POTENTIAL OF CRUDE GLYCEROL UTILIZATION IN BIOTECHNOLOGICAL PRODUCTION OF XANTHAN: A MINI REVIEW

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Abstract

Xanthan is extracellular secondary metabolite of bacteria from the genus *Xanthomonas* and the most important microbial polysaccharide as well as significant industrial biopolymer. Commercial xanthan is relatively expensive due to usage of glucose or sucrose as carbon sources in the xanthan production media. On the other hand, the production of biodiesel in the world is in the rise. The main effluent generated by the biodiesel industry is crude glycerol. The high costs of crude glycerol purification and degradation of environmental quality caused by its disposal in untreated form are the reasons for the increase in research about alternative methods that can be potentially used to convert crude glycerol into a valuable product. The promising alternative is microbial conversion of crude glycerol. The purpose of this study is to discuss data from available scientific and professional literature related to the possibility of using crude glycerol as a substrate for the production of xanthan.

Keywords: Biodiesel industry, crude glycerol, waste utilization, biotechnological xanthan production

Introduction

Fossil fuels in limited reserves are concentrated in certain regions of the world that are shortening day by day. They are being consumed more rapidly than they are being created so they are considered as non-renewable [1]. The rise in oil prices and the enlarge substitution of liquid fossil fuels with renewable energy sources have led to an increase of biodiesel production worldwide. Biodiesel presents a diesel-equivalent, processed fuel derived from biological sources such as plant and animal oils [2]. There are four primary ways to produce biodiesel but transesterification of animal fats or vegetable oils is the most commonly used method for biodiesel production [3]. The main advantages of biodiesel as a diesel biofuel are its portability, availability, renewability, higher combustion efficiency, lower sulfur and aromatic content, higher biodegradability and environmentally friendliness. Another important advantage of biodiesel lies in the fact that the risks of handling, transporting, and storing biodiesel are much lower than those associated with petro diesel [2]. Considering the environmental concerns about pollution coming from the fossil fuels and all advantages of biodiesel, it is evident why biodiesel has become a developing area of high concern. The world's biodiesel supply grew from 3.9 billion liters in 2005 to 18.1 billion liters in 2010 and is expected to reach 41.4 billion liters in 2025 [4].

Expansion of biodiesel production results in the accumulation of significant amount of effluents such as wastewater used to wash the catalyst from the ester phase, unused catalyst, crude glycerol, methanol, soaps, as well as peptides, lecithin, proteins and phospholipids in small quantities [5, 6]. Considering that crude glycerol is generated in the amount of 10-20% of the total volume of biodiesel produced, it presents the main by-product of the biodiesel industry [7]. The glycerol derived from biodiesel production is impure and requires some form of treatment prior to discharging into environmental receptors. The significant cost of crude glycerol purification that increases the cost of biodiesel production and health risks prevents its use in food and pharmaceutical industries [5]. Thus, new effective and environmentally safe methods for the utilization of crude glycerol are urgently needed.

The composition of crude glycerol depends on several factors, which is why it is difficult to define it precisely. Glycerol generated during biodiesel production is usually about 55-90% purity and contains residues such as unconverted triacylglycerol, methanol, ethanol, biodiesel, soap and other compounds [8]. The results of several studies indicate that glycerol represents an excellent carbon source for numerous bioconversions into value added products such as succinic acid, ethanol, hydrogen, polyhydroxybutyrate polymers, clavulanic acid, citric acid and xanthan [9, 10]. Among all aforementioned value added products, xanthan stands out due to its extraordinary characteristics.

Xanthan is a non-toxic, biocompatible and biodegradable microbial biopolymer. This water soluble heteropolysaccharide is biosynthesized by *Xanthomonas campestris* and by other *Xanthomonas* species on a culture medium of appropriate composition. Xanthan is industrially produced using the reference strain *Xanthomonas campestris* ATCC 13951 under optimal conditions, discontinuously, by submerged aerobic biosynthesis in bioreactors with a volume of 100,000 L [11]. The chemical structure of xanthan is composed of repeated units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.0:2.0:1.0. [9]. Molecular weight of xanthan macromolecules ranges from $2 \cdot 10^6$ Da to $20 \cdot 10^6$ Da and its distribution depends on the association between chains, i.e. formed aggregates of several individual chains [12].

