Bioresources and Environment 2025, 3(3) 1-9.

Content list available at BioEnv

Bioresources and Environment

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Sustainable Bioproduction of Xanthan Gum by *Xanthomonas campestris* Using Fructose and Black Soldier Fly Larvae as a Novel Nitrogen Source

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Received March 11, 2025, Accepted in revised form September 18, 2025
Available online October 28, 2025
DOI: 10.24191/bioenv.v3i3.95

ABSTRACT. Xanthan gum is a microbial exopolysaccharide produced commercially through the fermentation of *Xanthomonas campestris* using carbon sources. It is widely used in industries such as food, medicine, textiles, and oil due to its properties as a stabilizer, thickener, and emulsifier. However, the high cost of xanthan production and its environmental impact, particularly when relying on traditional carbon sources like glucose, pose significant challenges. This study explores the use of agricultural waste as a cost-effective carbon source and investigates the potential of Black Soldier Fly Larvae (BSFL) as an alternative nitrogen source for xanthan production. *Xanthomonas campestris* ATCC 13951 was cultivated in media containing fructose (40 g/L) as the carbon source and varying concentrations of BSFL (2, 4, 6, 8, and 10 g/L) as the nitrogen source. The results demonstrated that the medium containing 2 g/L of BSFL yielded the highest xanthan production at 5.29 g/L, indicating the potential of BSFL as a sustainable nitrogen source for xanthan synthesis. This study highlights the feasibility of using alternative, low-cost substrates to improve the economic and environmental sustainability of xanthan production.

Key words: Xanthan gum, Black Soldier Fly Larvae, Sustainable bioproduction, *Xanthomonas campestris*

1. INTRODUCTION

The production of biopolymers has emerged as a major area of intense research and industrial interest, driven by the growing demand for sustainable, bio-based materials. Among these biopolymers, xanthan gum—a microbial exopolysaccharide produced by the Gram-negative bacterium *Xanthomonas campestris*—has gained significant attention due to its versatile applications across various industries. Its unique properties as a stabilizing, gelling, emulsifying, and thickening agent make it indispensable in sectors such as food, pharmaceuticals, cosmetics, textiles, and even enhanced oil recovery (Cancella et al., 2024; Kalogiannis et al., 2003; Nejadmansouri et al., 2020; Niknezhad et al., 2016; Rashidi et al., 2023; Zakeri et al., 2017).

Xanthan gum is primarily produced by *Xanthomonas* species, including *Xanthomonas campestris*, *Xanthomonas phaseoli*, and *Xanthomonas malvacearum* (Bhat et al., 2022; Nejadmansouri et al., 2020). However, *Xanthomonas campestris* is the most widely used strain due to its high yield and superior product quality. The polymer's main structure consists of repeating pentasaccharide units, each comprising two glucose molecules, two mannose molecules, and one glucuronic acid molecule (Nordin et al., 2020). Xanthan gum exhibits exceptional solubility,

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resilience, and durability in acidic or alkaline environments, as well as stability in the presence of salts and resistance to enzymatic degradation. These properties make it a dominant polymer in the food industry and other applications (Kalogiannis et al., 2003; Nordin et al., 2020; Zakeri et al., 2017).

Despite its widespread use, the conventional production of xanthan gum is costly and resource-intensive, relying heavily on high-purity carbon and nitrogen sources such as glucose and peptone (Palaniraj & Jayaraman, 2011; Nordin et al., 2020). This dependence not only limits production efficiency but also raises environmental concerns, prompting the exploration of more sustainable and cost-effective alternatives. Organic nitrogen sources, such as black soldier fly larvae (BSFL), align with sustainable production practices. Black Soldier Fly Larvae (BSFL) also known as *Hermetia illucens* have been widely used as they can convert a variety of organic waste for example spent mushroom substrate (SMS) and wet distiller's grains (WDG) that have the potential to increase the waste generation caused by industrialization and population growth which increase the demand of edible mushroom and will increase the organic waste production every year (Wei et al., 2024). In addition, the biomass of black soldier fly larvae (BSFL) is rich in proteins, lipids, and bioactive compounds, making it a suitable feed ingredient for poultry, livestock, and aquaculture. BSFL has gained global recognition as a viable feed source for poultry and aquaculture, as supported by recent studies (Lu et al., 2022; Quan et al., 2023). This alternative has the potential to reduce dependence on traditional, high-cost nitrogen sources such as yeast or peptone, thereby enhancing the economic viability and environmental sustainability of xanthan gum production.

