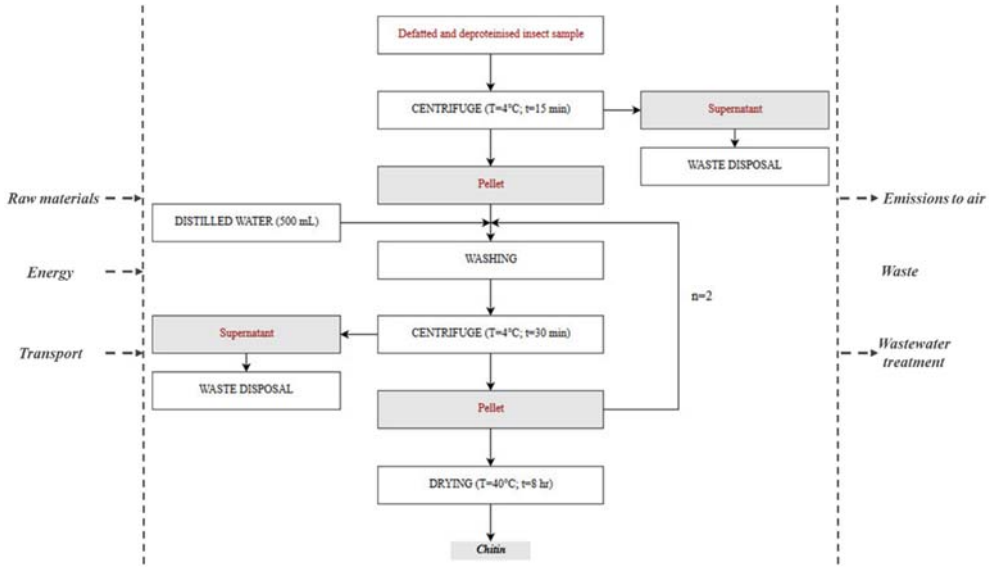

Figure 4: Flowchart of chitin extraction protocol, showing the system boundaries considered in the LCA study.

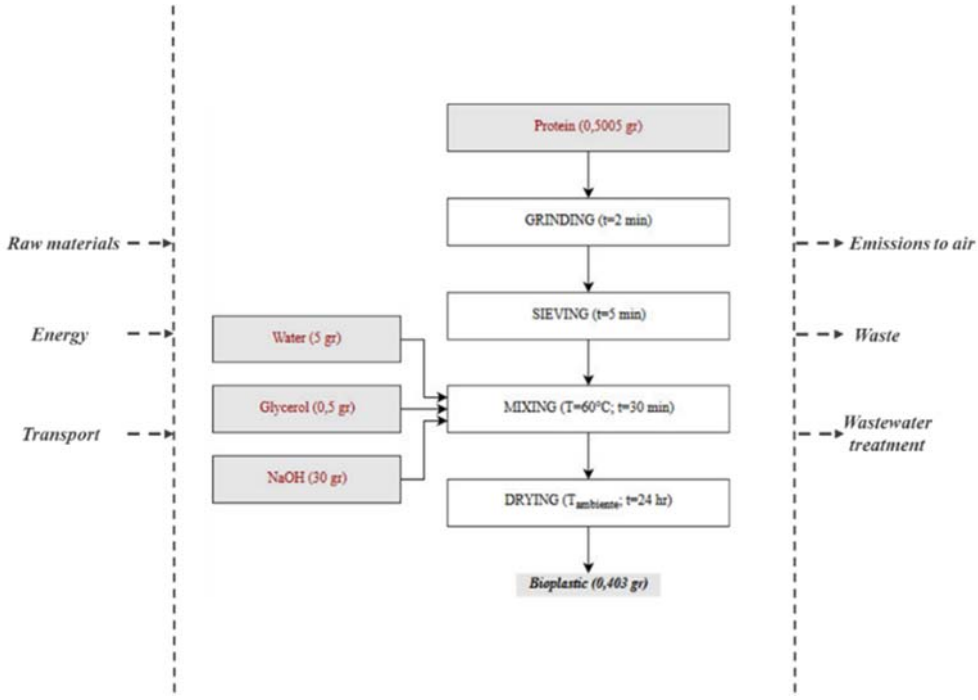

Figure 5: Flowchart of bioplastic production, showing the system boundaries considered in the LCA study.

2.2.4.3 (c) Production of bioplastic

The protein fraction is ground and then sieved. A mixture is prepared with distilled water, proteins, glycerol and NaOH. The mixture is heated at 60°C for 30 min and stirred at 200 rpm. The solution is poured into an aluminum wrapper and incubated under a fume hood for 24 hours at room temperature (Fig. 5).

