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Optimization of fermentation parameters conditions for xanthan production and standardization of product recovery process

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Abstract
Xanthan gum is an industrially important biopolymer synthesized by Gram-negative bacteria of the genus *Xanthomonas*. Its distinctive rheological and stabilizing properties make it widely applicable in the food, cosmetic, pharmaceutical, petroleum, and agricultural industries. The high cost of fermentation media has been a persistent bottleneck in its large-scale production. This study focuses on optimizing fermentation conditions, evaluating refined and agro-waste substrates as carbon and nitrogen sources, and standardizing recovery processes for xanthan gum. The optimal conditions were identified as pH 7, temperature 30 °C, and agitation at 300 rpm. Sucrose (40 g/L) and molasses (150 g/L) were the most effective carbon sources, while ammonium sulphate (1.5 g/L) and soymeal provided the best nitrogen support. Isopropyl alcohol (3:1 v/v) achieved maximum recovery yields. Scale-up studies in a bioreactor validated the optimized parameters and demonstrated the feasibility of cost-effective xanthan gum production.

Keywords: Xanthan gum, fermentation optimization, agro-waste substrates, *Xanthomonas campestris*, bioreactor scale-up, product recovery

Introduction
Xanthan gum is a high-value extracellular polysaccharide produced by the Gram-negative bacterium *Xanthomonas campestris*. Owing to its unique rheological properties, including high viscosity, shear-thinning behavior, and stability across a broad range of pH and temperatures, xanthan gum has gained wide applications in the food, cosmetic, pharmaceutical, petroleum, and agricultural industries [1-5]. The global xanthan gum market continues to expand, reflecting the growing demand for biopolymer-based additives [2, 3].

Commercial xanthan production relies on aerobic submerged fermentation, typically using refined sugars as carbon sources [6-9]. However, the high cost of such substrates remains a key limitation for large-scale production. Consequently, agro-industrial by-products, such as molasses, cheese whey, and fruit processing residues, have been explored as alternative substrates, offering both cost reduction and environmental benefits [10-15]. Optimizing fermentation parameters, including pH, temperature, agitation, and nutrient composition, is crucial for maximizing yields [16-19].

In addition to upstream process optimization, downstream recovery plays a vital role in overall yield and cost-effectiveness. Alcohol precipitation, particularly with isopropanol, remains the most widely used and effective method for xanthan recovery [20, 21]. Recent studies have also focused on scaling up optimized processes to validate their industrial feasibility [22-25].

The present study aimed to optimize fermentation parameters for xanthan gum production using both refined and agro-waste substrates, to evaluate the efficiency of different nitrogen sources, and to standardize recovery methods. Furthermore, scale-up experiments were conducted in a bioreactor to validate the reproducibility of optimized conditions, thereby demonstrating a sustainable strategy for cost-effective xanthan production.

Materials and Methods
Four bacterial strains were isolated from black rot of cabbage, bacterial blight of rice, bacterial blight of French bean, and citrus canker.

These were screened for efficient xanthan gum production and compared with the reference culture *Xanthomonas campestris* (NCIM 2954). Fermentation parameters such as pH, temperature, agitation, carbon sources, and nitrogen sources were optimized in 250 mL flasks containing 100 mL XPM broth. The optimized conditions were then scaled up in a 7 L Applikon Bio Console ADI 1025 fermentor. Recovery of xanthan gum was carried out using alcohol precipitation with ethanol, methanol, or isopropanol at 3:1 (v/v), followed by centrifugation and oven drying.

Results

Optimization of Fermentation Parameters

The optimal conditions for xanthan production were established at pH 7.0 (Figure 1), a temperature of 30 °C (Figure 2), and agitation at 300 rpm (Figure 3). All five test cultures achieved maximum biomass and xanthan yields under these conditions, in agreement with earlier studies [9-11].

Carbon Sources

Among refined carbon sources, sucrose was the most effective, supporting higher xanthan yields than glucose or starch (Figure 4). At 40 g/L sucrose, both biomass accumulation and xanthan production peaked (Figure 5). Among agro-based sources, molasses (40 g/L) produced the highest yield, followed by cheese whey and soya oil, consistent with prior reports validating industrial by-products as substrates (Figure 6 & 7) [12-15].