In this study, BSFL is utilised as an alternative nitrogen source due to its potential to promote environmental sustainability, cost-effectiveness, and ease of availability. However, research on the use of BSFL as an alternative nitrogen source for xanthan gum production remains scarce and underexplored. This study addresses this gap by systematically evaluating BSFL's efficacy, offering a scalable model for sustainable bioproduction. By adopting BSFL as an alternative source of nitrogen, this could make xanthan production viable for small-scale industries in developing regions. Therefore, this research aims to evaluate the feasibility of using fructose as a carbon source and BSFL as a nitrogen source for the bioproduction of xanthan gum by *Xanthomonas campestris*. Furthermore, the study investigates the impact of varying BSFL concentrations on xanthan gum yield.

2. METHODOLOGY

This study investigates the feasibility of using fructose as a carbon substrate and Black Soldier Fly (BSF) larvae as an alternative substrate in xanthan gum production by *Xanthomonas campestris* ATCC 13951. The methodology was designed to evaluate the impact of these carbon and alternative nitrogen sources on xanthan yield, aiming to see what the best concentration is needed to produce more xanthan. To achieve this, Figure 1 shows that the process comprises stages including substrate preparation, fermentation setup, sampling, and analysis. Each stage was carefully designed to optimize conditions for microbial growth and xanthan synthesis, following standardized protocols while adjusting variables relevant to the alternative substrates.

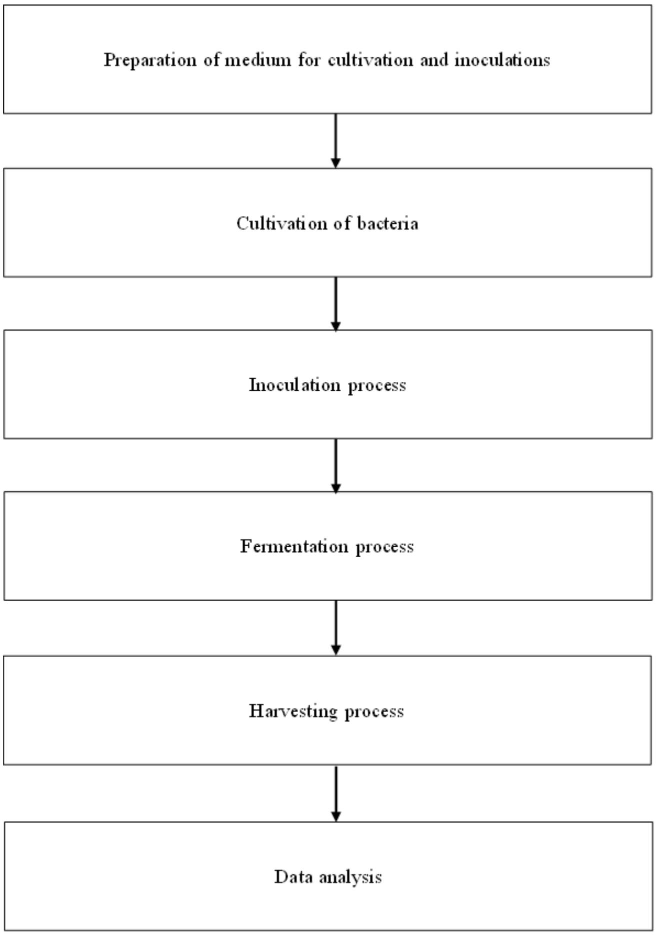

Figure 1. Process flowchart of xanthan production

2.1. Materials and chemicals

The chemicals used in this experiment were glycerol (Qrec), fructose (HmbG), potassium chloride (Bendosen), di-potassium hydrogen phosphate (Bendosen), zinc sulphate heptahydrate (HmbG), ethanol (Vchem), nutrient agar (Bendosen), nutrient broth (Bendosen), sodium hydroxide (Merck), hydrochloric acid (Qrec), BSFL (local supplier) and distilled water. These chemicals were obtained from the Bioprocess Laboratory and the Environment Laboratory of UiTM Johor Branch, Pasir Gudang Campus.