We are still in the process of performing an agronomic evaluation of compost and biomaterials (crop productivity, weeds control, duration, biodegradability). In this first stage of the study, a composting process has been hypothesized as bioplastic end of life.

2.2.5 Impact assessment methodology (LCIA)

The life cycle impact assessment (LCIA) results were modelled using the IMPACT 2002+ method [32] with Simapro 8.3 [33] to determine the environmental impact. This impact assessment method covers more impact categories than other methods, includes more substances. Since it is midpoint and endpoint oriented, it provides a complete overview of the environmental performance. However, the following additions and modifications were implemented to describe the system considered in a more representative manner i.e. modification to land use (different types of land transformations were considered) and mineral extraction categories (additional resources were added) and radioactive waste (radioactive waste and its occupied volume were evaluated) [34]. The LCIA results were derived from both midpoint and endpoint levels. However, for the sake of brevity we report only the endpoint results. These are usually shown as the impact on human health, ecosystem quality, climate change and resource depletion. We decided to report only the endpoint results

as the interpretation of these results does not require extensive knowledge of the environmental effects. Moreover, midpoint results can be more difficult to interpret because they consider a large number of impacts which are often difficult to understand.

3 RESULTS AND DISCUSSION

The analysis of results (Fig. 6) shows that the total damage associated to 1 gr bioplastic production process is equal to 3.4296 mPt. Furthermore, the main environmental impact is mainly due to energy consumption (63%), in particular, due to the energy consumption of aspiration system (93%) because of the incubation phases under aspiration hoods. In fact, the extraction process of the three fractions (lipid, protein, chitinic) and the bioplastic production provide for incubation steps under the aspiration hood whose duration is very high. Table 2 reports the environmental performance at end-point level (damage categories). Therefore, Human health, Resources and Climate change categories afflict the total damage for 31.48%, 32.06% and 26.60% respectively. In particular, in Human health the major contribute to the total impact is due to Particulates, < 2.5 μm emission in air; Resources is mainly affected by natural gas and the emission that mainly contributes to the Climate change is Carbon dioxide, fossil. All these emissions are due to electric energy production used by the aspiration system. Occupation, forest, intensive affects Ecosystem quality category (9.85% on the total damage) and is generated by wood used for the activated carbon filter production (filter typology installed in the aspiration system).

4 CONCLUSIONS

The results show that the energy consumption of aspiration system is high. This consumption should be reduced in order to minimize the environmental impacts but it is absolutely necessary to aspire to avoid emissions of dangerous substances. This environmental impact

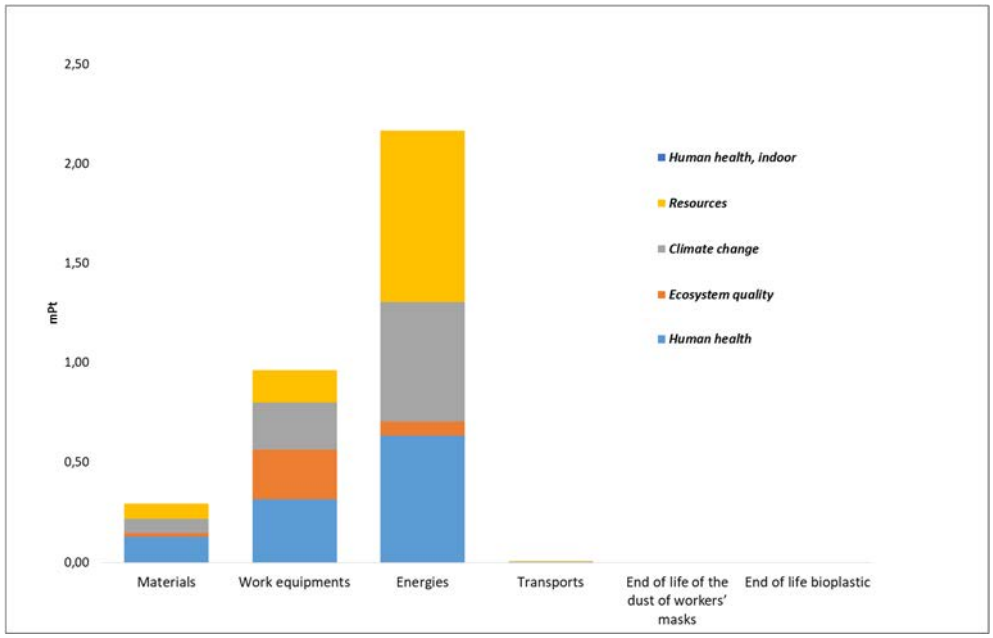

Figure 6: Environmental damage by single score-bioplastic production process (1 gr).

