



# Industrialization of the Use of Mealworm and Black Soldier Fly in Urban Waste Management Utilizing Probiotics

Nasrin Taghikhani<sup>1</sup>, Amirreza Shaebani Darejazi<sup>2\*</sup>, Mahsa Abedi<sup>3</sup>, Ghazale Maghsoudi<sup>3</sup>

<sup>1</sup>Imam Khomeini Higher Education Center, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran;

<sup>2</sup>Department of Chemical Engineering, Brunel University, London, United Kingdom; <sup>3</sup>Department of Microbiology, Islamic Azad University, Karaj, Iran

## ABSTRACT

In the face of increasing global demands for sustainable waste management and alternative protein sources, this study explores the industrialization potential of utilizing mealworms (*Tenebrio molitor*) and black soldier flies (*Hermetia illucens*) in urban waste management with the aid of probiotics. These insect species, rich in protein content, are recognized as valuable substitutes for traditional livestock and poultry in the search for more sustainable protein sources.

The study investigates the use of beneficial insects, specifically mealworms and black soldier flies, to process damp waste efficiently. Black soldier flies, known for their rapid growth and reproduction rates, have demonstrated their ability to thrive on organic waste materials, providing an alternative to soy-based feeds in livestock and fish farming. Challenges in scaling up the use of beneficial insects on an industrial level, including decomposition rates, survival rates, protein content, and product toxicity, have prompted research into the potential benefits of probiotics in waste digestion processes. This study explores the impact of fermenting wastewater with a combination of *Lactobacillus* strains (VSL#3) on decomposition rates, protein content, and toxicity of the resulting products.

The findings reveal that the addition of probiotics to waste significantly increases the protein content of insects. Furthermore, the study highlights the greater effect of VSL#3 probiotics in comparison to *Lactobacillus casei*, particularly in enhancing the protein content of black soldier fly larvae. These results suggest that the combination of probiotics has the potential to expedite the transition from a linear economy to a circular one, emphasizing the importance of waste pre-processing, especially for large volumes.

This research signifies a positive direction for addressing the challenges associated with urban waste management and the sustainable production of protein sources. It underscores the potential of utilizing mealworms and black soldier flies, with the aid of probiotics, as efficient tools in managing organic waste while concurrently producing high-quality protein. The study contributes to the ongoing discourse on sustainable waste utilization and circular economy practices.

**Keywords:** Meal worms; Black soldier flies; Urban waste management; Probiotics; Sustainable protein sources; Circular economy

**Correspondence to:** Amirreza Shaebani Darejazi, Department of Chemical Engineering, Brunel University, London, United Kingdom, E-mail: Amirreza.ShaebaniDarejazi@brunel.ac.uk

**Received:** 10-Oct-2023, Manuscript No. JFPT-23-23525; **Editor assigned:** 13-Oct-2023, PreQC No. JFPT-23-23525 (PQ); **Reviewed:** 27-Oct-2023, QC No. JFPT-23-23525; **Revised:** 03-Nov-2023, Manuscript No. JFPT-23-23525 (R); **Published:** 10-Nov-2023, DOI: 10.35248/2157-7110.23.14.1061

**Citation:** Taghikhani N, Darejazi AS, Abedi M, Maghsoudi G (2023) Industrialization of the Use of Mealworm and Black Soldier Fly in Urban Waste Management Utilizing Probiotics. J Food Process Technol. 14:1061.

**Copyright:** ©2023 Taghikhani N, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## INTRODUCTION

As the global population burgeons and the demand for protein escalates, exploring unconventional yet sustainable alternatives to traditional livestock and poultry farming practices has become imperative [1]. Within this context, the utilization of insects as a novel protein source has gained significant traction. Among the myriad of insect species, two hold particular promise in terms of economic viability and their ability to process organic residues: the yellow mealworm (*Tenebrio molitor*) and the black soldier fly (*Hermetia illucens*) [2].