2.2. Microorganism

Xanthomonas campestris ATCC 13951 was obtained from the American Type Culture Collection (ATCC) as the reference strain and is being used as the producing microorganism for this study. The strain was cultured in a standard nutrient broth and stored at -30°C in a vial tube containing glycerol for further use.

2.3. Cultivation process

A nutrient broth was prepared in a 250 mL conical flask and sterilized by autoclaving to eliminate any existing bacteria in the solution. Subsequently, 50 mL of the sterilized nutrient broth was inoculated with *Xanthomonas campestris*. The bacterial culture was then incubated in a shaker incubator at 30°C and 200 rpm for 24 hours to facilitate growth.

2.4. Preparation of fermentation medium

In this experiment, fructose served as the carbon source for xanthan production, with a constant concentration of 40 g/L in each medium. The media contained five different nitrogen concentrations, each prepared in separate conical flasks. Black Soldier Fly Larvae (BSFL) were utilized as an alternative nitrogen source for xanthan production. A nitrogen solution derived from BSFL was prepared at five distinct concentrations: 2 g/L, 4 g/L, 6 g/L, 8 g/L, and 10 g/L. The pH of both media was adjusted to approximately 7 and then sterilized by autoclaving at 120°C for 20 minutes.

2.5. Xanthan gum production

The fermentation medium was inoculated with 5% (v/v) of a pre-prepared inoculum containing *Xanthomonas campestris* and then cultured in an incubator shaker at 30°C and 150 rpm for 24 hours.

2.6. Determination of cell dry weight (CDW) and xanthan gum

The fermentation broth contained in conical flasks was centrifuged in Falcon tubes at 7,000 rpm and 4°C for 15 minutes to separate the cells. The resulting liquid, known as the supernatant, was transferred to a new Falcon tube for xanthan precipitation. The separated cells were dried in an oven at 80°C for 24 hours and then weighed to determine their dry weight. Next, the supernatant was mixed with ethanol in a 1:3 ratio (supernatant to ethanol) in a Falcon tube. The mixture was refrigerated for 24 hours and then centrifuged again at 7,000 rpm and 4°C for 15 minutes to collect the precipitate. The precipitate, which contained xanthan gum, was dried in an oven at 60°C for 24 hours and subsequently weighed.

3. RESULTS AND DISCUSSION

3.1. Cell dry weight (CDW) analysis

Figure 2 illustrates the impact of varying nitrogen concentrations on cell dry weight (CDW), a measure of biomass production, using black soldier fly larvae (BSFL) as an alternative nitrogen source and fructose as the carbon source. The nitrogen concentrations tested in this experiment were 2 g/L, 4 g/L, 6 g/L, 8 g/L, and 10 g/L. At the lowest concentration of 2 g/L, the CDW produced was 1.29 g/L, which was the lowest among all concentrations tested. This indicates minimal biomass formation at this nitrogen level. When the concentration was increased to 4 g/L, the CDW rose to 1.55 g/L, reflecting a noticeable increase in biomass production. Further increasing the nitrogen concentration to 6 g/L resulted in a CDW of 1.66 g/L, demonstrating continued growth in biomass. The highest CDW of 2.46 g/L was observed at 8 g/L, representing the peak biomass production in this study. However, at the highest concentration tested (10 g/L), the CDW decreased slightly to 2.00 g/L, suggesting a decline in biomass yield beyond the optimal nitrogen level. These findings align with trends observed in other studies.