Table 2: Environmental damage by single score-bioplastic production process (1 gr).

Damage category	Materials	Work equipment	Energies	Transports	End of life of the dust of workers' masks	End of life of bioplastic
Human Health DALY	1,29E-01	3,16E-01	6,32E-01	2,21E-03	1,60E-10	5,96E-06
Ecosystem quality PDF*m^2*yr	1,93E-02	2,46E-01	7,16E-02	9,83E-04	2,05E-11	8,16E-07
Climate change kg CO_2 eq.	7,07E-02	2,37E-01	6,03E-01	1,89E-03	1,20E-10	3,61E-06
Resources MJ primary	7,70E-02	1,60E-01	8,61E-01	2,08E-03	5,54E-11	3,53E-06
Human Health Indoor DALY	3,32E-07	–	–	–	–	1,55E-07
Total (mPt)	2,96E-01	9,59E-01	2,167E+00	7,16E-03	3,57E-10	1,41E-05

is perhaps due to the production process is a laboratory-scale process and not yet an industrial one. Therefore these results will help to the ecodesign of industrial production of innovative bioplastics in order to minimize these environmental hotspots.

ACKNOWLEDGEMENT

This work has been financially supported by the Emilia Romagna Region, Italy, POR-FESR 2014-2020.

REFERENCES

[1] Newton, L., Sheppard, C., Watson, D.W. & Burtleg, D.R., Using the Black Soldier Fly, Hermetia illucens, as a Value-Added Tool for the Management of Swine Manure. Report, Waste Management Center, North Carolina State University: Raleigh, NC, 2005.

[2] De Foliart, G.R., The human use of insects as food and as animal feed. *Bulletin of the Entomological Society of America*, **35**(1), pp. 22–36, 1989. DOI: 10.1093/besa/35.1.22.

[3] Bukkens, S.G., The nutritional value of edible insects. *Ecology of Food and Nutrition*, **36**(2–4), pp. 287–319, 1997. DOI: 10.1080/03670244.1997.9991521.

[4] Allotey, J. & Mpuchane, S., Utilization of useful insects as food source. *African Journal of Food, Agriculture, Nutrition and Development,* **3**(2), pp. 112–121, 2003.

[5] Rumpold, B.A. & Schlüter, O.K., Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, **17**, pp. 1–11, 2013. DOI: 10.1016/j.ifset.2012.11.005.

[6] Van Huis, A., Potential of insects as food and feed in assuring food security. *Annual Review of Entomology,* **58**(1), pp. 563–583, 2013. DOI: 10.1146/annurev-ento-120811-153704.

[7] Kumar, N. & Sharma, P.B., Jatrophacurcus: A sustainable source for production of biodiesel. *Journal of Scientific and Industrial Research,* **64**(11), p. 883, 2005.
[8] Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z. & Zhou, S., From organic waste to biodiesel: black soldier fly, Hermetia illucens, makes it feasible. *Fuel,* **90**(4), pp. 1545–1548, 2011. DOI: 10.1016/j.fuel.2010.11.016.
[9] Zheng, L., Li, Q., Zhang, J. & Yu, Z., Double the biodiesel yield: rearing black soldier fly larvae, Hermetia illucens, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. *Renewable Energy,* **41**, pp. 75–79, 2012. DOI: 10.1016/j.renene.2011.10.004.
[10] Manzano-Agugliaro, F., Sanchez-Muros, M.J., Barroso, F.G., Martínez-Sánchez, A., Rojo, S. & Pérez-Banòn, C., Insects for biodiesel production. *Renewable and Sustainable Energy Reviews*, **16**(6), pp. 3744–3753, 2012.
[11] Yang, S. & Liu, Z., Pilot-scale biodegradation of swine manure via Chrysomya megacephala (Fabricius) for biodiesel production. *Applied Energy,* **113**, pp. 385–391, 2014. DOI: 10.1016/j.apenergy.2013.07.056.
[12] Oonincx, D.G. & De Boer, I.J., Environmental impact of the production of mealworms as a protein source for humans: a life cycle assessment. *PLoS One,* **7**(12), p. e51145, 2012. DOI: 10.1371/journal.pone.0051145.
[13] De Boer, H.C., Van Krimpen, M.M., Blonk, H. & Tyszler, M., Replacement of Soybean Meal in Compound Feed by European Protein Sources e Effects on Carbon Footprint. (University and Research Centre) Livestock Research, Wageningen, Wageningen UR. Livestock Research Report 819, 2014.
[14] Van Zanten, H.H.E., Mollenhorst, H., Oonincx, D.G.A.B., Bikker, P., Meerburg, B.G. & De Boer, I.J.M., From environmental nuisance to environmental opportunity: housefly larvae convert waste to livestock feed. *Journal of Cleaner Production,* **102**, pp. 362–369, 2015. DOI: 10.1016/j.jclepro.2015.04.106.
[15] Salomone, R., Saija, G., Mondello, G., Giannetto, A., Fasulo, S. & Savastano, D., Environmental impact of food waste bioconversion by insects: Application of Life Cycle Assessment to process using Hermetia illucens. *Journal of Cleaner Production* **140**, pp. 890–905, 2017. DOI: 10.1016/j.jclepro.2016.06.154.
[16] Banks, I., To assess the impact of black soldier fly (Hermetia illucens) larvae on faecal reduction in pit latrines. PhD thesis, 2014.
[17] Diener, S., Zurbrügg, C. & Tockner, K., Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management Research,* **27**(6), pp. 603–610, 2009. DOI: 10.1177/0734242x09103838.
[18] Kalova M. & Borkovcova, M., Voracious larvae Hermetia illucens and treatment of selected types of biodegradable waste. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* **61**(1), pp. 77–83, 2013. DOI: 10.11118/actaun201361010077.
[19] Sheppard, C., Newton, G.L., Thompson, S.A. & Savage, S., A value added manure management system using the black soldier fly. *Bioresour. Technology*, **50**(3), pp. 275–279, 1994. DOI: 10.1016/0960-8524(94)90102-3.
[20] St-Hilaire, S. et al., Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. *Journal of the World Aquaculture Society,* **38**(1), pp. 59–67, 2007. DOI: 10.1111/j.1749-7345.2006.00073.x.
[21] Van Huis, A. et al., 2013. Edible Insects e Future Prospects for Food and Feed Security, FAO Forestry, Paper 171.

