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The Production of Xanthan from Brewer's Spent Grain

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Sugar or dextrose increases the cost of production of xanthan gum by *Xanthomonas campestris*. Brewers' Spent Grain (BSG) was chosen as a source of fermentable sugars. BSG is a significant industrial by-product generated in large quantities from the breweries. Primarily used as animal feed due to its high fiber and protein content, BSG holds great potential as an economically and ecologically sustainable substrate for fermenting biomolecules. This study explores BSG's potential as a cost-effective carbon source for producing xanthan, utilizing *Xanthomonas campestris* NCIM 2961. An aqueous extract was prepared from BSG and inoculated with the bacterium under standard fermentation conditions. After fermentation, xanthan gum was purified using a standard protocol. The xanthan yield from BSG media was compared to that from MGYP media (control). The fermentation parameters, including pH, temperature, agitation and duration were optimized for maximum xanthan gum yield by varying them at different levels. Following fermentation, the xanthan gum was purified from the broth by alcoholic precipitation and then dried. The weight of the dried gum was measured. The obtained xanthan from BSG under standard conditions and commercial food-grade xanthan were characterized using FTIR. The highest xanthan yields were achieved at 32℃, pH 6.0, and 72 h of fermentation at 200 rpm using BSG media. The FTIR spectra of xanthan from BSG media closely resembled that of commercial food-grade xanthan. The results confirm the potential of BSG as a cost-effective alternative carbon source for xanthan production, thereby reducing production costs and solid waste.

Keywords: **Xanthan gum, Brewers' Spent Grain (BSG), value addition, waste management, solid waste**

Introduction

Xanthan gum is a biopolymer obtained as a product via the fermentation of various strains of *Xanthomonas campestris* under optimum reaction parameters. Xanthan gum displays excellent solubility, thermal resilience, pH stability, high viscosity, pseudoplasticity, and cross-linking, which makes it an ideal candidate for various industrial applications, especially in the food and cosmetic industries [1]. A distinguishing feature of xanthan gum contrary to other natural biopolymers is its ability to be used in organic solvents, acids, bases, and even saltwater environments without altering viscosity [2]. Xanthan gum consists of repeating units of D-Glucose as the main chain and two side chains of mannose and glucuronic acid. The mannose side chains are attached to the main chain via alpha-1,2 glycosidic bonds and the other side chain is composed of glucuronic acid units linked to the main chain via beta-1,3 glycosidic bonds. The mannose side chains provide elasticity to xanthan, while the glucuronic acid side chains contribute to its anionic character [1]. A thorough investigation was carried out to assess the production capacity of *X. arboricola, X. axonopodis, X. campestris, X. citri, X. Fragaria, X. gummisudans, X. juglandis, X. phaseoli*, and *X. vasculorium* bacteria [3]. Among these bacterial strains, *X. campestris* demonstrated the highest level of efficiency, achieving an approximate conversion rate of 80% and consequently, it is commonly regarded as the preferred choice for gum production [4]. The industrial fermentation of xanthan involves a cascade of sequential

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steps. The first phase of commercial production of xanthan involves the fermentation of *Xanthomonas* sp. on a suitable substrate along with a carbon source (generally glucose) and supplemented with nitrogen, salts, and trace elements under optimum reaction conditions. After fermentation, the resulting broth contains xanthan gum which is generally recovered by alcoholic precipitation (using ethanol, isopropanol, etc.) due to the generation of reverse charges [1]. However, other methods like enzymatic degradation [5] and ultrafiltration [6] have shown similar results over the years.

However, the industrial carbon source used in xanthan fermentation is very expensive and consequently increases the production costs and results in the end product being expensive [7, 8]. This calls for an economical alternative carbon source/substrate. Various researchers have experimented on different substrates, including industrial wastes, agricultural wastes, and lignocellulosic substrates over the years to reduce the manufacturing expenses of Xanthan. A maximum Xanthan yield of 10.03 g/l was obtained using Confectionery Wastewater as fermentation media enriched with sucrose and yeast extract in a 2:1 ratio [9]. Similar results were obtained using Rose Wine and White Wine effluents from Wine Industry as an alternative substrate for xanthan production [10]. Date palm juice (Phoenix dactylifera) by-products have also been used for xanthan fermentation utilizing *X. campestris* NRRL B-1459 with the goal of lowering the product's cost [11].