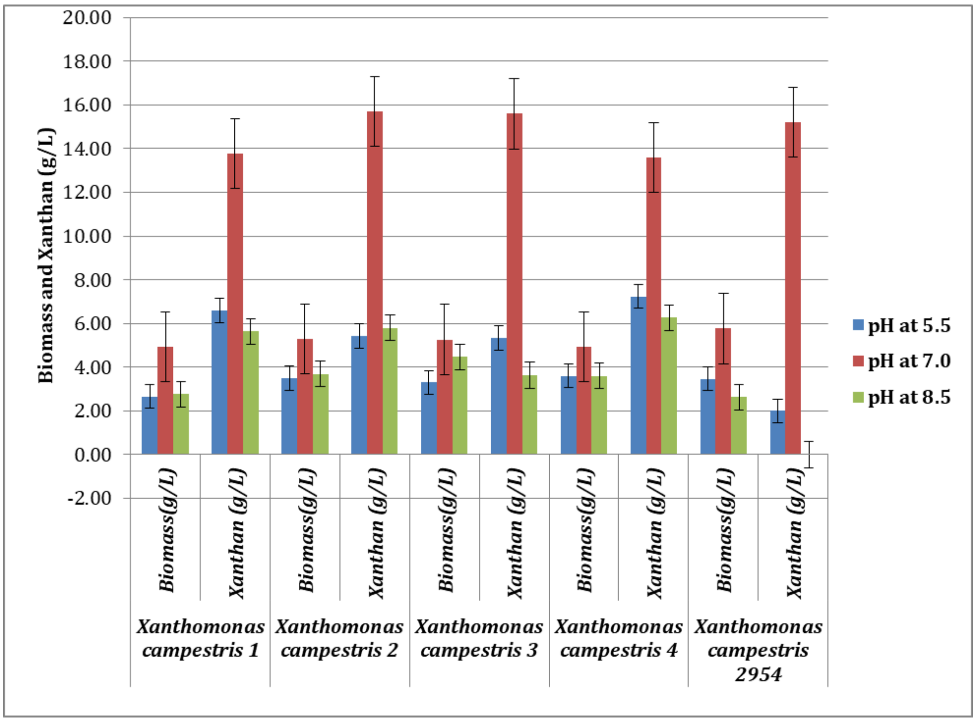

Fig 1: Effect of pH on biomass and xanthan production

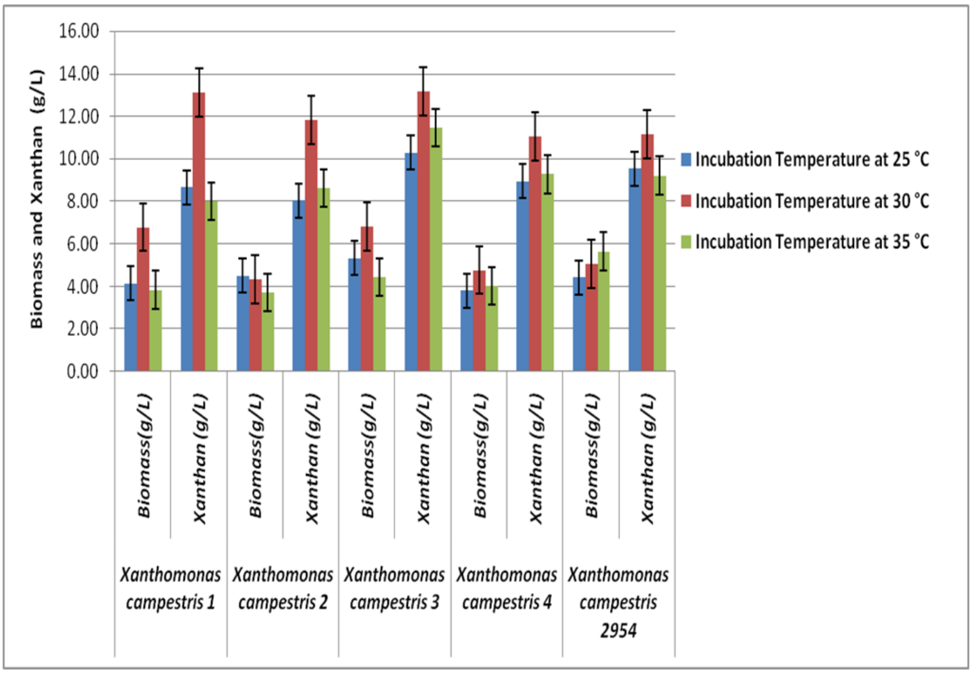

Fig 2: Effect of different levels of incubation temperature on biomass and xanthan production

