XANTHAN PRODUCTION ON GLYCEROL-BASED MEDIA: A MINI REVIEW

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Abstract
Xanthan is the most important microbiological polysaccharide. This biopolymer has widespread commercial applications as a viscosity enhancer, emulsifier and stabilizer in the food, pharmaceutical and petrochemical industries. The yield and properties of the produced xanthan are largely dependent on the producing microorganism, medium composition and process parameters. In industrial conditions, a pure bacterial culture is used to produce xanthan on a medium containing glucose or sucrose as a carbon source. However, the increasing market price and demand for these sugars indicate the need for implementation of an alternative raw material. Hence, many studies are focused on finding cheap and available, alternative raw materials, rich in carbon sources, as a replacement for mentioned sugars. Results from several studies indicate that certain strains of bacteria of the genus *Xanthomonas* possess the ability to biosynthesize xanthan on a medium with glycerol as the sole carbon source. The aim of this study is to discuss data from available scientific literature related to the xanthan production on glycerol-based media.

Introduction
Xanthan is non-toxic, biocompatible and biodegradable polysaccharide of microbial origin [1]. There are only a few microbial exopolysaccharides that are commercially available and xanthan is one of the most important [2]. Outstanding rheological properties of xanthan solutions contribute to its wide-range of applications as a suspending, stabilizing, and/or thickening agent in the food industry and its use as an emulsifier, thickening agent for oil recovery [3]. Xanthan is approved by the United States Food and Drug Administration (FDA) for use as a food additive without any specific quantity limitations and European Economic Community approved xanthan under the number E415 as a permitted thickener and stabilizer [4]. Currently leading xanthan manufacturers are Jungbunzlauer (Switzerland), ADM (USA), Cargill (USA), CP Kelco (USA), Deosen Biochemicals (China) and Meihua Group (China) [5]. The demands for xanthan are constantly growing and according to GlobeNewswire it is estimated that xanthan production will reach 1403 million US dollars by 2026.

This extracellular heteropolysaccharide has a primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0 [6]. The structure of xanthan macromolecule is composed of linear chain consisting with 1,4-linked β-D-glucose backbone with two tri-saccharide units pairing the side chain on every glucose unit. The other, side chain is formed by a D-glucuronic acid unit-linked between two D-mannose units [7]. Structural characteristics directly affect xanthan molecular weight. The molecular weight of xanthan is usually from $2 \cdot 10^5$-10^6 Da [8].

Despite the fact that many different strains as *Xanthomonas arboricola*, *Xanthomonas axonopodis*, *Xanthomonas campestris*, *Xanthomonas citri*, *Xanthomonas fragaria*, *Xanthomonas gummisudans*, *Xanthomonas juglandis*, *Xanthomonas phaseoli* and *Xanthomonas vasculorium* have the ability to produce xanthan, *X. campestris* is the most

commonly strain employed for industrial production of xanthan [1]. On industrial scale, production of xanthan is usually performed by aerobic submerged batch cultivation of reference strain *X. campestris* ATCC 13951 on the appropriate medium under optimal conditions [9]. Glucose, sucrose, starch, sugarcane molasses and corn syrup are most commonly used as carbon sources in biotechnological production of xanthan, but the industrial production of xanthan is mainly based on the usage of glucose or sucrose containing media. However, rising prices and increasing demand for these sugars indicate the necessary exploitation of alternative, carbon rich substrates of lower market value that are available in sufficient amount in order to reduce xanthan production costs caused by high price of glucose and sucrose [10].

Recent research related to xanthan production has been focused on the possibility of use of substrates that meet aforementioned criteria, such as sugarcane molasses, whey, glycerol, kitchen waste and olive mill wastewaters [11]. Among examined substrates, glycerol proved to be one of the most promising for xanthan biosynthesis. The aim of this review study is to discuss data from available scientific literature related to the possibility of xanthan production on a glycerol-based media using different *Xanthomonas* strains.

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

Research related to the development of a biotechnological process of the production of xanthan on glycerol-based media are new and still in initial stages, due to impaired metabolic activity of the applied producing microorganism [12]. Only a few studies focused on xanthan production on a medium containing commercial glycerol have been reported.This study is focused on research with commercial glycerol so that it could potentially be replaced by cheaper raw material, crude glycerol, the main by-product of the biodiesel industry. As the demand and production of biodiesel grow exponentially, the utilization of the glycerol becomes an urgent topic [13].

First study focused on the xanthan production on glycerol-based media is the study of Serbian research team where the effect of different initial glycerol concentrations in the medium on the success of biopolymer biosynthesis by *X. campestris* ATCC 13951 was examined [14]. In this study, commercial glycerol in concentrations of 10.00 g/L, 20.00 g/L, 30.00 g/L, 40.00 g/L, 50.0 g/L and 60.00 g/L was added to the production media. The biosynthesis was carried out in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30^{o}C and agitation rate of 150 rpm for 168 h. The results obtained in applied experimental conditions suggest that xanthan production by cultivation of reference strain on all six media was successful. The values of xanthan concentration in media at the end of bioprocess indicate that the biosynthesis of the desired biopolymer increased with the increase in the initial glycerol concentration in the medium. The highest xanthan concentration of 15.81 g/L was obtained in the media with a glycerol content of 60.00 g/L and the lowest xanthan concentration of 9.82 g/L was obtained in the media with a glycerol content of 10.00 g/L. The average molecular weights of the xanthan biosynthesized in this research ranged from $2.64 \cdot 10^5$ Da to $3.17 \cdot 10^5$ Da. According to the obtained results there is no significant difference in the quality of the produced xanthan and it is concluded that different initial glycerol concentrations in the cultivation medium do not significantly affect the quality of the biopolymer under the applied experimental conditions. The results obtained in this study

were the basis for development of xanthan production on glycerol-based media in order to increase the xanthan yield and its quality.