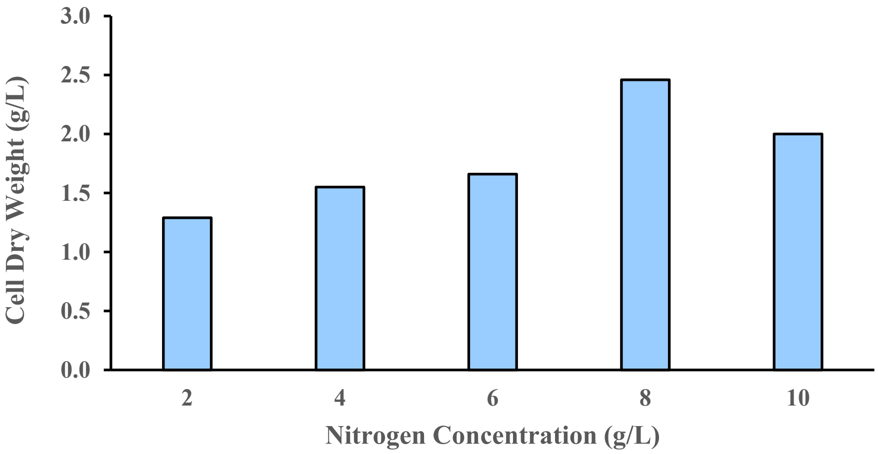

Figure 2. Effect of different concentrations of nitrogen source on cell dry weight (CDW)

For instance, Rashidi et al. (2023) reported a similar pattern in biomass production when using ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$) as the nitrogen source and glucose as the carbon source. Their study showed that biomass production increased up to a nitrogen concentration of 12 g/L but declined when the concentration exceeded this threshold. Additionally, Rui et al. (2023) emphasized the critical role of nitrogen as a limiting factor in cell growth, noting that different nitrogen concentrations and forms can significantly influence biomass production. For example, they found that the microalga *Conticribra weissflogii* achieved maximum growth and biomass production at a nitrogen concentration of 150 mg/L, which was identified as the optimal level for enhancing growth rate and biomass yield. In conclusion, this study demonstrates that CDW production increases with nitrogen concentration from 2 g/L to 8 g/L but declines at 10 g/L. The highest CDW was achieved at 8 g/L, while the lowest was observed at 2 g/L. These results suggest that 8 g/L is the optimal nitrogen concentration for maximizing biomass production under the given experimental conditions.

3.2. Xanthan gum analysis

Figure 3 illustrates the effect of varying concentrations of nitrogen source, using Black Soldier Fly Larvae (BSFL) as an alternative nitrogen source, on xanthan production. At the lowest concentration of 2 g/L BSFL, xanthan production reaches its peak at 5.29 g/L, indicating that this concentration yields the highest xanthan output compared to other concentrations tested. However, at 4 g/L, xanthan production decreases significantly to 1.76 g/L, suggesting a negative impact at this concentration. Interestingly, at 6 g/L, xanthan production rises to 3.39 g/L, showing a partial recovery in yield compared to the 4 g/L concentration. This trend, however, does not persist at higher concentrations. At 8 g/L, xanthan production drops to 1.47 g/L, and at 10 g/L, it further declines to 1.13 g/L, the lowest yield observed in the study. Based on these results, the optimal nitrogen concentration for maximizing xanthan production is 2 g/L.