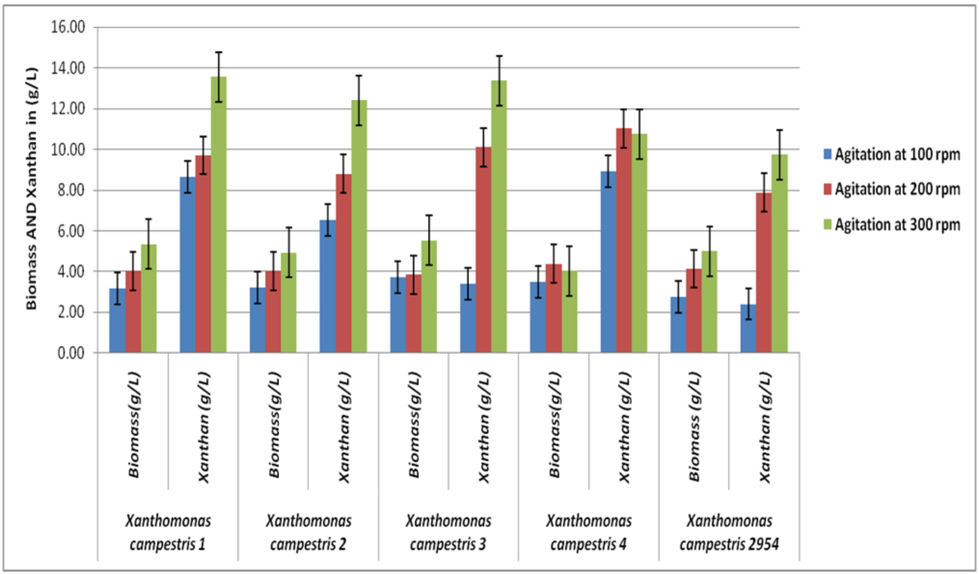

Fig 3: Effect of agitation at different levels on biomass and xanthan production

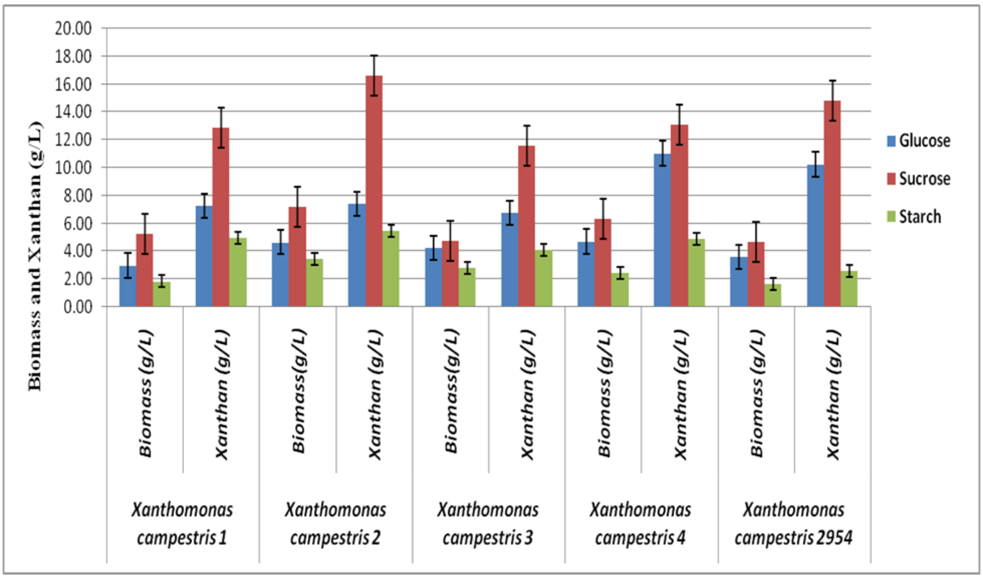

Fig 4: Effect of different carbon source on biomass and xanthan production