[22] Tomberlin, J.K., Selected Life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial Diets. *Annals of the Entomology Society of America,* **95**(3), pp. 379–386, 2002. DOI: 10.1603/0013-8746(2002)095[0379:slhtob]2.0.co;2.
[23] Furman, D.Y., Hermetia illucens (Linnaeus) as a factor in the natural control of musca domestica linneaus. *Journal of Economic Entomology,* **52**(5), pp. 917–921, 1959. DOI: 10.1093/jee/52.5.917.
[24] Leclercq, M., Á propos de Hermetia illucens (Linnaeus, 1758) ("soldier fly") (Diptera Stratiomyidae: Hermetiinae). *Bulletin et Annales de la Societe Royale Belge d'Entomologie,* **133**, pp. 275–282, 1997.
[25] Booth, D. &. Sheppard, C., Ovoposition of the black soldier fly, hermetia illucens (L) (diptera: stratiomydae): eggs, masses, timing, and site characteristics. *Environmental Entomology*, **13**(2), pp. 421–423, 1984. DOI: 10.1093/ee/13.2.421.
[26] Newton, G.S., Soldier fly benefits: house fly control, manure volume reduction, and manure nutrient recycling. *USA Animal and Dairy,* p. 8, 1995.
[27] Stefan, D., Studt Solano, N.M., Gutiérrez, F.R., Zurbrügg, C. & Tockner, K., Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization,* **2**(4), pp. 357–363, 2011. DOI: 10.1007/s12649-011-9079-1.
[28] ISO 14040, Environmental management life cycle assessment principles and framework. Geneva, 2006.
[29] ISO 14044, Environmental management life cycle assessment requirements and guidelines. Geneva, 2006.
[30] Ecoinvent Centre, Ecoinvent data v3.0. Swiss Centre for Life Cycle Inventories, St. Gallen. www.ecoinvent.org.20.
[31] Oonincx, D.G.A.B., Volk, N., Diehl, J.J.E., Van Loon, J.J.A. & Belušič, G., Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (Hermetia illucens) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, **95,** pp. 133–139, 2016. DOI: 10.1016/j.jinsphys.2016.10.006.
[32] Jolliet, O. et al., IMPACT2002+: A new life cycle impact assessment methodology. *International Journal of Life Cycle Assessment,* **8**(6), pp. 324–330, 2003. DOI: 10.1007/bf02978505.
[33] PRé, SimaPro 8 Multi user. PRé Consultants bv, Stationsplein 121, 3818 LE Amersfoort, The Netherlands 2014.
[34] Spinelli, R., Gamberini, R., Neri, P., Pini, M. & Ferrari, AM., Comparative Life Cycle Assessment of innovative and traditional adhesives for the laying of ceramic tiles. *IJOQM*, **20**(3), pp. 227–241, 2014. ISSN: 10821910.