The main objective of this study was to evaluate the ability of Brewer's Spent Grain (BSG) to serve as an economical, eco-friendly carbon source for xanthan fermentation. In 2021, global beer production was approximately 1.86 billion hectoliters (Conway J. 2023. Global beer production 1998–2022. Available from https://www.statista.com/statistics/270275/worldwide-beer-production/. Accessed 7th September, 2023) which provides a peek into the global production of BSG. About 20 kg of BSG is produced per hectoliter of brewed beer [12]. Based on this data, America (North and South), Asia, and Europe collaboratively produced approximately 33.34 million tonnes of BSG in 2021 [13]. The problem of BSG generated as a solid waste from brewery is huge. Earlier there have been attempts to solve the enormity of BSG waste; BSG is mostly used as feed for cattle and poultry due to its high fiber and protein content and can be potentially utilized as a cheap, eco-friendly alternative substrate for the production of a wide range of products through fermentation. In addition, BSG is a potential source of prebiotics that can optimize the balance in the gut microflora of cattle, poultry, etc. when BSG is added to the feed [13]. Some of the biggest barriers to the wider application of BSG include the price of transporting BSG, the drying process, and the requirement of a pretreatment procedure that decreases its resistance [14]. BSG is also employed in the food sector to manufacture various food products such as bread, cookies, muffins, pasta, cereal bars, chips, and yogurt [15–21]. Supplementing BSG to wheat flour cookies resulted in a significant increase in the Total Dietary Fibre content in the cookies [21]. In a study, it was found that dried form of BSG in combination with wheat semolina was a suitable extrusion raw material [22]. BSG has been also used as a substrate for the production of different enzymes like xylanase by different microorganisms via solid-state fermentation using different microorganisms - *Penicillium janczewskii*, *Mucor* sp., *P. brasilianum, Aspergillus niger*, etc. [23–26]. BSG hydrolysate has also been used for Lactic Acid fermentation using *Lactobacillus rhamnosus* as a carbon and nitrogen source [27]. Biovalorised BSG has been used to produce laccase and polyphenols via solid state fermentation using *Trametes versicolor* [28].

BSG is a lignocellulosic solid phase by-product during the production of alcohol and is generally composed of hemicellulose, cellulose, lignin, proteins, and polysaccharides [29]. BSG is rich in protein but its concentration varies and typically present at levels of ~20% per dry weight basis [30]. However, the concentrations of the phenolic compounds and lignocellulosic components in BSG vary depending on the end-product produced e.g., light malt, dark malt, beer, etc. BSG also contains phenolic compounds that include - ferulic acid, p-coumaric acid, catechin, 4-hydroxybenzoic acid, sinapic acid, syringic acid, protocatechuic acid, and caffeic acid [31, 32].

In a previous study, the potential of Spent Grain Liquor to produce xanthan was evaluated [33]. They enhanced the SGL with specific salts and added maltose. However, the current study did not enhance the Brewer's Spent Grain (BSG) based media with external inputs. The maximum xanthan yield achieved was 18.9 g/l after 120 h of incubation [33]. In view of the past

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use of BSG as a carbon source in fermentative production of biomolecules, the present authors designed a study on utility of BSG (obtained from CMJ Breweries, Byrminghat, Meghalaya) as a starting material for fermentative xanthan production. The aim of this study is to recycle the solid waste of brewery to an important value-added product, xanthan. The objective of this research was to employ BSG as a fermentation medium of *X. campestris* to produce xanthan gum. The increase in the yield of xanthan was also studied by varying the fermentation parameters.

