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**BIOPRODUCTION OF XANTHAN BY
XANTHOMONAS CAMPESTRIS USING
FRUCTOSE AND BLACK SOLDIER FLY LARVAE
AS ALTERNATIVE NITROGEN SOURCES**

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ABSTRACT

Xanthan gum is a microbial exopolysaccharide that has been produced commercially from carbon sources by fermentation using *Xanthomonas campestris*. Xanthan is a natural polysaccharide that is currently used for variety of applications in industry such as food, medical, textile and oil industries. Many industries used xanthan because of its properties meet the industry's needs. It is use as a stabilizer, thickener, and emulsifier agent in various food and industrial products. However, xanthan production is very costly and can cause the effect to the environmental sustainability if the production is relying only on the traditional source and material. Production of xanthan is commonly using glucose as the carbon substrate for the commercial manufacture, which effect to increasing cost of the xanthan synthesis. By using a less expensive substrate for example like agricultural waste, it is one of the techniques that can reduce the cost of the production. Besides, suitable nitrogen source also plays a crucial role for the production by using the optimal concentrations, to help maximize the synthesis of xanthan. The bacteria were cultivated at five different concentration of nitrogen source which are 2, 4, 6, 8, and 10 g/L. Therefore, this study will be focusing on the effect of different nitrogen concentrations which use Black Soldier Fly Larvae (BSFL) as the alternative nitrogen source and its potential on the production of xanthan. Fructose will be use as the carbon source with the fixed concentration of 40g/L for each medium with different nitrogen concentration. And the bacteria that will be use for the fermentation process is *Xanthomonas campestris* ATCC 13951. At the end, the result shows that the medium containing 2 g/L of BSFL successfully produced the highest xanthan production that is 5.29 g/L. This is proven that BSFL has a potential in producing xanthan.

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CHAPTER 1

BACKGROUND

1.1 Introduction

Biopolymers production has become one of the major areas of intense research and industrial interest, carried by highly growing demand for sustainable, bio-based materials. Xanthan gum, a microbial exopolysaccharide that is produced by a gram-negative bacteria named *Xanthomonas campestris*, is one of the biopolymers that has been known as the source of numerous applications and plays a crucial role across various industries, for example, in the food industry, pharmaceuticals, cosmetics, textile, and more recently, in the improvement of oil production. Due to its ability and speciality that can be used as a stabilizing, gelling, emulsifying and thickening agent make this polysaccharide is used in many industries because of its biopolymers properties that meet the industry's needs (Cancella et al., 2024; Kalogiannis et al., 2003; Nejadmansouri et al., 2020; Niknezhad et al., 2016; Ramli Rashidi et al., 2023; Zakeri & Pazouki, 2017)

There are many types of *Xanthomonas* spp bacteria that is used to produce the xanthan gum such as *Xanthomonas campestris*, *Xanthomonas phaseoli* and *Xanthomonas malvacearum* (Bhat et al., 2022; Nejadmansouri et al., 2020). But the most commonly used for xanthan production is *Xanthomonas campestris* because of its high yield and high-quality product. Xanthan polymer main structure consist of repeated units of pentasaccharide where every unit has two units of glucose, two units of mannose and one unit of glucuronic acid (Zahidah Nordin et al., 2020). Xanthan has a high solubility, resilience and durability when in contact with acid or alkaline environments, stability of xanthan gum in the presence of salts and has the potential of resistance to enzymes make xanthan gum as the one of the main polymers that been used in the food industry and plays a dominant role (Kalogiannis et al., 2003; Zahidah Nordin et al., 2020; Zakeri & Pazouki, 2017)

However, conventional production of xanthan gum is expensive and resource intensive which is typically relying on high purity of carbon and nitrogen source like glucose and peptone (Palaniraj & Jayaraman, 2011; Zahidah Nordin et al., 2020). This reliance on the materials will not only limit the production efficiency but also will affect the environmental concerns, pushing for more sustainable and cost-effective substrate explorations. Use of organic nitrogen source is aligned with sustainable production practices and BSFL is one of the sources that can be used. BSFL or also known as *Hermetia illucens* is known for its ability to feed on organic waste and convert it into high quality protein, offering a rich nitrogen source that can be integrated into microbial culture media (*Black Soldier Flies_ Inexpensive and Sustainable Source for Animal Feed*, 2020). This alternative can help in reducing the dependency on traditional, high-cost nitrogen sources, such as yeast or peptone, making production of xanthan becomes more economically and environmentally viable.

This study focuses on investigating the feasibility of using fructose as carbon source and BSF larvae as an alternative nitrogen source for the bioproduction of xanthan gum by *Xanthomonas campestris*. Plus, to demonstrate on how different concentrations of nitrogen source would affect to the production of xanthan.

1.2 Literature Review

1.2.1 Traditional Xanthan Production and Substrate Used

Xanthomonas campestris is generally fermented in batches using glucose or sucrose as a carbon source and yeast as a nitrogen source in a traditional way of producing xanthan. Under specific conditions, it has been demonstrated that this combination maximizes bacterial growth and xanthan yield. Because of its purity, simplicity of fermentation, and reliable yield, glucose and sucrose are recommended. Although they considerably raise the cost of production, glucose-based substrates can produce up to 40 g/L of xanthan (Bhat et al., 2022).

Yeast extract and peptone are good source of nitrogen because they are high in vitamins and amino acids that promote cell development. Findings proves that peptone is the most effective nitrogen source that can be used for xanthan production (Mohsin et al., 2021). However, usage of peptone is a bit costly for the industrial scale and can