The yellow mealworm, often abbreviated as YM, represents the larval stage of a species of beetle, undergoing a remarkable metamorphic journey from egg to larva, pupa, and finally adult beetle [1]. Notably, these larvae, characterized by their protein-rich composition, flourish in controlled environments on wheat bran beds, rendering them a valuable reservoir of high-quality protein. In parallel, the Black Soldier Fly (BSF) stands as a testament to nature's efficiency, as this species possesses an extraordinary capacity to consume discarded organic materials while exhibiting rapid growth rates and prolific reproductive capabilities [2]. With the ability to efficiently convert organic waste into valuable biomass, BSF presents a compelling solution to recycling and sustainable protein production. Consequently, the cultivation of these insects for livestock and fish feed holds significant potential as a sustainable alternative to conventional soy-based diets [3].

However, the industrial-scale implementation of beneficial insects like YM and BSF in urban waste management and protein production is overloaded by a multiple challenges. Central among these challenges are concerns related to the rate of waste decomposition, insect survival rates, protein yield, and product toxicity [2]. Recent scientific endeavours have explored the potential of probiotics in addressing these challenges by enhancing the growth of beneficial insects [4]. While these studies have yielded produce positive results, substantial barriers to full-scale industrialization remain.

Probiotic bacteria, a class of microorganisms known for their beneficial role in promoting optimal food digestion, have collected significant attention in recent years. These microorganisms, referred to as probiotic bacteria, not only facilitate the process of digestion but also exhibit the capacity to synthesize intricate molecules and compounds, including vitamins and various antibiotics, which offer substantial advantages to consumers [5,6]. Recognizing their potential, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have jointly defined probiotics as "live microorganisms which, when administered in sufficient amounts, are beneficial to the host" [3]. The utilization of probiotics presents an encouraging solution to address the challenges associated with food digestion and processing. Moreover, probiotics offer an appealing alternative to commercial enzymes and provide additional benefits such as antioxidant and antimicrobial effects, along with enhancements in nutrient digestion. One notable probiotic

including *Streptococcus thermophilus* (BT01), *Bifidobacterium breve* (BB02), *Bifidobacterium longum* (BL03), *Bifidobacterium infantis* (BI04), *Lactobacillus acidophilus* (BA05), *Lactobacillus plantarum* (BP06), *Lactobacillus paracasei* (BP07), and *Lactobacillus delbrueckii subsp. Bulgaricus* (BD08), each of which is present in an active form at a concentration ranging from  $10^{10}$  to  $10^{11}$  colony-forming units per cubic centimeter (CFU/cm<sup>3</sup>) [7]. This formulation's robust microbial composition underscores its potential utility in various applications within the realm of food science and nutrition.

Given these considerations, this research undertakes a comprehensive examination of the impact of wastewater fermentation through a combination of *Lactobacillus* strains, particularly VSL#3, on critical factors including waste decomposition rates, protein content, and product toxicity. By elucidating the potential of probiotics to augment insect protein content while mitigating concerns surrounding waste processing, this study not only advances the discourse on sustainable urban waste management but also holds the promise of expediting the transition from a linear economic model to a circular one.

## MATERIALS AND METHODS

### Materials

The materials and methods employed in this study were carefully designed to investigate the potential of probiotics in enhancing the utilization of mealworms (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae for urban waste management. Yellow Mealworms (YM) and Black Soldier Flies (BSF) were procured from the Insect Breeding Center, ensuring a consistent source of these beneficial insects [1,2]. Two essential probiotic strains, namely *Lactobacillus casei* strain NZ98 and the commercially available VSL#3, were selected for the study. These probiotics were employed in the treatment of wastewater to assess their impact on waste decomposition and insect development.

To meet the nutritional requirements of the beneficial insects, a source of nutrients was obtained from local fruit and vegetable waste, providing an organic and sustainable feedstock for the larvae. The equipment utilized in the experimental setup included a 5-liter agitator tank for waste fermentation, ensuring proper mixing and contact between the waste substrate and probiotic strains. Additionally, specialized containers were employed for rearing and breeding the insects. For yellow mealworms, a box with dimensions of 124060 cm<sup>3</sup> was used, while black soldier flies were reared in a 144060 cm<sup>3</sup> box. To facilitate the breeding process, an industrial meat grinder was employed on-site to prepare the feed for the beneficial insects [3].