Materials and Methods

Media and bacterial strain

A pure bacterial culture of *X. campestris* NCIM 2961 was sourced from the National Collection of Industrial Microorganisms (NCIM), Pune, and used for all the experiments throughout this study. MGYP medium (Malt extract = 0.3 g/l, Glucose = 1 g/l, Yeast extract = 0.3 g/l, and Peptone = 0.5 g/l in 100 ml of distilled water) was used as the culture media. The bacterial culture was subcultured at regular intervals in MGYP agar plates (2% agar-agar) to avoid culture degradation and maintain consistency in the experiments.

Liquid media culture

Standard MGYP media. 100 ml of MGYP liquid medium was prepared in distilled water to serve as the standard media. The pH of the prepared media was adjusted to 6.8 using 1N HCl and 1M NaOH solutions. The media was then divided equally into two parts (50 ml each) and added to two Erlenmeyer flasks and autoclaved prior to inoculation and subsequent fermentation.

Alternative BSG-based media. For this study, an aqueous extract was prepared from BSG. 50 g of BSG was grounded in 200 ml of distilled water in a mixer-grinder (Inalsa) and then filtered through a muslin cloth to obtain the extract. The reducing sugar concentration was determined in the BSG extract by the DNSA method. The extract was then adjusted to a pH of 6.8 using 1N HCl and 1M NaOH solutions and then autoclaved at 121℃ and 15 psi for 15 min prior to inoculation. For experiments on variation of pH, media was adjusted to the suitable pH.

Fermentation

Fermentation was initiated by introducing a loopful of the original culture into the sterile standard and BSG-based media. Prior to inoculation, the media were divided equally into 50 ml aliquots and transferred to 250 ml Erlenmeyer flasks which were specifically selected for their headspace to allow optimal gaseous exchange during fermentation.

Optimization of fermentation parameters

To study the influence of pH, temperature, agitation, and incubation time, these parameters were varied at different levels and optimized in different experiments for each parameter (pH - 6, 6.8, and 7; Temperature - 28℃, 30℃, and 32℃; Agitation - 100, 150, and 200 rpm; Incubation - 24 h, 48 h, and 72 h) to determine the best fermentation conditions for xanthan production. Each of the experiments were performed in triplicates to avoid any errors. The aqueous extract of BSG was divided into 50 ml aliquots and the pH was adjusted to different levels (pH = 6.0, 6.8, and 7.0). The media was then inoculated and incubated for 24 h at 32℃, and 100 rpm agitation. The best pH was determined based on the highest biopolymer yield. Next, the temperature for fermentation of BSG extract was varied at three different levels 28℃, 30℃, and 32℃'keeping the pH same to observe the temperature for maximal xanthan production from BSG. Agitation was varied from 50 rpm to 200 rpm at three levels (50 rpm, 100 rpm, and 200 rpm) to maximize the mass transfer in the media at 32℃, pH = 6.8, and incubation time of 24 h. The incubation time for fermentation of BSG extract was varied at three different levels (24 h, 48 h, and 72 h) to observe the maximal xanthan yield at 32℃, 100 rpm, and pH = 6.8. The results described in the study are depicted as the average of the individual values and the computations were performed and the data was represented graphically using Labplot 2.0.

Xanthan gum recovery

After incubation, the biomass was separated from the fermentation broth by centrifuging the fermentation broth at 492 ×*g* for 2 min in 100-ml polypropylene centrifuge tubes using a cooling centrifuge (Remi C-24BL). The precipitate was discarded, and the supernatant was added to double the volume of Isopropyl Alcohol (IPA)

and chilled in the refrigerator for 1 h and then centrifuged at 12298 ×*g* for 10 min to separate out the biopolymer from the fermentation broth. After centrifugation, the supernatant was discarded and the pellet was dried in a hot air oven at 70℃ overnight. The dried xanthan gum was then weighed in an analytical weighing balance (Mettler Toledo) and the weight was recorded.