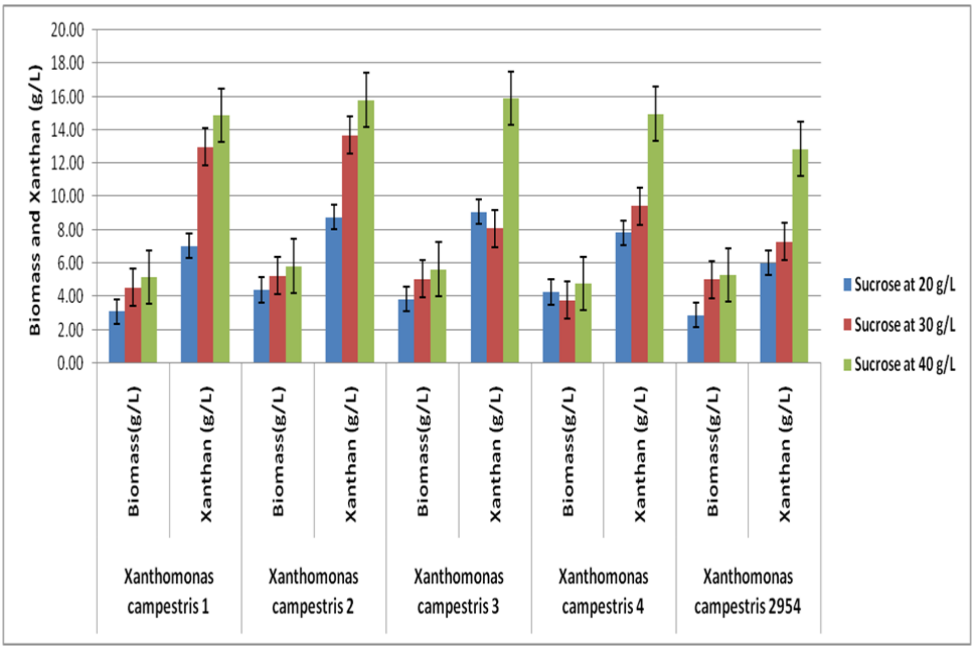

Fig 5: Effect of different levels of sucrose on biomass and xanthan production

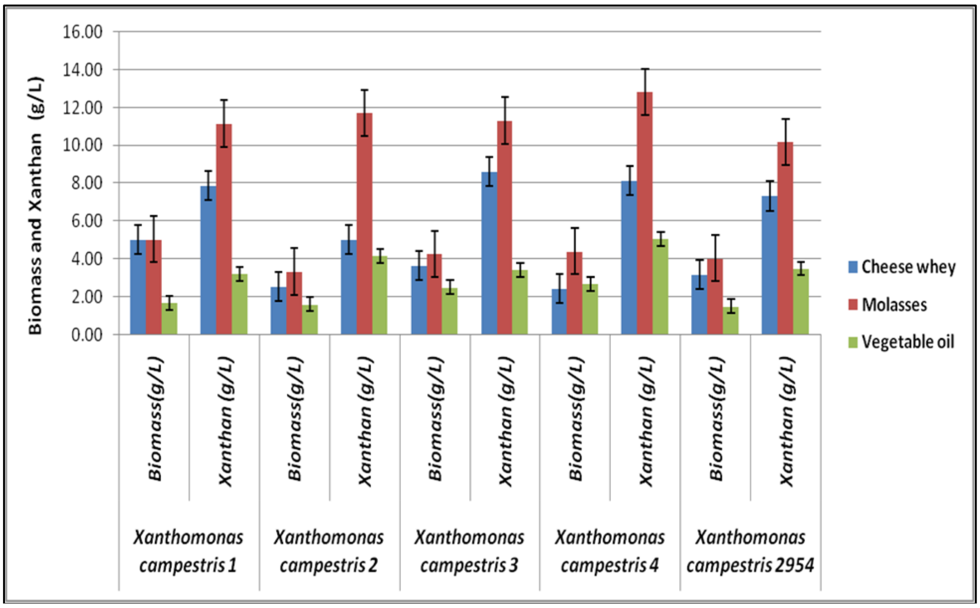

Fig 6: Effect of different raw carbon source on biomass and xanthan production