### Methods

The research methodology adopted in this study built upon the work of Chang, with necessary modifications to suit the specific objectives of the investigation [8].

## Waste preparation

Fresh waste residue from the day's collection was processed using an industrial meat grinder. Subsequently, this waste was placed in an agitator tank equipped with a continuous stirrer to ensure uniform mixing.

## Probiotic treatment

To explore the impact of probiotics, two separate treatments were conducted. In one tank, a bacterial strain, *Lactobacillus casei*, was introduced, while in another tank, VSL#3 was added. Both probiotic treatments involved the addition of 2% of the respective probiotic strains to the waste. The tanks were then stirred continuously for 12 hours at ambient temperature, with the pH maintained at 5.5 to create an optimal environment for probiotic activity.

## Control group

To establish a consistent baseline, a control sample was maintained without the addition of any probiotic strain. This control sample was subjected to the same 12-hour stirring period.

## Insect rearing

Following waste processing and probiotic treatment, each sample was transferred to storage boxes designed for insect rearing. Yellow mealworms and black soldier fly larvae were placed in separate boxes, each containing 1 kg of treated waste. The rearing environment was maintained at a temperature of 25°C in darkness to facilitate optimal insect growth and development.

## Biodegradation assessment

The biodegradation of industrial waste was quantified by monitoring the residual weight of the waste at regular intervals. Measurements were taken every 4 hours over a 36-hour period to assess the effectiveness of waste decomposition.

## Protein content analysis

The protein content of the insects was determined using the Kjeldahl method, allowing for the measurement of protein levels in both yellow mealworms and black soldier fly larvae [9-11].

## Toxicity evaluation

To assess the potential toxicity of the insect larvae resulting from waste processing, tests were conducted for the presence of *Salmonella* and *Escherichia coli* using specialized kits [12].

# RESULTS AND DISCUSSION

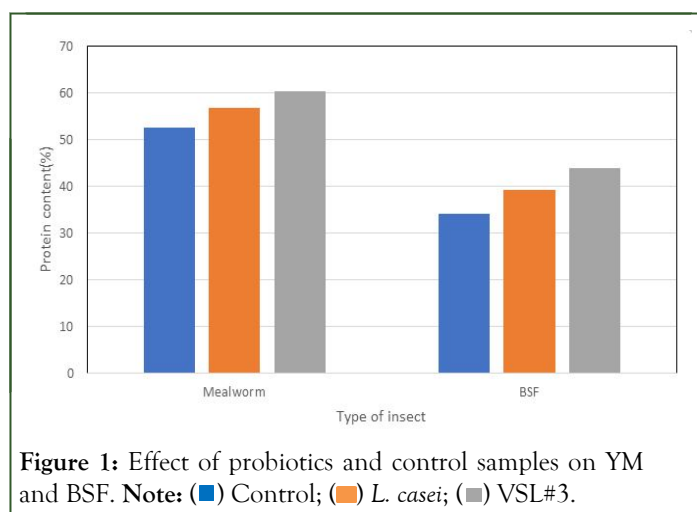
## The amount of protein

The investigation into protein content reveals a noteworthy impact of probiotics on the protein levels of both Yellow Mealworms (YMs) and Black Soldier Fly (BSF) larvae. When

probiotics were introduced to the experimental setup, it became evident that these beneficial microorganisms played a pivotal role in enhancing the protein content of the insects. Specifically, *Lactobacillus casei*, one of the selected probiotic strains, exerted a positive influence on the protein content of both YMs and BSF larvae. In the case of YMs, the addition of *Lactobacillus casei* resulted in a 4% increase in protein content, underscoring its potential to elevate the nutritional quality of these insects.

However, the impact of VSL#3 probiotics, a commercially available probiotic formulation, was even more pronounced. VSL#3 demonstrated its effectiveness by leading to a substantial 5% increase in the protein content of BSF larvae. Remarkably, the protein content reached an impressive 63% for YMs and 48% for BSF when VSL#3 probiotics were incorporated into the experimental conditions. To contextualize these findings, it is worth noting that previous research has reported that the crude protein content of *Tenebrio molitor* larvae averages around 52.4% [13]. Similarly, studies on optimizing the protein content of BSF have indicated a range of 30%-45% [14]. The results obtained in this study not only affirm the potential of probiotics to enhance protein content but also showcase the remarkable protein-rich composition achieved, particularly with the use of VSL#3 probiotics.