Characterization of xanthan gum using FTIR

Samples of commercial food-grade xanthan gum (Urban Platter Professional Xanthan Gum Powder, India) and xanthan gum obtained from BSG were powdered and subjected to FTIR at Guwahati Biotech Park Incubation Centre (GBPIC) for spectral analysis. The FTIR was performed on K-Br pellets of the samples using Thermo Nicolet iS10 FTIR Spectrometer (Thermo Scientific, USA).

Results

Estimation of reducing sugars by DNSA method

A standard curve correlating absorbance with glucose concentration was used to estimate the levels of reducing sugar in the BSG extract. The concentration of reducing sugars in BSG was estimated to be 3 g/l after analysis.

Observation of parameters

pH variation. Experiments using *X. campestris* NCIM 2961 revealed the maximal yield in standard MGYP media at 32℃, 100 rpm agitation to be at pH = 6.8. However, the BSG-based media requires pH optimization for which the pH was varied at three different levels (6.8, 6.0, and 7.0) to assess the ideal pH as shown in Fig. 1. In addition, the standard MGYP media fermentations were also carried out at different pH levels to compare with that of the alternative medium. The maximum xanthan yield obtained from the BSG media and MGYP media were 2.67 g/l and 2.11 g/l at pH = 6.0 and pH = 6.8 respectively at 32℃, agitation at 100 rpm after 24 h of incubation which indicates a 1.26-fold increase in xanthan yield from the BSG media relative to the control MGYP media.

Temperature variation. The temperature for fermentation of BSG and standard MGYP media was varied in three levels to optimize the parameter for the highest xanthan yield. The average yield of xanthan was calculated at different temperatures and error bars were added on the basis of the standard deviation. The maxi-

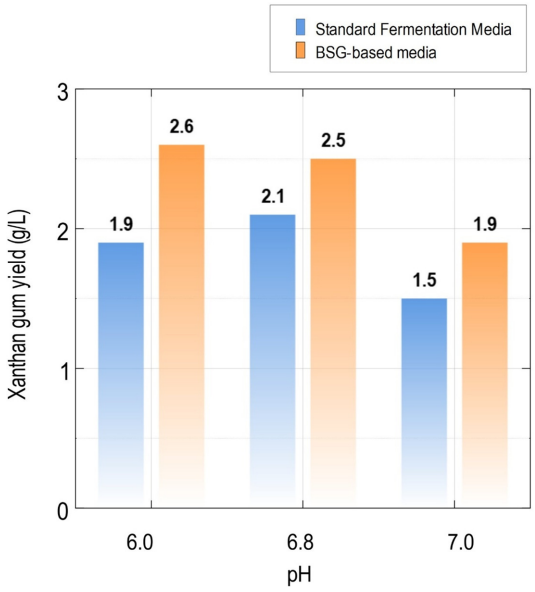

Fig. 1. Comparison of xanthan yield from standard MGYP media and BSG-based media at different pH (6.0, 6.8, and 7.0) at 32℃, 100 rpm agitation, and 24 h of incubation.

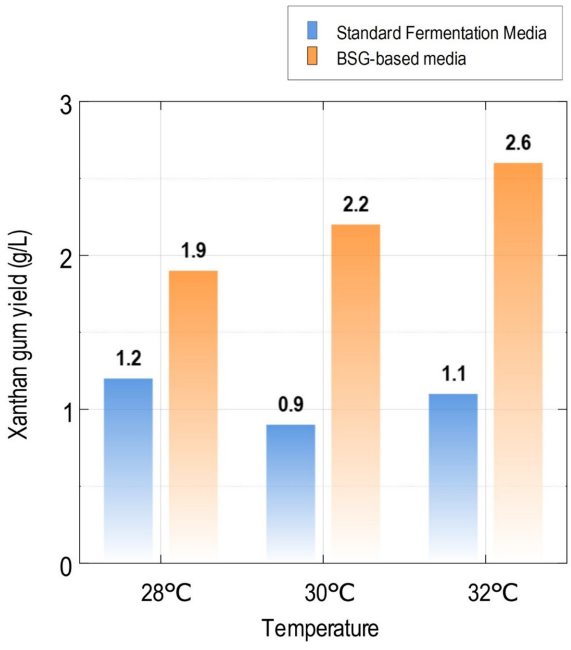

Fig. 2. Comparison of xanthan yield from standard MGYP media (pH 6.8) and BSG-based media (pH 6.0) at different temperatures (28℃, 30℃, and 32℃) at 100 rpm agitation, and 24 h of incubation.