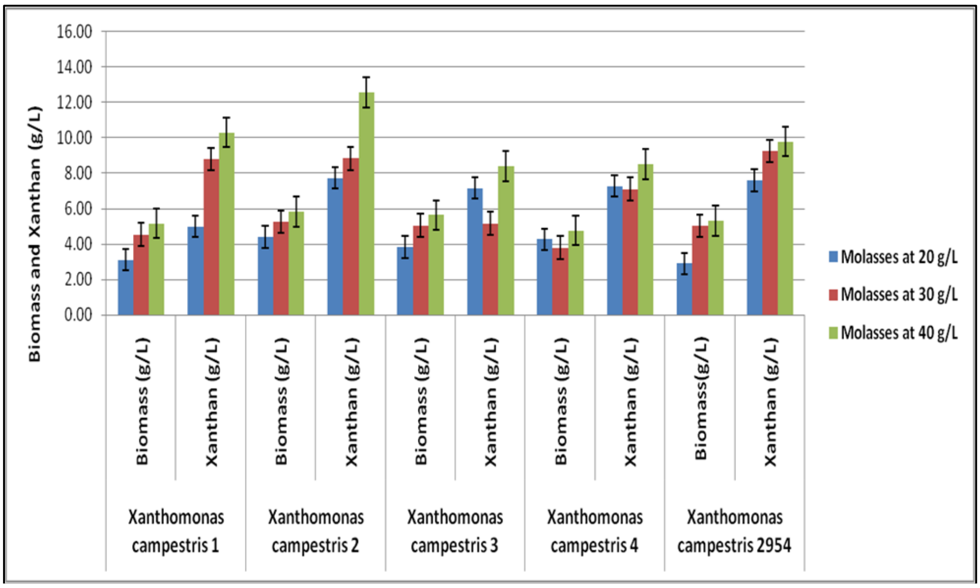

Fig 7: Effect of different levels of molasses on biomass and xanthan production

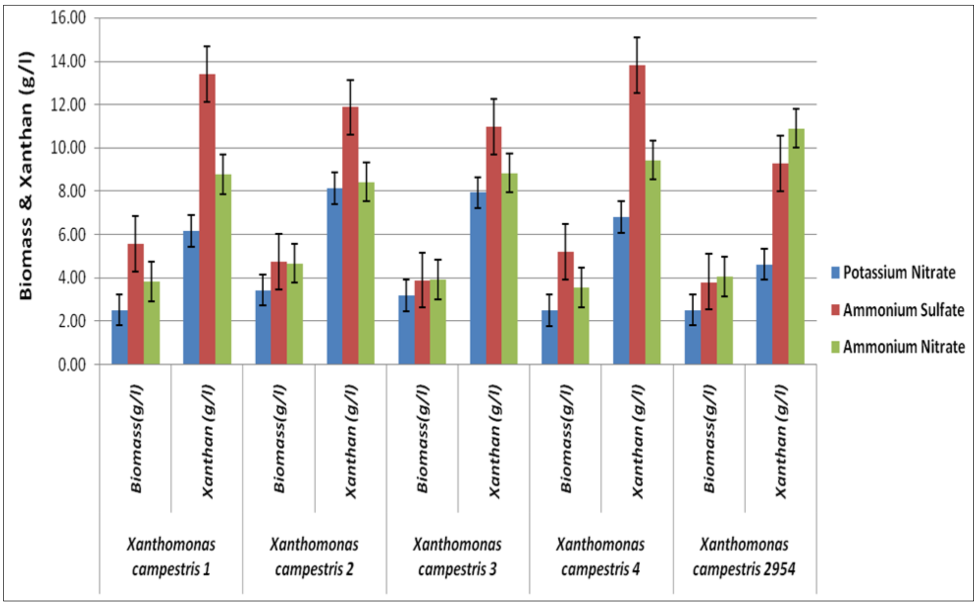

Fig 8: Effect of different inorganic nitrogen sources on biomass and xanthan production

