Dependence of the xanthan exopolysaccharide synthesis on the composition of the nutritional medium

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Abstract. The possibility of changing the composition of the feed by replacing glucose with equivalent amounts of sucrose, starch, maltose was investigated. It was found that in the case of using sucrose, the kinematic viscosity of the culture liquid increased by more than 3 times. The replacement of ammonium sulphate in the composition of the nutrient medium with glycine, histidine and asparagine did not stimulate the growth of the producer and its synthesis of exopolysaccharide. At the same time, the replacement of citrate with fumarate made it possible to increase the concentration of biomass by 3 times and the kinematic viscosity of the samples by 8.5 times compared with the control. Based on the data obtained, it was proposed to optimize the composition of the nutrient medium by replacing citrate with fumarate and introducing sucrose-based feed instead of glucose. The study of the structural-dynamic state of EPS samples obtained during cultivation on an optimized nutrient medium using NMR relaxometry made it possible to detect a change in the xanthan structure.

1 Introduction

Polysaccharides have recently attracted the attention of many researchers. They are often used as thickeners, stabilizers, gelling agents in various fields of human activity. The most widely used among them are exopolysaccharides (EPS) [1, 2]. Traditionally, the main consumer of EPS was the oil industry. Hydrocolloids are widely used in the food industry. They are also applied in the textile and leather industries. Many environmental projects also involve the use of naturally occurring polysaccharides [3]. Recently, EPS are considered as promising components in the technology of pharmaceuticals production [4, 5]. The spectrum of promising polysaccharides is constantly expanding. Research is being actively conducted on polysaccharides of plant origin. However, polysaccharides of microbial origin have a number of advantages. They actively grow, accumulating large amounts of exopolysaccharides. Changes in environmental conditions do not affect their development.

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Among microbial polysaccharides, xanthan occupies a special place, which is actively used in industry due to its unique rheological properties. Xanthan is a by-product of the metabolism of the bacteria *Xanthomonas campestris*. It is known that cultivation conditions have a significant effect on the growth of the producer and its synthesis of EPS [6]. In this regard, the aim of the work was to find a correlation between the composition of the nutrient medium for cultivation of *Xanthomonas campestris* and the processes of accumulation of microbial biomass and xanthan, as well as the structure of the resulting polysaccharide.

2 Materials and methods

Xanthomonas campestris VKM B-611, a producer of xanthan exopolysaccharide, was used as the object of study. The producer was grown under fed-batch rocking chair culture conditions. During the entire cultivation process, the temperature was maintained at 24-26°C. First, the producer was grown on a nutrient medium in order to achieve high concentrations of biomass, using a regulated nutrient medium of the following composition (g/l): glucose - 50, K_2HPO_4 - 5.0, $(NH4)_2SO_4$ - 3.0, citric acid - 2.0, H_3BO_3 – 0.006, ZnO – 0.006, $FeCl_3 \cdot 6H_2O$ – 0.0024, $CaCO_3$ – 0.02 [3]. The cultivation process was carried out for 72 hours. Then a feed was introduced and the process continued for another 2 hours. The feed was a 5% (vol.) solution of carbohydrates. In the control variant, the feed included a glucose solution. In experimental media, glucose was replaced by maltose, sucrose, or starch. The introduction of feeding ensured the presence (accessibility) of an organic substrate for the synthesis of xanthan. During and after the cultivation, the microbiological purity was controlled by microscopy. At the end of the fermentation, the biomass concentration and kinematic viscosity were analyzed. The biomass concentration was determined by the nephelometric method. The kinematic viscosity of the culture liquid was analyzed using a VPZh-2 capillary viscometer. The standard package of statistical programs of the Microsoft Office Excel editor was used for statistical processing of experimental data. The significance of the difference in the mean values of a number of repetitions was established by the value of the Student's criterion with a significance level of 0.05, adopted in biological studies.

3 Results and discussion

Tables 1 and 2 present the results of the effect of changing the composition of the feed on the growth of *Xanthomonas campestris VKM B-611* and the release of xanthan. One of the carbohydrates (glucose, sucrose, maltose or starch) was introduced into the composition of the feed. The concentration of carbohydrates in all experiments was 5 vol%.

