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Evaluation of Fructose Concentration on Xanthan Gum Bioproduction Using Black Soldier Fly Larvae as a Sustainable Nitrogen Source

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ABSTRACT

Xanthan gum is a valuable polysaccharide used in the food and pharmaceutical industries, but its conventional production relies on costly, unsustainable glucose and commercial nitrogen sources. This study evaluates the use of Black Soldier Fly Larvae (BSFL) as an alternative nitrogen source and investigates the effect of different fructose concentrations on xanthan gum production by Xanthomonas campestris ATCC 13951. Experiments were conducted at 30°C and 150 rpm with varying fructose concentrations, and biomass yield (CDW) and xanthan production were analyzed after 24 hours. The results indicated that a fructose concentration of 30 g/L produced the highest xanthan yield (3.80 g/L), while higher concentrations led to metabolic inhibition. This indicates that optimizing fructose levels is essential for maximizing yield. This study also highlights the potential of BSFL as a sustainable alternative to nitrogen, contributing to cost-effective and environmentally friendly bioprocessing. These findings support the integration of waste-based resources in microbial fermentation, aligning with Sustainable Development Goal 12 (Responsible Consumption and Production) by promoting circular economy principles through waste valorization. Future research should systematically investigate the synergistic effects of critical fermentation parameters to optimize xanthan yield and quality.

Keywords: Black Soldier Fly Larvae (BSFL) hydrolysate, *Xanthomonas campestris* ATCC 13951, Fructose concentration, Xanthan gum yield, Sustainable fermentation.

1. INTRODUCTION

Xanthan gum possesses the qualities that make it a versatile exopolysaccharide produced by *Xanthomonas campestris* bacteria. Its robust applications in industries such as food, cosmetics, pharmaceuticals, and oil recovery were supported by its rheological structure, which made it a suspending, stabilizing, and thickening agent [1, 2]. The demand for their use is getting bigger in the current global market. This means that the production cost must be effective during xanthan gum production and support environmental sustainability [3]. The fermentation process for xanthan gum production in industry requires a carbon source and nitrogen as the main components of the medium. While high-purity carbon sources like sucrose or glucose and expensive nitrogen sources such as peptone or yeast extract may seem effective, they contribute to significant economic and environmental difficulties. The carbon footprint of the intensive processing and land use required to produce refined sugar indicates that reliance on these sources could contribute to environmental problems and elevate production costs in the sugar sector [4]. Identifying cost-effective, alternative substrates derived from waste to produce

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nitrogen has become important to addressing the current issue of excessive energy use in generating conventional nitrogen. This is a pivotal approach to maintaining the sustainability of bioprocesses.

Numerous studies related to the production of fermentable sugars, such as glucose, arabinose, and xylose, from agro by-products and industrial wastes have highlighted the success in the literature. In light of this, the search for alternative nitrogen sources for long-term supply has been ongoing, which is equally important. One promising source is insect biomass, which is rich in essential amino acids, provides substantial protein, and, most importantly, is not harmful to the environment. Recently, the Black Soldier Fly larvae (BSFL) have gained attention for their ability to convert organic waste and biomass into nutrient-rich larvae. BSFL typically contains about 25.6% protein and 35.5% lipids, and the protein hydrolysate yields free amino acids including lysine (8.0%), leucine (7.7%), and valine (7.2%) [5]. This circular economy strategy makes BSFL hydrolysate a fantastic, long-lasting, and cheap supply of nitrogen for microbial fermentation [6, 7]. The use of BSFL and fructose in making xanthan gum has tremendous promise to boost environmental sustainability. On the one hand, BSFL has been shown to convert organic waste substrates into nutrient-dense by-products that could minimize the use of synthetic fertilizers, thereby reducing environmental pollution and impacts to human health [8, 9]. On the other hand, fructose acts as a renewable carbon source during fermentation, further improving sustainability [10]. This is the perfect arrangement to establish a circular economy, reducing environmental degradation and CO_2, while converting organic residues into valuable by-products such as frass, a biodegradable fertilizer [11, 12]. These strategies thus move toward a more sustainable form of production with a concomitant reduction in environmental impact.