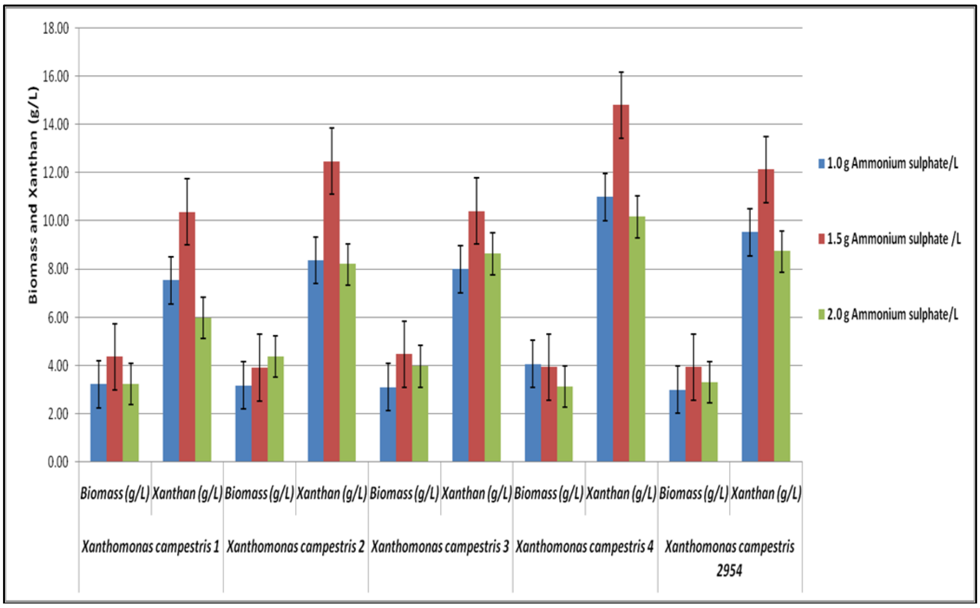

Fig 9: Effect of different concentrations of ammonium sulphate on biomass and xanthan production

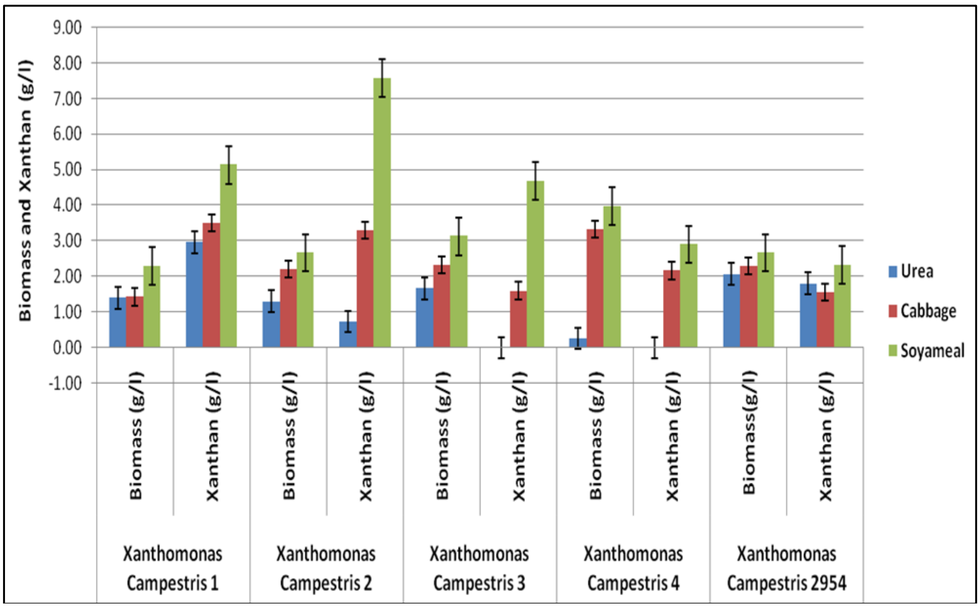

Fig 10: Effect of different sources of organic nitrogen on biomass and xanthan production