mum yield of xanthan gum from BSG (2.67 g/l) was observed at 32℃, however, the maximum xanthan yield from the fermentation of standard MGYP media (1.26 g/l) was observed at 28℃ which is the standard temperature for industrial xanthan production from MGYP media. Xanthan yield increased steadily with the increase in temperature from 28℃ to 32℃ at pH = 6.8 BSG media, agitation of 100 rpm, and 24 h of incubation (Fig. 2). The xanthan gum yield was maximum (2.67 g/l) at 32℃ from BSG fermentation but the greatest xanthan yield from standard MGYP (1.26 g/l) was observed at 28℃. The xanthan gum obtained from the BSG media showed approximately a twofold increase in its yield compared to that obtained from the standard MGYP media.

Variation of agitation. Agitation during the fermentation of BSG was varied at three different rates - 50 rpm, 100 rpm, and 200 rpm to optimize the condition for the greatest xanthan yield from BSG. As earlier, the average xanthan yield was calculated at varying agitation and error bars were added on the basis of the standard deviation. It is evident from Fig. 3, that the greatest xanthan yield was observed at an agitation rate of 200 rpm at 32℃, pH = 6.0, and an incubation period of 24 h for BSG media. This is also reflected in the case of xanthan production from standard MGYP media under the same conditions. Xanthan yield in both standard and BSG media showed similar yields at 50 rpm and 100 rpm. However, the yield significantly increases and becomes maximal at 200 rpm for BSG and MGYP media. The xanthan obtained from the BSG media (5.34 g/l) at 200 rpm shows a 1.4-fold increased yield compared to the xanthan obtained from MGYP media (3.75 g/l).

Variation of the incubation period. The incubation time period of the fermentation of BSG was varied at three different levels - 24 h, 48 h, and 72 h to optimize the incubation period and obtain the highest xanthan yield. The average xanthan yield was calculated at different incubation periods and the error bars were plotted on the basis of standard deviation. As depicted in Fig. 4, the highest xanthan yield from BSG-based media (5.71 g/l) was observed during fermentation at 72 h. The xanthan yield from standard MGYP media followed the same trend. It was observed from the graph that the xanthan gum yield decreased after 48 h incubation but increased almost two-fold after 72 h of incubation.

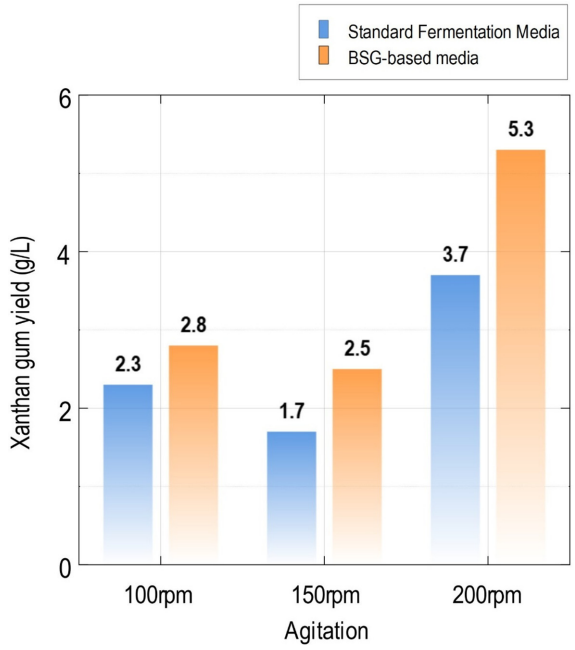

Fig. 3. Comparison of xanthan yield from standard MGYP media (pH 6.8) and BSG-based media (pH 6.0) at different agitations (50, 100, and 200 rpm) at 32℃, and 24 h of incubation.