Figure 1 in the article graphically illustrates the effect of probiotics and control samples on both YMs and BSF, further emphasizing the significant protein enhancement achieved through probiotic intervention (Figure 1).



## Insect growth rate

The exploration of insect growth rates under varying conditions is pivotal to comprehend the transformative effects of probiotics on the development of Yellow Mealworms (YMs) and Black Soldier Fly (BSF) larvae. In this section, we delve into the intricacies of insect development and how probiotics play a central role in shaping their growth trajectories. The data presented in Figure 2, reveals interesting insights into the growth patterns observed in these beneficial insects. Notably, it highlights that BSF larvae exhibit both the highest growth rates and the most significant response to probiotic fermentation. This finding suggests that probiotics exert a more pronounced influence on BSF when compared to YMs. In the absence of

probiotics, BSF larvae exposed to waste without fermentation display a higher mortality rate than a growth rate. It's intriguing to note that, in this scenario, the number of live YMs nearly diminishes after 24 hours. However, when probiotics, specifically *Lactobacillus casei* and VSL#3, are introduced to the waste environment, a remarkable transformation unfolds. After just 10 hours, BSF larvae subjected to waste treated with *L. casei* exhibit a 1.75-fold increase in growth rate, while those exposed to VSL#3 experiences an impressive 2.5-fold boost in their growth rate. The subsequent reduction in growth rate after 10 hours can be attributed to the depletion of backlog resources. While the influence of probiotics is also observed in YMs, it is less pronounced compared to that on BSF. The application of VSL#3 probiotics significantly enhances the growth rate of BSF, while a similar effect is not observed in the case of YMs. These findings significantly surpass previous reports related to the growth rates of BSF and YMs, highlighting the potential of probiotics, particularly VSL#3, to expedite insect growth and development. Notably, Schebeck et al. (2022) reported a growth rate range of 42% for YMs based on temperature [15], and Kim et al. (2021) noted a 36-hour survival rate of 86% for BSFs [16]. However, our results reveal even more substantial enhancements (Figure 2).

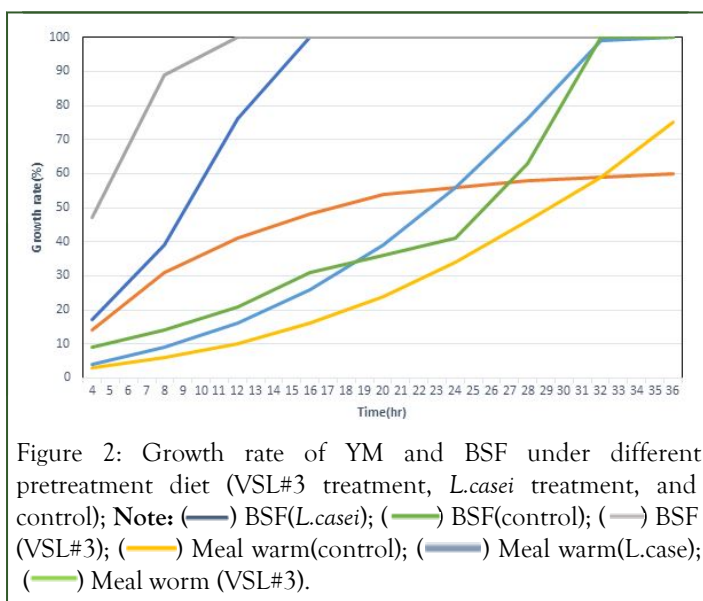


Figure 2: Growth rate of YM and BSF under different pretreatment diet (VSL#3 treatment, *L.casei* treatment, and control); **Note:** (—) BSF(*L.casei*); (—) BSF(control); (—) BSF (VSL#3); (—) Meal warm(control); (—) Meal warm(*L.casei*); (—) Meal worm (VSL#3).