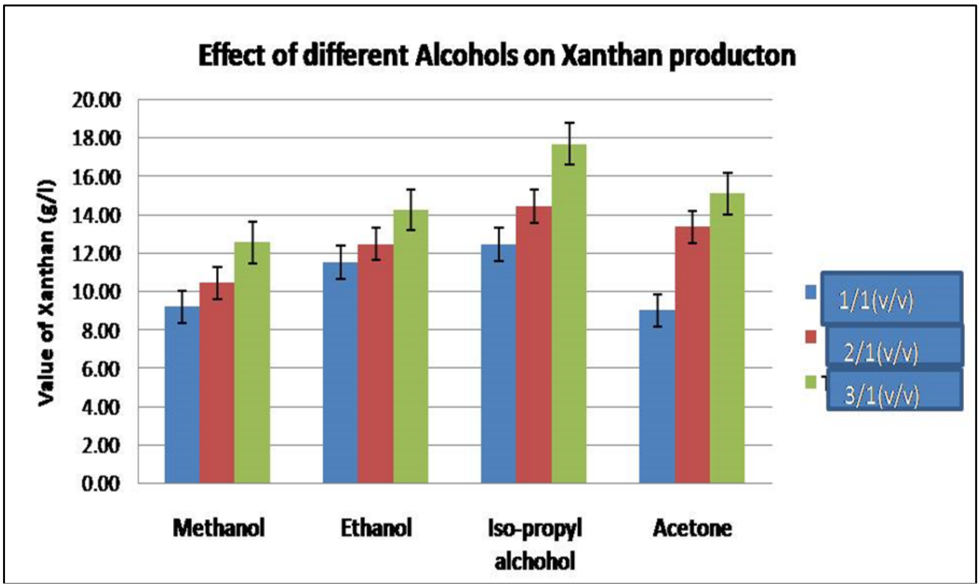

Fig 11: Effect of different Alcohols on recovery of xanthan gum

Table 1: Scale-up production of xanthan gum in fermentor

Fermentation conditions	Standardized conditions	Standardized Agrowaste conditions
Strain	*Xanthomonas campestris* 3	*Xanthomonas campestris* 3
Carbon	Sucrose	Molasses
Amount of Carbon source (g/L)	40	150
Nitrogen source	Ammonium sulphate	Soya meal
Amount of Nitrogen source (g/L)	1.5	20
pH	7	7
Temperature (° C)	30	30
Agitation (rpm)	300	300
Xanthan produced	143 g	118 g