After the confirmation that xanthan production on medium containing commercial glycerol as a sole carbon source by reference strain *X. campestris* ATCC 13951 is possible, previously mentioned researchers conducted another investigation to compare the producibility of *X. campestris* strains isolated from the environment with the reference strain [15]. In this research, xanthan biosynthesis was carried out by cultivation of reference strain and eight strains isolated from the infected leaves of several different cruciferous plants, such as cabbage (I2, I4, I6, I8), kale (I1, I7) and cauliflower (I3, I5) on glycerol-based medium in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 120 h. The obtained results show that the xanthan production in applied experimental conditions was influenced by the used strain. Xanthan concentration in media at the end of performed bioprocesses was in the range from 1.68 g/L to 7.24 g/L, while the highest values of this parameter was obtained when reference strain was used. The findings of this study indicate that glycerol is appropriate carbon source for xanthan biosynthesis in the applied experimental conditions by all tested *Xanthomonas* strains. The authors concluded that further research should be focused on improvement of production process in order to increase the bioprocess efficiency.

Results from research performed in China indicate that xanthan can be produced by a mutant strain *X. campestris* CCTCC M2015714 on medium with glycerol as the sole carbon source [16]. In this study, xanthan was produced on medium with 40 g/L commercial glycerol. The bioprocess was performed in a 7 L bioreactor (4 L working volume) under aerobic conditions at temperature of 30°C and agitation rate of 400 rpm for 84 h. Characterization of xanthan biosynthesized in applied experimental conditions through FT-IR and NMR resulted in conclusion that chemical structure of produced xanthan is similar to that of the commercial xanthan. The molecular weight of xanthan produced by mutant strain *X. campestris* CCTCC M2015714 on glycerol-based medium was $3.0 \pm 0.14 \cdot 10^6$ Da. The results obtained in this study suggest that xanthan produced in applied experimental conditions could be used as dietary fiber potentially in food industry because of the low viscosity.

Optimization of a glycerol-based medium for xanthan production by *X. campestris* ATCC 13951 was performed in 2020 in Serbia [17]. The xanthan biosynthesis was carried out simultaneously in 300 mL Erlenmeyer flasks (100 mL working volume) in batch mode under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. Glycerol content in cultivation medium was varied from 15 g/L to 45 g/L. Response surface methodology was used for the optimization of this bioprocess. The results of optimization obtained in this research suggest that the maximal xanthan concentration from 10.71 g/L to 11.25 g/L can be achieved when biosynthesis is performed by cultivation of producing strain in media containing glycerol in concentration of 32.96 g/L. The average molecular weight of the xanthan biosynthesized in applied experimental conditions is in range from $3.28 \cdot 10^5$ Da to $3.32 \cdot 10^5$ Da. According to the authors, commercial glycerol represents an appropriate replacement for traditionally used carbon sources responsible for quantity and quality of the biopolymer. Additionally, it is proven that commercial glycerol can be substituted with crude glycerol generated by a biodiesel industry, as a cheap alternative substrate, but further research is necessary in order to determine the optimal process parameters for xanthan biosynthesis and to examine the success of application of novel producing strains.

Recent study focused on the possibility of using glycerol as substrate for xanthan production by different *Xanthomonas* strains was performed also in Serbia [18]. The reference strain *X. campestris* ATCC 13951, eight *Xanthomonas* strains isolated from crucifers and five *Xanthomonas* strains isolated from pepper leaves were used as producing microorganisms in this research. Xanthan production was carried out in 300 mL Erlenmeyer flasks (100 mL working volume) under aerobic conditions at temperature of 30°C and agitation rate of 150 rpm for 168 h. The obtained results indicate that xanthan production in applied experimental conditions was possible by all examined strains. According to the determined biopolymer quantity, xanthan concentration varied from 5 g/L to 7 g/L when biosynthesis was performed by *Xanthomonas* strains isolated from crucifers and from 8 g/L to 10 g/L when *Xanthomonas* strains isolated from pepper leaves were used. This indicates that glycerol potentially may be suitable carbon source for industrial xanthan production by *Xanthomonas* strains isolated from pepper leaves. On the other side, the molecular weight of xanthan produced in applied experimental conditions varied from $2 \cdot 10^5$ Da to $5 \cdot 10^5$ Da when *Xanthomonas* strains isolated from crucifers was used and from $5 \cdot 10^5$ Da to $8 \cdot 10^5$ Da when *Xanthomonas* strains isolated from pepper leaves was producing microorganisms. The obtained results suggest that glycerol is suitable carbon source not only for the xanthan production in large quantities but also for the biosynthesis of good-quality biopolymer. It is concluded that *Xanthomonas* strains isolated from pepper leaves have the greatest potential for application in biotechnological production of xanthan on glycerol-based medium.

Conclusion

This paper provides valuable information about the possibility of biotechnological xanthan production on a glycerol-based medium by different *Xanthomonas* strains. According to the results discussed above, glycerol has great potential for the efficient production of xanthan, but in order to achieve successful xanthan biosynthesis it is necessary to select appropriate *Xanthomonas* strains. Data presented in this review study can be useful for the selection of producing strain, formulation of medium composition and optimization of process conditions for successful xanthan production on glycerol-based medium. Results presented in this research may be also a suitable background for future investigations and development of the economically justified production of xanthan on glycerol-based medium.

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