Xanthan gum production relies on glucose from food crops, making it costly and unsustainable [13]. Additionally, commercial nitrogen sources also contribute to high costs and environmental impact. For example, yeast extract works great, but it's expensive and energy-intensive to produce [14]. Moreover, corn steep liquor and soybean meal are inconsistent batch to batch [15, 16]. What BSFL offers is a more consistent protein profile, plus it can be grown locally on waste, so the carbon footprint is lower. To address this, sustainable alternatives such as Black Soldier Fly Larvae (BSFL) are being explored for their ability to convert organic waste into nutrients, providing a viable nitrogen source [17, 18, 19]. However, research on its impact on xanthan gum yield and quality remains limited. This study evaluates the effect of varying fructose concentrations and BSFL on xanthan gum production to develop an effective medium formulation that enhances microbial growth and xanthan yield. In addition, this study aligns with SDG 12 (Responsible Consumption and Production) by reducing dependence on conventional agricultural resources and with SDG 9 (Industry, Innovation, and Infrastructure) through sustainable bioprocessing innovation, ultimately contributing to a circular bioeconomy.

2. MATERIAL AND METHODS

2.1 Microorganisms

The reference strain, *Xanthomonas campestris* ATCC 13951, was sourced from the American Type Culture Collection (ATCC) and served as the production microorganism. It was grown in a standard nutrient broth and preserved at -70°C in glycerol-containing vials for future applications.

2.2 Materials and chemicals

Macronutrients used in the fermentation process include fructose as a source of carbon and black soldier fly larvae (BSFL) as a substitute source of nitrogen. In addition, micronutrients such as zinc sulfate ($ZnSO_4$), dipotassium hydrogen phosphate anhydrous (K_2HPO_4), and boric acid

(H_3BO_3) are used to support bacterial growth and production. For the xanthan harvest process, 95% ethanol is used.

2.3 Development of a working cell bank

A reliable working cell bank of *Xanthomonas campestris* ATCC 13951 was then cultured in controllable conditions. The 2-ml vial containing bacteria was transferred to 50 mL of sterilized nutrient broth and then incubated in an incubator shaker at 30°C, 200 rpm for 24 hours for cultivation. Later, the bacteria were streaked onto nutrient agar plates and incubated at 30°C for 24 h to allow growth. When the biomass was sufficient, the culture was supplemented with sterile glycerol at a ratio of 15% v/v to protect the cells from ice damage during freezing. Portions of 1.5 mL were aseptically transferred into sterile vial tubes and stored at -80°C. A periodic viability test of samples was performed by reviving cultures stored on nutrient agar plates and incubating them at 30°C to confirm their growth capacity and functionality. The method is designed to ensure long-lasting effectiveness and serves as an inoculum source for future experiments that adhere to established microbial preservation protocols [20]

2.4 Medium preparation

To prepare the fermentation medium, fructose was dissolved in distilled water at concentrations of 30 g/L, 40 g/L, 50 g/L, 60 g/L, and 70 g/L, while black soldier fly larvae served as the nitrogen source at a fixed amount of 4 g/L. Other micronutrients were also prepared, such as 4 g/L di-potassium hydrogen phosphate, 0.06 g/L zinc sulphate, and 0.06 g/L boric acid. The pH of the medium was adjusted to 7 using NaOH or HCl to optimize growth for *X. campestris*, which resides in this pH range. The medium was autoclaved at 121°C for 15 minutes after pH adjustment. All sterilized media were distributed to multiple sterilized conical flasks, which were plugged with cotton and stored in a sterile environment until being used.

2.5 Fermentation process

The prepared fermentation media were inoculated with bacterial strains after initial cultivation. Inoculated flasks were incubated at 30°C and agitated at 150 rpm in a rotary shaker for 24 hours. Agitation ensured proper nutrient mixing and adequate oxygenation, both of which are critical for bacterial growth and xanthan gum production [21]. Preliminary findings indicated significant bacterial activity and polysaccharide production within 24 hours [22]. However, longer fermentation durations (up to 144 hours) were shown to enhance xanthan gum accumulation [23]. The shorter fermentation time was selected for its time- and cost-efficiency, aligning with the goals of sustainable production.

2.6 Harvesting

The fermentation broth was centrifuged after a 24-hour cultivation to measure cell dry weight (CDW) and recover xanthan gum. The fermentation broth was transferred into Falcon tubes for centrifugation at 7000 rpm for 15 minutes at 4°C, separating the broth into two distinct layers: a precipitate containing bacterial cells and a supernatant. For CDW determination, the precipitate was collected, washed, and dried in an oven at 80°C for 24 hours to remove residual moisture. Once dried, the precipitate was weighed on an analytical balance, and the mass was recorded. To recover xanthan gum, the supernatant was mixed with ethanol (1:3 ratio). The mixture was refrigerated at 4°C for 24 hours to allow xanthan gum to precipitate. After refrigeration, the Falcon tubes were centrifuged again at 7000 rpm for 15 minutes at 4°C, resulting in the xanthan precipitate settling to the bottom of the tubes. The liquid supernatant was carefully discarded, leaving only the xanthan gum. The precipitated xanthan gum was then dried in an oven at 80°C for 24 hours to remove any residual moisture. Afterwards, the xanthan gum was weighed using an analytical balance, and its yield was calculated. Recovering a component with ethanol and subsequent drying, as described here, is consistent with methods used by previous researchers [24, 25].