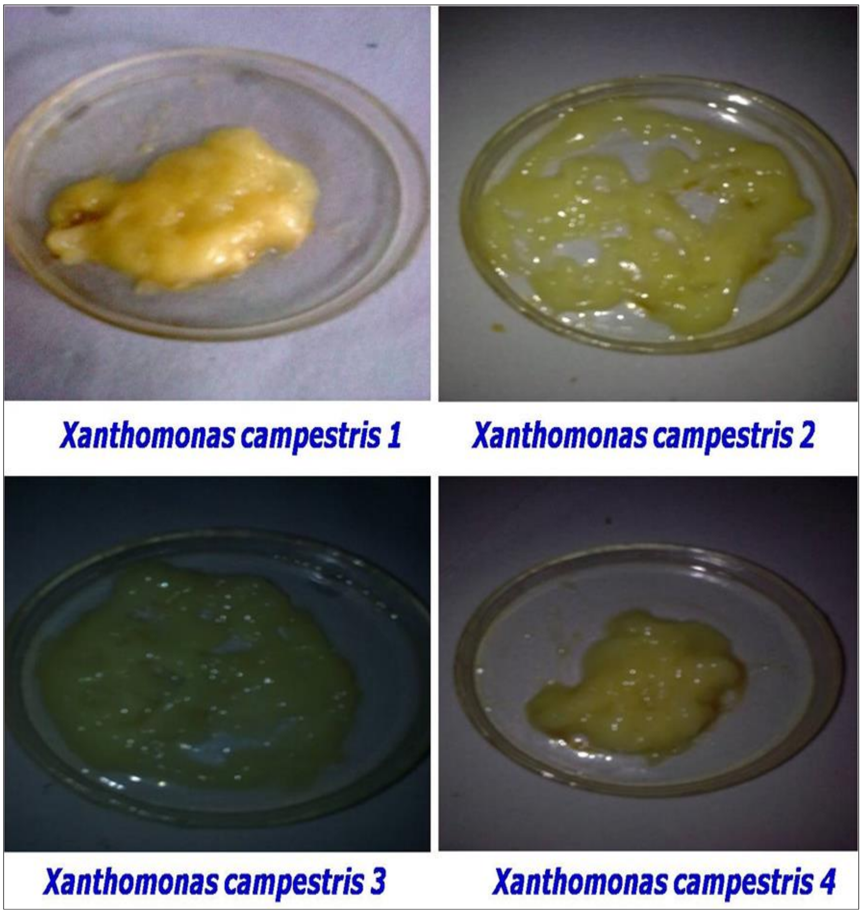

Plate 1: Xanthan gum extracted from isolates of *Xanthomonas campestris*

Nitrogen Sources

Ammonium sulphate at 1.5 g/L was the most effective inorganic nitrogen source, outperforming ammonium nitrate and potassium nitrate (Figure 8 & 9). Among organic sources, soymeal supported superior xanthan production compared to urea or cabbage extract (Figure 10). Although inorganic nitrogen generally enhanced growth and yield, soymeal provided a promising and sustainable alternative [16-19].

Product Recovery

Isopropanol (3:1 v/v) was identified as the most efficient alcohol for xanthan precipitation, yielding higher recovery rates than ethanol or methanol. Scale-up experiments further validated the optimized conditions, with xanthan production reaching 143 g/L under refined substrates and 118 g/L with agro-waste inputs. Although yields were lower with agro-wastes, their reduced cost highlights their industrial potential (Figure 11 and Plate 1).

Discussion

The findings of this study reaffirm that fermentation parameters play a decisive role in xanthan gum yield and quality. A neutral pH (7.0) proved most favorable, consistent with reports that *X. campestris* grows optimally under near-neutral conditions [9]. Similarly, 30 °C was confirmed as the ideal temperature, aligning with previous studies showing that 28-30 °C supports maximum productivity [10, 11]. Agitation at 300 rpm enhanced oxygen transfer, which is critical for aerobic metabolism, echoing earlier observations [12].

Among the carbon sources, sucrose and molasses emerged as superior substrates. Molasses, an abundant by-product of the sugar industry, continues to be validated as a cost-effective raw material for xanthan production [13-15]. The results also highlighted that sugar concentration must be carefully optimized: While moderate levels (40 g/L sucrose and 150 g/L molasses) maximized productivity, excessive sugar exerted an inhibitory effect, consistent with C/N ratio optimization studies [16].

Regarding nitrogen nutrition, ammonium sulphate (1.5 g/L) enhanced xanthan synthesis, supporting prior evidence that inorganic salts promote biomass accumulation [17]. Soymeal, though less efficient than inorganic salts, offered a promising organic alternative, reflecting recent findings that agro-waste-derived nitrogen sources can reduce production costs while maintaining productivity [18, 19].

For downstream recovery, isopropanol precipitation proved superior to ethanol and methanol, providing the highest yields. This observation agrees with contemporary advances in xanthan gum recovery strategies [20, 21]. Although agro-waste substrates produced slightly lower yields than refined counterparts, their economic and sustainability advantages strengthen their industrial relevance [22].

Overall, the study demonstrates that a combination of optimized fermentation conditions and strategic use of low-cost substrates offers a practical pathway to efficient, scalable, and sustainable xanthan gum production.

Conclusion

This study successfully optimized the key fermentation parameters, substrate sources, and recovery process for xanthan gum production. The results demonstrate that maintaining neutral pH, moderate temperature, and sufficient agitation significantly enhances yield. Sucrose and molasses were validated as effective carbon sources, while ammonium sulphate and soymeal provided efficient nitrogen supplementation. Isopropyl alcohol was identified as the most effective precipitant for xanthan recovery. Furthermore, scale-up in a bioreactor confirmed the reproducibility and industrial feasibility of the optimized conditions. These findings offer a sustainable and cost-effective strategy for xanthan gum production, supporting its application in diverse industries.

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