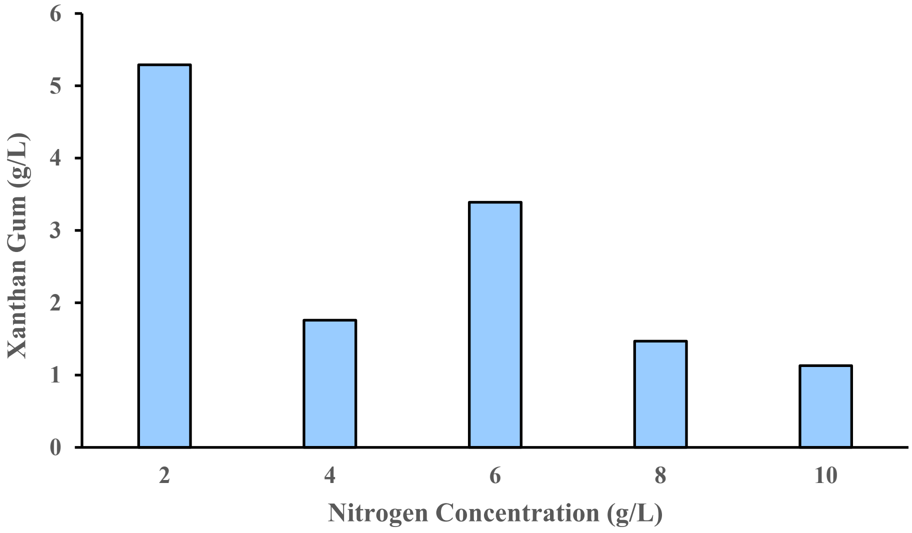

Figure 3. Effect of different concentrations of nitrogen source on xanthan production.

Comparatively, other studies using different nitrogen sources, such as ammonium dihydrogen phosphate ($NH_4H_2PO_4$) with glucose as a carbon source, have reported contrasting trends. For instance, Rashidi et al. (2023) observed that xanthan production increases with nitrogen concentration up to 12 g/L, after which it declines. This suggests that the optimal nitrogen concentration for xanthan production may vary depending on the nitrogen source used. A higher carbon-to-nitrogen (C/N) ratio generally promotes increased xanthan production. Conversely, elevating the concentration of nitrogen sources tends to reduce xanthan yield. A similar study conducted by Ozdal et al. (2019) revealed that the highest xanthan yield was achieved after 24 hours of fermentation. The study also demonstrated that varying concentrations of chicken feather peptone, used as a nitrogen source, resulted in different amounts of xanthan production. Additionally, Ashour (2000) highlighted that limited nitrogen conditions favour xanthan formation, with 5.0% corn steep liquor yielding the highest xanthan production. Higher concentrations of corn-steep liquor were found to reduce xanthan output, further emphasizing the importance of nitrogen concentration in xanthan production. In conclusion, this study demonstrates that xanthan production is highest at a nitrogen concentration of 2 g/L when using BSFL as the nitrogen source. This concentration proves to be the most effective for achieving a high yield of xanthan, while higher concentrations (e.g., 10 g/L) result in significantly lower production. These findings underscore the critical role of nitrogen concentration in optimizing xanthan production and highlight the need to carefully select and regulate nitrogen sources for industrial applications.

4. CONCLUSION

It can be concluded that the objective of this study has been successfully achieved. Different concentrations of nitrogen sources significantly influenced and affected xanthan production. The best concentration of nitrogen source, which used Black Soldier Fly Larvae (BSFL) as the alternative nitrogen source, was 2 g/L, producing the highest xanthan yield of 5.29 g/L compared to other concentrations. Meanwhile, for cell dry weight (CDW) or biomass production, the highest production was 2.46 g/L at a nitrogen concentration of 8 g/L. However, when comparing the data collected to other research, the data obtained shows a different production trend between cell dry weight (CDW) and xanthan. This may have occurred due to errors during the experiment or other potential issues that may have arisen. This study demonstrates that BSFL at 2 g/L maximizes xanthan yield while minimizing resource input, offering a template for sustainable biopolymer production. By replacing conventional nitrogen sources, industries could reduce reliance on synthetic substrates, lower production costs by an estimated 20–40%, and contribute to waste-to-value supply chains. Future work should explore BSFL's amino acid profile to tailor fermentation media for higher efficiency and the ideal process parameters to improve xanthan production.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Faculty of Chemical Engineering, UiTM Johor Branch, Pasir Gudang Campus, for providing the necessary facilities and support, which were instrumental in the successful completion of this research.

AUTHOR CONTRIBUTIONS

Muhammad Safiy Hafiz was responsible for conducting the research and writing the articles, while Ahmad Ramli and Mohd Zaki were the driving force behind the research idea, contributing both theoretical insights and practical expertise.

FUNDINGS

Not applicable

DATA AVAILABILITY

If there is a data set associated with the paper, please provide information of where the data supporting the results or analyses presented in the paper can be found.

COMPETING INTEREST

The authors confirm that there are no conflicts of interest to disclose.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable

SUPPLEMENTARY MATERIAL

Not applicable

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