2.7 Analysis

The CDW was calculated using the weight of the dried bacterial cells, while the xanthan yield was determined by weighing the dried xanthan gum. This ethanol precipitation method, followed by drying, ensured reproducibility of results, consistent with previously established protocols for xanthan gum production [24, 25]. The data were recorded in duplicate.

3. RESULTS AND DISCUSSION

3.1 Cell dry weight (CDW)

Cell dry weight (CDW) indicates the growth of *Xanthomonas campestris* ATCC 13951 at various fructose concentrations. The results were visualized in Figure 1. The data indicate that as the fructose concentration increases, so does the CDW, which reaches a maximum of 1.25 g/L at 70 g/L. At lower fructose concentrations (30 and 40 g/L), the CDW remained low due to a limited carbon source to support high bacterial growth. Moreover, the marginal increase in CDW between 60 g/L and 70 g/L indicates that bacterial growth potential is saturated at higher carbon levels.

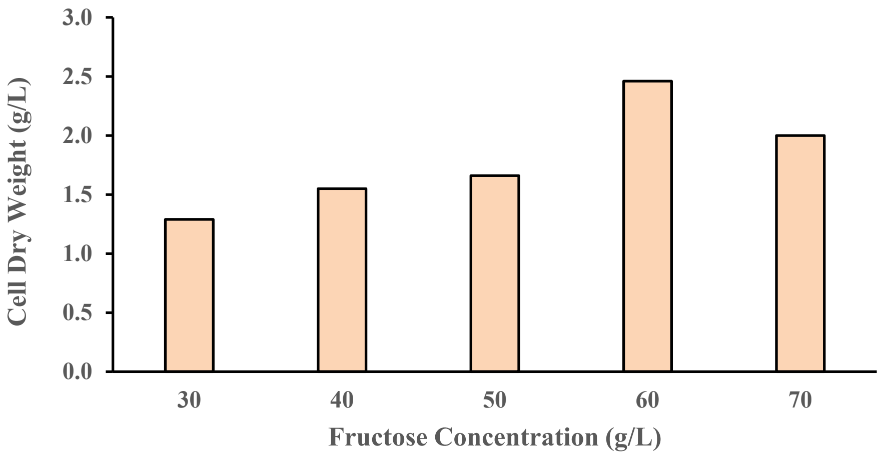

Figure 1: Cell dry weight (CDW) using *Xanthomonas campestris* with different fructose concentrations.

Earlier works with longer fermentation periods have shown relatively higher CDW values; for example, Elsayed [26] reported a CDW of 4.05 g/L when the fermentation period was extended beyond 24 hours. Moreover, extended fermentation periods significantly affect bacterial biomass accumulation; for instance, studies tracking *Xanthomonas campestris* during 96-hour fermentations have demonstrated substantial shifts in cell dry weight (CDW) with substrate concentration [27]. Another study by Asase [28] revealed that the kinetics of *Xanthomonas campestris* growth require careful monitoring, as biomass and target yields are heavily influenced when the fermentation period is extended beyond the initial 24-hour logarithmic growth phase up to 72 hours. However, the fermentation period in this study of 24 hours was selected with an intention to maximize time efficiency and minimize operational expenses, although it is likely to restrict bacterial growth relative to longer times.

3.2 Xanthan gum production

Figure 2 shows the results of xanthan production. At 30 g/L fructose, the highest average yield was 3.80 g/L; however, at 70 g/L fructose, cell dry weight (CDW) showed substantially reduced xanthan production, likely due to osmotic stress or feedback inhibition from excess fructose. These findings are in agreement with other studies that have been able to show how high sugar concentrations negatively influence polysaccharide biosynthesis [29]. With nitrogen fixed at 4 g/L, the C/N ratio increased from about 7.5:1 at 30 g/L fructose to roughly 17.5:1 at 70 g/L fructose. A study investigating the direct effect of C/N ratio found that xanthan production was enhanced at specific ratios. For instance, production increased as the C/N ratio rose from 5 to 20, after which it began to decline at a ratio of 40 [30]. The C/N ratio significantly affects exopolysaccharide (EPS) synthesis, which primarily occurs under conditions of excess carbon and limited nitrogen availability. High C/N ratios generally result in increased specific EPS production per bacterial biomass. However, the reduction in nitrogen availability at the highest C/N ratio limited bacterial biomass formation, leading to lower total EPS production [31]. So the high C/N ratio likely favored bacterial growth over gum production. However, some variability in yield was noted, which may indicate minor experimental inconsistencies in recovery or drying. Despite this inconsistency, the data indicate that 30 g/L fructose supports xanthan synthesis more effectively than other concentrations tested.