Exceptional rheological characteristics and stability over a wide range of temperature and pH value make xanthan widely used in a broad range of industries, such as food, pharmaceutical, cosmetic, chemical, oil recovery and textile. Xanthan is reported as GRAS by United States drug and food administration on the basis of toxicology tests in human foods. Hence, the most advanced application of xanthan is in food industry as a suspending and thickening agent and in pharmaceutical industry in controlled drug delivery system [13, 14].

Glucose and sucrose have been used for decades as the most suitable carbon sources in cultivation media for xanthan production, but rising prices and increasing demand for these sugars indicate the necessary exploitation of alternative substrates of lower market value. Results from several researches indicate the possibility of using crude glycerol as a raw material for production of xanthan of satisfactory quality [5, 15]. However, the main disadvantage of the application of this significant waste stream in the production of xanthan is reflected in the limited ability of the reference strain to metabolize glycerol as a carbon source [9]. Thus, there is a need for isolation of new strains that exhibit tolerance against crude glycerol impurities and produce xanthan. The purpose of this review is to discuss the possibility of xanthan production on a medium containing crude glycerol by different strains of *Xanthomonas campestris.*

Material

The available scientific publications were used as a primary material for this paper. The collated data were selected, systematized, compared and critically discussed.

Discussion

Despite the fact that development and improvement of xanthan production have been studied during the years, there are a small number of studies based on the biotechnological xanthan production on crude glycerol containing media. One of the first studies focused on the possibility of using crude glycerol for xanthan production is performed by Reis et al. and aimed to investigate the possibility of using glycerol, obtained from biodiesel industry, as substrate for xanthan production by *Xanthomonas* C1 and *Xanthomonas* C9 strains isolated in Brazil [16]. The following carbon sources were used in cultivation medium: only sucrose, only glycerol or a glycerol-sucrose mixture in the ratio of 50:50%. Incubation was performed at 28±2°C with stirring at 180 rpm for 96 h. The obtained results showed that crude glycerol as unique carbon source is not a media adequate for xanthan production in applied experimental conditions. However, when supplemented with sucrose were not detectable significance differences with the medium containing only sucrose. The highest yield of xanthan of 0.0038 g/L·h among the three media was obtained using glycerol with supplementation of the sucrose (25:25, w/w) by the wild-type isolate *Xanthomonas* C1 and C9. The best results of productivity and apparent viscosity were accomplished when the media containing glycerol was used. Therefore, the results obtained in this study suggest that the crude glycerol is an adequate substrate for the production of xanthan.

One more confirmation that *Xanthomonas* isolates can be employed to convert crude glycerol is obtained by Brazlilan team of researchers. Brandão et al. also investigated the possibility of using residual crude glycerol from biodiesel production as an alternative substrate for xanthan biosynthesis by *X. campestris mangiferaeindicae 2103* using a fermentation medium supplemented with 0.01% urea and 0.1% K_2HPO_4 [5]. Batch fermentation was carried out at 250 rpm and 28±2°C for 120 h. The amount of xanthan obtained using crude glycerol as substrate in applied experimental conditions was 7.23 g/L. This value is approximately 70% higher than the production obtained from the conventional substrate sucrose (4.21 g/L) under the same operating conditions. The solutions of obtained xanthan (2.0% w/v) exhibited a pseudoplastic rheological behavior, with a viscosity of up to 642.57 mPa·s at 25°C. The viscosity was 30% higher than the viscosities found for the solution of xanthan obtained from the sucrose media. The xanthan's molecular weight varied from $28.2 \cdot 10^6$ Da to $36.2 \cdot 10^6$ Da. From the obtained results it can be noted that the molecular weight of xanthan produced from glycerol presented a similar value to xanthan obtained from sucrose. The findings of this study indicate that crude glycerol presents great promise as a substrate for the efficient production of xanthan.