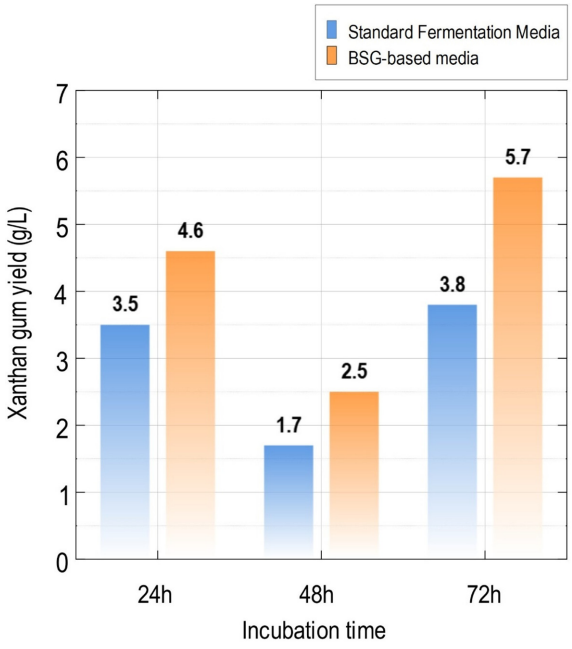

Fig. 4. Comparison of xanthan yield from standard MGYP media and BSG-based media at different incubations (24 h, 48 h, and 72 h) at pH = 6.8, 32℃, and 100 rpm agitation.

Table 1. Optimal reaction parameters for maximum xanthan production obtained from standard and BSG-based media.

Optimal Reaction Parameters	Standard MGYP media	BSG-based media
pH	28 ℃	32 ℃
Temperature	6.8	6.0
Agitation	200 rpm	200 rpm
Incubation period	72 h	72 h

After the completion of all the parameter variation experiments, the best parameters were obtained for the maximum production of xanthan gum from a BSG-based medium. Table 1 depicts the best reaction conditions for the highest end-product yield from MGYP media and BSG-based media.

Analysis of xanthan gum using FTIR

The FTIR spectra of the Xanthan gum were compared with that of commercial food-grade Xanthan gum (Urban Platter Professional Xanthan Gum Powder) to validate the results. There are many peaks in the spectral graph which shows that the analyzed compound is a complex chemical compound. The peaks contain a single bond area (2500–4000 cm^{-1}). No other peaks were found between 3000 and 3200 cm^{-1} indicating the absence of any aromatic groups. No specific peak for aldehyde was found between 2700 and 2800 cm^{-1}. No C triple bond region was found between 2000 to 2500 cm^{-1}. Regarding the double bond region (1500 to 2000 cm^{-1}), a sharp peak was detected at 1632 cm^{-1} that indicates the presence of carbonyl double bonds. In the fingerprint region (600 to 1500 cm^{-1}), multiple strong signals were detected at 1023 cm^{-1}, 1243 cm^{-1}, and 1404 cm^{-1}. Spectral data from FTIR of commercial food-grade xanthan shows close similarity to that for xanthan extracted from the BSG-based medium as shown in Fig. 5. As a result, xanthan from BSG-based media and standard commercial xanthan gum have almost identical spectral characteristics that validate their identity.

Table 2. Yields of xanthan gum obtained at varying reaction parameters (* represents the maximum xanthan yield).