As can be seen from the presented data, all biomass concentration and viscosity values are statistically significant. The change in the composition of the feed had a diverse effect on the growth of biomass. In the presence of maltose, growth is even slightly inhibited compared to glucose. The largest increase in biomass (21%) of all the studied options is observed when sucrose is added. Feeding with sucrose also stimulated an increase in the viscosity of the culture liquid by a factor of 3 compared with glucose. It should be noted that close values of kinematic viscosity were also recorded when feeding with starch. However, it should be borne in mind that starch solutions also have an increased viscosity. Thus, the viscosity of a 5% starch solution is 1.2 times higher than the viscosity of a sucrose solution of the same concentration. Attention is drawn to the fact that there is a correlation between the increase in biomass and the kinematic viscosity of the culture liquid, however, the absolute values of the growth of these parameters are different. Thus, when sucrose was added, the biomass concentration increased by 1.21 times, while the viscosity increased by 3 times. Obviously, this is due to a change in the molecular structure of the produced EPS. Comparison of the data presented in Table 1 allows us to conclude that it is necessary to use sucrose (or starch) as part of the feed, which makes it possible to achieve better results in terms of the concentration of the biomass of *X. campestris* and the synthesis of EPS. Therefore, all subsequent experiments were carried out using these carbohydrates.

Table 1. Influence of the carbohydrate composition of the feed on the growth of X. campestris culture and the kinematic viscosity of the culture medium.

Nutrient medium	Composition of the feed	Biomass concentration, units of opt. density	Confidence level	Kinematic viscosity, ml^2/s	Confidence level
Synthetic medium with glucose	Glucose	18.15±0.1	-	4.91±0.04	-
	Sucrose	22.1±0.3	0.020	15.10±0.05	8.44E-08
	Starch	19. 6±1.2	0.003	4.32±0.07	2.1E-07
	Maltose	17.5±0.2	0.037	5.35±0.01	0.02

The data available in the literature on this issue are contradictory. Some authors consider glucose as the optimal source of carbon in the nutrient medium for cultivation of *X. campestris* [6]. Others believe that the maximum yield of the polysaccharide is possible on a medium containing sucrose [7]. Perhaps this is due to the species specificity of the producer. In addition, the ratio of carbon and nitrogen in the nutrient medium, as well as the nitrogen source itself, is of great importance in the synthesis of a polysaccharide.

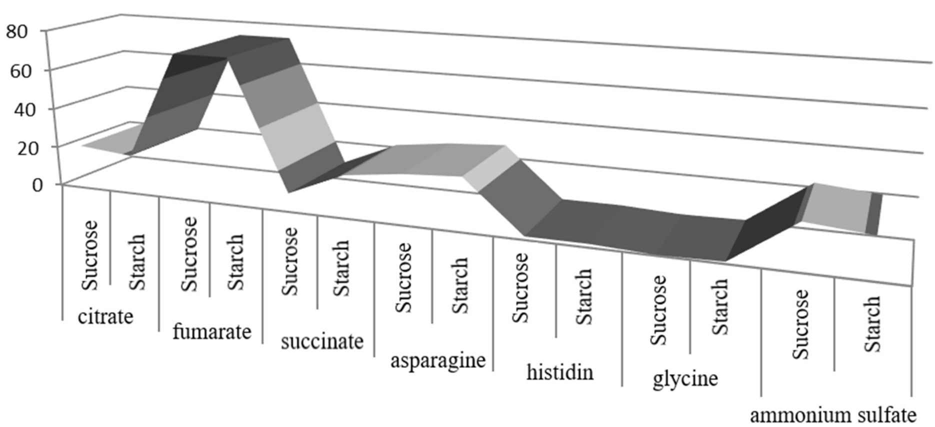

Fig. 1. Influence of organic acids and sources of nitrogen on the growth of *X. campestris* culture.

Both inorganic and organic compounds can serve as a source of nitrogen nutrition for the Xanthomonas campestris. Ammonium sulphate is included in the standard bacterial culture medium. We have studied the effect of amino acids as a nitrogen source. The following amino acids were used as nitrogen sources: asparagine, histidine, glycine (ammonium sulphate in the nutrient medium was replaced by the corresponding amino acid). For the adequacy of comparison of the results, the amount of added acids was determined by converting nitrogen equivalents to the content of ammonium sulfate. As can be seen from Figure 1, the largest increase in biomass (measured in units of optical density) compared to the control is observed when using asparagine, while the introduction of starch as part of the feed turned out to be more preferred. In this case, the increase in biomass concentration was 72%. However, the

https://doi.org/10.1051/e3sconf/202339002023

increase in the viscosity of the resulting solutions was only 19%. The use of other organic nitrogen sources even led to a decrease in kinematic viscosity (Figure 2).