### Toxicity

A remarkable finding emerges from our study yellow mealworm larvae, irrespective of the treatment group (control, *Lactobacillus casei*, or VSL#3), displayed an impressive resistance to toxicity. Even in the presence of probiotic-treated waste, YMs exhibited complete resilience to the presence of toxic agents. Notably, neither *Salmonella* nor *Escherichia coli* had a detrimental impact on the health of YM larvae. These findings underscore the inherent resistance of YMs to potential health hazards, even in scenarios where probiotics are introduced. These results are consistent with previous research findings.

In stark contrast, the Table 1 considerably different when it comes to BSF larvae BSF larvae subjected to unfermented waste displayed susceptibility to toxicity, as evidenced by the presence of both *Salmonella* and *Escherichia coli*. This observation aligns

with prior studies that have identified BSFs as vulnerable to these pathogens [17]. However, the introduction of *Lactobacillus casei*-treated waste did exhibit some improvement, particularly regarding resistance to *E. coli*. Nevertheless, susceptibility to *Salmonella* persisted (Table 1).

Tests	YM			BSF		
	Control	<i>L. casei</i>	VSL#3	Control	<i>L. casei</i>	VSL#3
<i>Salmonella</i>	-	-	-	+	+	-
<i>E. coli</i>	-	-	-	+	-	-

**Note:** YM- Yellow Mealworms; BSF- Black Soldier Fly; *L. casei*- *Lactobacillus casei*; VSL#3- Probiotics.

**Table 1:** Effect of *Salmonella* and *E. coli* on each of YM and BSF samples.

Perhaps the most intriguing revelation of our study pertains to the remarkable impact of VSL#3 probiotics on enhancing the resistance of BSF larvae to *Salmonella*. When VSL#3-treated waste was introduced, BSF larvae exhibited a notable resistance to this pathogen, a phenomenon not observed in the other treatment groups. This underscores the pivotal role of specific probiotic formulations in mitigating health concerns associated with BSF-derived products.

### CONCLUSION

In conclusion, the industrialization of mealworms (*Tenebrio molitor*) and black soldier flies (*Hermetia illucens*) for urban waste management, with the integration of probiotics, emerges as an encouraging avenue to address the growing challenges of sustainable waste disposal and protein production. The study has highlighted on several key findings that underline the potential of this approach. First and foremost, the introduction of probiotics, particularly VSL#3, has demonstrated a remarkable capacity to enhance the protein content of both Yellow Mealworms (YMs) and Black Soldier Fly (BSF) larvae. The substantial increase in protein levels, reaching 63% for YMs and 48% for BSF, showcases the significant impact of probiotic intervention. These protein-rich insect species offer a sustainable solution to protein production, particularly in comparison to traditional livestock and poultry farming.

Furthermore, the research has revealed the differential growth rates of YMs and BSF under various conditions. BSF larvae, in particular, exhibited the highest growth rates, and the introduction of probiotics, especially VSL#3, led to a remarkable acceleration in their growth. This finding underscores the potential of probiotics to expedite insect development, making them more efficient tools for organic waste management. One of the most notable findings of this study is the impressive resistance of yellow mealworm larvae to toxicity, regardless of the treatment group. Even in the presence of probiotic-treated waste, YMs displayed resilience to toxic agents such as *Salmonella* and *Escherichia coli*. This inherent resistance highlights the robustness of YMs in mitigating health hazards, even in

scenarios involving probiotics. Conversely, BSF larvae exhibited susceptibility to toxicity when subjected to unfermented waste, aligning with prior research that identified BSFs as vulnerable to these pathogens. However, the introduction of *Lactobacillus casei*-treated waste did offer some improvement, particularly in resistance to *E. coli*. Nevertheless, susceptibility to *Salmonella* persisted. The most intriguing revelation pertains to the remarkable impact of VSL#3 probiotics on enhancing the resistance of BSF larvae to *Salmonella*. This specific probiotic formulation proved effective in conferring notable resistance to this pathogen, a phenomenon not observed in the other treatment groups. This underscores the critical role of specific probiotic formulations in addressing health concerns associated with BSF-derived products.