Another study focused on the production of xanthan on crude glycerol media was performed in Brazil. de Jesus Asis et al. investigated xanthan production from crude glycerol biodiesel (CGB) by *Xanthomonas campestris mangiferaeindicae* 2103 [17]. The xanthan was produced from CGB in a 4.5 L bioreactor containing production medium consisting of 2.0 % (v/v) CGB, 0.01 % (w/v) $(NH_2)_2CO$, 0.1 % (w/v) KH_2PO_4, and 0.1 % (v/v) antifoam at 28°C for 120 h. The obtained results showed that low agitation speed increases the production of xanthan, biomass concentration, apparent viscosity of xanthan solutions, glucose and pyruvic acid concentrations in the xanthan chain, and molecular mass in applied experimental conditions. The results also showed that decreasing the aeration contributes to increased xanthan production and higher concentrations of glucose and mannose in the polymeric chain. According to the results obtained in this study it can be concluded that the highest content of xanthan of 5.59 g/L was achieved when biosynthesis was performed on medium with crude glycerol at aeration of 0.97 vvm and agitation speed of 497.76 rpm. The findings of this research indicate that the studied crude glycerol have strong potential and promising properties for the efficient and cost-effective production of xanthan.

In the work of Trinidade et al. the possibility of crude glycerol (81.92%) utilization for xanthan biosynthesis by *Xanthomonas campestris* pv. *mangiferaeindicae* IBSBF 1230 was examined [18]. Following the idea of Reis et al. [16], sucrose and crude glycerol were used as single and mixed (1:1) carbon sources in cultivation media. The highest amount of xanthan of 4.98 g/L was obtained when crude glycerol was used as the only carbon source in cultivation media in applied experimental conditions. Similar to the results obtained in research performed by Reis et al. [16], results from this study indicate that the use of sucrose and a mixture of sucrose and crude glycerol did not represent significant differences in xanthan yield. Thus, it was shown that crude glycerol is a potential alternative carbon source for substituting sucrose in the biotechnological production of xanthan.

Research performed in Serbia in 2015 confirmed that commercial glycerol is viable as a carbon source in the applied experimental conditions using the reference strain *Xanthomonas campestris* ATCC 13951 and eight strains isolated from different vegetables grown in Vojvodina for the production of xanthan [15]. The authors suggested that further research should encompass the optimization of the glycerol-based media in order to increase yield and quality of the desired product. This research was great background for the later research conducted in Serbia in 2019 [19] and 2020 [20]. Yield of xanthan of 6.68 g/L was achieved when *Xanthomonas campestris* ATCC 13951 was cultivated for 168 h on crude glycerol containing medium under aerobic conditions. This value is higher comparing to yield achieved in several previous studies [17, 18]. Later, the same authors examined the possibility of xanthan biosynthesis using four *Xanthomonas campestris* strains isolated from different cruciferous plants (CB, CF, 12-2 and Xp3-1) on a crude glycerol-based medium. Xanthan production was carried out in a batch mode under aerobic conditions (an air flow rate of 1 vvm in the first 48h, and 2 vvm afterwards) for 168 h. In the first 48 h, the temperature was 25°C and the agitation rate was 200 rpm, which thereafter increased to 30°C and 300 rpm, respectively. The results obtained in this study indicate that all the strains considered can be used for xanthan production on a crude glycerol-based medium. The values of produced xanthan were in range from 5.22 g/L to 7.67 g/L. The values of xanthan yield obtained in this research are the highest values ever reported when *Xanthomonas* spp. were cultivated on crude glycerol-based media. High values of all the indicators of bioprocess success suggest that *Xanthomonas campestris* Xp 3-1 isolate represents the most appropriate producing strain for xanthan biosynthesis on crude glycerol-based media under the set experimental conditions.

Conclusion

This paper provides valuable information on the possibility of biotechnological xanthan production on a medium containing crude glycerol as a carbon source by different strains of *Xanthomonas campestris*. The results discussed above indicate that crude glycerol has strong potential for the efficient and cost-effective production of xanthan. All the results presented in this study can be used for selecting the producing strain and optimum medium composition for economically justified xanthan production. Besides the significance for industrial process development, studies focused on crude glycerol usage in biotechnological xanthan production are also a precious tool for great social, economic and environmental benefits for society.

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