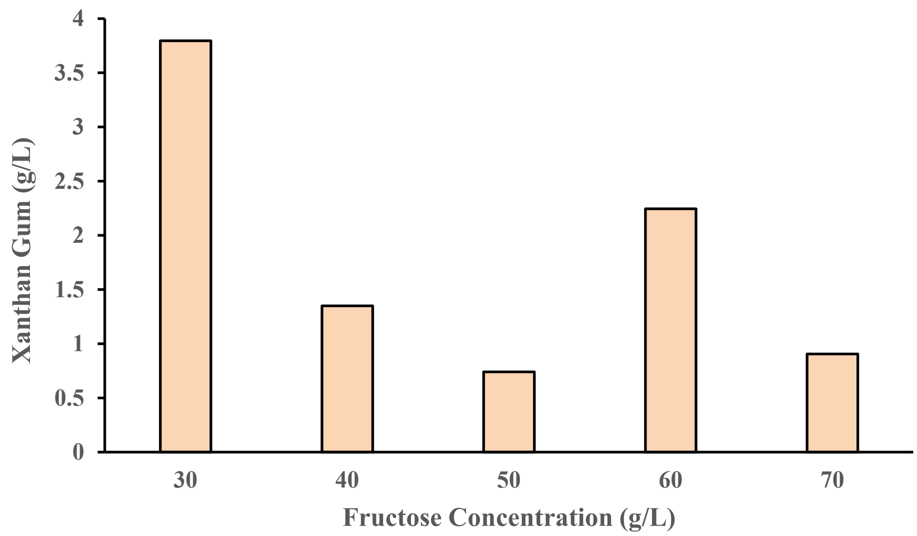

Figure 2: Xanthan gum production using *Xanthomonas campestris* with different fructose concentrations.

At 60 g/L, the xanthan yield was lower, indicating stability but reduced production compared to 30 g/L. A higher CDW at 60 g/L (1.12 g) compared to 0.78 g at 30 g/L suggests increased bacterial growth at higher fructose levels, but this did not translate into higher xanthan synthesis. This finding indicates that while higher fructose concentrations support cell growth (CDW), they do not necessarily enhance xanthan production, possibly due to metabolic regulation or substrate inhibition effects. Higher yields were reported in other studies with longer fermentation durations, such as Mei-xia [32], who achieved over 21 g/L after 72 hours using glucose, and Mohsin [33], who obtained 16-18 g/L after 80 hours using sucrose. In contrast, this study used a 24-hour fermentation period, which was efficient but may have sacrificed yield due to the shorter incubation time. However, for rapid screening or small-scale applications, 24 hours could save energy, labour, and production turnaround time.

4. CONCLUSION

In conclusion, this study investigated the impact of different fructose concentrations on xanthan gum production by *Xanthomonas campestris* ATCC 13951, using Black Soldier Fly Larvae (BSFL) as an alternative nitrogen source. The findings highlight the importance of optimizing substrate concentration to enhance xanthan biosynthesis. The highest xanthan yield of 3.80 g/L was achieved at 30 g/L fructose within a 24-hour fermentation period, emphasizing the balance between substrate availability and metabolic efficiency. Additionally, the results show the promising potential of BSFL as a sustainable nitrogen source, supporting its application in microbial fermentation. However, proper validation against conventional media is still needed. The full compositional characterization (amino acid profile and mineral content) of BSFL should be conducted to unlock its full biotechnological potential and to optimize its use as a sustainable nitrogen source in xanthan gum production. In addition, measuring the residual fructose and calculating the yield coefficients will provide further insight into substrate limitation and inhibition. This study also contributes to the understanding of xanthan gum production under controlled conditions, emphasizing the important role of fermentation parameters such as time-course studies (24, 48, 72, 96 hours), agitation rates, and the temperature and pH of the medium in achieving optimal yield for future work.

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