Fermentation Parameters	Variation levels	Standard MGYP media (g/l)	BSG-based media (g/l)
pH	6.0	1.9	2.6*
	6.8	2.1*	2.5
	7.0	1.5	1.9
Temperature	28 ℃	1.2*	1.9
	30 ℃	0.9	2.2
	32 ℃	1.1	2.6*
Agitation	100 rpm	2.3	2.8
	150 rpm	1.7	2.5
	200 rpm	3.7*	5.3*
Incubation period	24 h	3.5	4.6
	48 h	1.7	2.5
	72 h	3.8*	5.7*

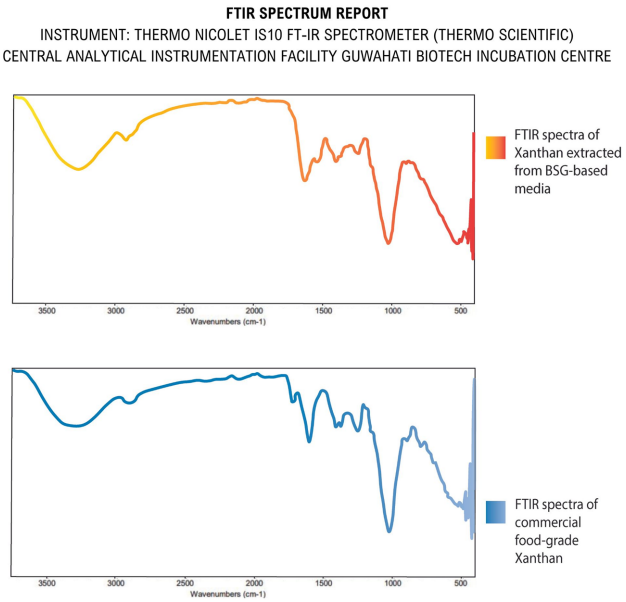

Fig. 5. FTIR spectra of xanthan gum extracted from BSG-based media and Commercial food grade xanthan gum (Urban Platter Professional Xanthan Gum Powder).

Discussion

With industrial expansion, the amount of waste generated also increases, which poses a huge challenge in waste management and generates environmental concerns. However, the usage of industrial waste as an alternative source of fermentation can be a solution to these issues along with the decrease in production costs consequently bringing down the price of the end product.

The results obtained in this study show the possibility of using unmodified BSG from Brewery Industries to produce xanthan gum by *X. campestris* NCIM 2961,

representing a method of reusing industrial wastes in addition to reducing the production costs of biopolymers like xanthan. Fermentation of BSG-based media used in this study produced a higher amount of xanthan gum compared to conventional MGYP media under standard reaction conditions. The spectral data obtained from FTIR characterization of xanthan produced from BSG-based media and food-grade xanthan, show very similar characteristics that establish the purity of xanthan produced from BSG-based media. In addition, the concentration of reducing sugars in BSG was estimated to be 3 g/l. A similar study was conducted by (Dodić *et al*., 2011) to study the potential of Spent Grain Liquor (SGL) to produce xanthan by enhancing the SGL with salts. However, the BSG-based media used in the current study was not enhanced with any external inputs like salts, N, or C additives. The xanthan yield obtained by (Dodić *et al*., 2011) was maximum at 18.9 g/l after 120 h of incubation, and the highest xanthan yield obtained in this study after the optimization of reaction parameters (5.71 g/l) was obtained at pH = 6.8, agitation of 100 rpm and incubation for 72 h. The lower xanthan yield observed in the BSG-based medium compared to the yields obtained by (Dodić *et al*., 2011) from Spent Grain Liquor can be attributed to the absence of any enhancing agent, such as malt extract or Glucose. However, by varying the components of the medium and adjusting the reaction parameters, it is possible to achieve a significant increase in the end-product yield.

The findings in this study confirm the viability of utilizing BSG as a sustainable, environmentally friendly, and cost-effective substitute substrate for aerobic fermentation with *X. campestris* NCIM 2961 at best fermentation conditions. This approach also effectively addresses the issue of waste management associated with BSG in the overall production process of alcoholic beverages. The large amounts of BSG produced annually are mostly used as fodder for cattle and most of it is wasted without utilizing its potential. This study describes an alternate use of BSG as an eco-friendly and cost-effective substrate that can reduce the production costs of xanthan by adding industrial value.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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