In summary, this research signifies an encouraging direction for addressing the challenges associated with urban waste management and the sustainable production of protein sources. It underscores the potential of utilizing mealworms and black soldier flies, with the aid of probiotics, as efficient tools in managing organic waste while concurrently producing high-quality protein. These findings contribute significantly to the ongoing discourse on sustainable waste utilization and circular economy practices, opening the path for more environmentally friendly and economically viable solutions in the terms of waste management and protein production.

## CONFLICT OF INTEREST

We proclaim that we have no conflict of interest.

## REFERENCES

1. Toviho OA, Bársony P. Nutrient composition and growth of yellow mealworm (*Tenebrio molitor*) at different ages and stages of the life cycle. *Agriculture*. 2022;12(11):1924.
2. Heussler CD, Insam H, Walter A, Steiner BS, Steiner FM, Klammsteiner T. Life-history traits of black soldier fly reared on agro-industrial by-products subjected to three pre-treatments: a pilot-scale study. *J Insects as Food and Feed*. 2023;9(5):545-556.
3. Thrastardottir R, Olafsdottir HT, Thorarinsdottir RL. Yellow mealworm and black soldier fly larvae for feed and food production in Europe, with emphasis on Iceland. *Foods*. 2021;10(11):2744.
4. Ojha S, Bußler S, Schluter OK. Food waste valorisation and circular economy concepts in insect production and processing. *Waste Manage*. 2020;118(1):600-609.
5. Richard N, Rabot R, Beutin C, van Loon JJ. Live yeast probiotic can boost growth performances of yellow mealworm and black soldier fly larvae. Wageningen University: Wageningen, the Netherlands. 2019.
6. Selaledi L, Mbajiorgu CA, Mabelebele M. The use of yellow mealworm (*T. molitor*) as alternative source of protein in poultry diets: a review. *Trop Anim Health Prod*. 2020;52(1):7-16.
7. Ramedani N, Sharifan A, Gholam-Mostafaei FS, Rostami-Nejad M, Yadegar A, Ehsani-Ardakani MJ. The potentials of probiotics on gluten hydrolysis a review study. *Gastroenterol Hepatol Bed Bench*. 2020;13(1):S1.
8. Hu Xin J, Zhang G. Method for processing food waste by using black soldier fly larvae and material formula. 2012.
9. Hayes M. Measuring protein content in food: An overview of methods. *Foods*. 2020;9(10):1340.
10. Darejazi AS, Abedi M. Optimize collagen extraction from yellow-fin tuna (*Thunnus albacares*) fish skin based on yield, collagen content, and color. 2022.
11. Darejazi AS, Abedi M, Roostaazad R. Optimize gelatin extraction from yellow-fin tuna (*Thunnus albacares*) fish skin based on yield, collagen content, and color. 2022.
12. Wynants E. Microbiological dynamics and safety risks during rearing of insects for food and feed. 2019.
13. Hong J, Han T, Kim YY. Mealworm (*Tenebrio molitor* Larvae) as an alternative protein source for monogastric animal: A review. *Animals*. 2020; 10(11):2068.
14. Fuso A, Barbi S, Macavei LI, Luparelli AV, Maistrello L, Montorsi M, et al. Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. *Foods*. 2021; 10(8):1773.
15. Eberle S, Schaden LM, Tintner J, Stauffer C, Schebeck M. Effect of temperature and photoperiod on development, survival, and growth rate of mealworms, *Tenebrio molitor*. *Insects*. 2022; 13(4): 321.
16. Kim CH, Ryu J, Lee J, Ko K, Lee JY, Park KY, et al. Use of black soldier fly larvae for food waste treatment and energy production in Asian countries: a review. *Processes*. 2021;9(1):161.
17. Auza FA, Purwanti S, Syamsu JA, Natsir A. Antibacterial activities of black soldier flies (*Hermetia illucens*. l) extract towards the growth of *Salmonella typhimurium*, *E. coli* and *Pseudomonas aeruginosa*. In IOP Conf Ser Earth Environ Sci. 2020; 492(1): 012024.