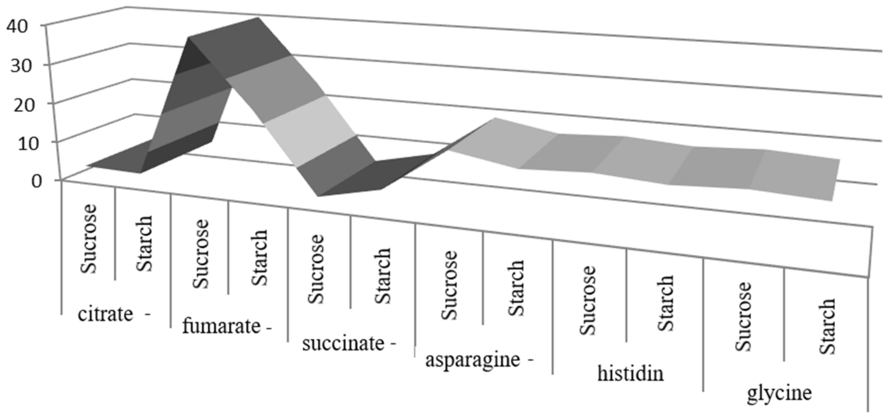

Fig. 2. Effect of organic acids and nitrogen nutrition sources on the kinematic viscosity of culture liquid of *Xanthomonas campestris/*

At the same time, there are data in the literature on the need for the presence of organic acids in the nutrient medium [6]. The composition of the standard regulated environment includes citric acid. We have replaced citric acid with an equivalent amount of fumaric and succinic acids. Data on the effect of various organic acids on the growth of the producer are presented in Figure 1. Analysis of the data shows that the most significant differences are observed when using fumaric acid, regardless of the type of feed (sucrose or starch). In this case, the biomass concentration increased by more than 3 times. In this variant, the maximum kinematic viscosity was also recorded. The use of sucrose as a feed on the medium with fumarate led to an increase in the kinematic viscosity of the post-fermentation medium by 8.5 times (Figure 2). A putative mechanism for stimulating xanthan formation in the presence of fumaric acid is discussed in [8]. Thus, it can be concluded that the standard nutrient medium in its composition is not optimal in terms of the source of organic acids and carbohydrate feed. The optimal nutrient medium for xanthan production should contain ammonium sulphate as a source of nitrogen nutrition, fumaric acid, and the feed should be based on sucrose instead of glucose.

The differences found in the values of kinematic viscosity when using nutrient media of different composition can be associated not only with a change in the amount of synthesized EPS, but also with a variation in its composition. A number of authors believe that changing the cultivation conditions lead to a variation in the composition of xanthan, which is manifested in its properties [9, 10]. Therefore, we subjected the obtained culture fluid samples to NMR relaxometric analysis. As a control, we used samples obtained by cultivating *Xanthomonas campestris* on a standard medium with glucose feeding. Prototypes were obtained by growing *Xanthomonas campestris* on a modified nutrient medium with citrate replaced by an equivalent amount of fumarate and fed with sucrose. The data are presented in Table 2.

Table 2. NMR relaxation parameters of xanthan EPS samples.

Sample	T21, ms	T22, µs	T23*, µs	P21, %	P22, %	P23, %
Experiment	19	37	16	2.5	23.5	74
Control	43	39	19.5	0.5	29.5	70

* due to the non-exponential form of the initial section of the SSI time T23 was determined by the change in the signal amplitude in e times

 https://doi.org/10.1051/e3sconf/202339002023

It follows from the data in the table that the presented samples of xanthans differ noticeably in molecular mobility (the relaxation times T2i serve as a criterion) and the proportion of structural-dynamic fragments corresponding to this mobility in the sample. The existence of the most mobile third phase can be explained by the peculiarities of the structure and structure of polymer macromolecules, associated with the presence of a large free space around the terminal fragments of macromolecules, which gives greater freedom of movement in the volume of the reaction medium. As can be seen from the NMR data in Table 2, the characteristics of this phase differ little from each other. NMR phases with shorter transverse relaxation times (T22 and T23) characterize the higher molecular components of the hydrocolloid. In this case, the intermediate relaxation times (T22) and the corresponding populations (P22) characterize the more mobile and (or) less densely packed fragments of the high-molecular phase, and the shortest relaxation times (T23) and the corresponding populations (P23) describe the behavior of the least mobile (more only in structure, but also in mobility)) of high-molecular fragments.

It is known that xanthan exopolymer has a rather complex structure [6]. The basis of the polymer is a polymer chain formed by D-glucose residues connected by α-(1→4) glycosidic bonds. Unlike cellulose in the xanthan molecule, the main polymer chain has side branches containing other carbohydrates. The side chains are built from residues of glucose, mannose, glucuronic acid, as well as pyruvic and acetyl groups. The viscosity of xanthan solutions largely depends on the amount of pyruvic groups. Their number can vary significantly depending on the cultivation conditions. Correlation of the obtained experimental results of NMR relaxometric analysis with the data on the structure of xanthan allows us to suggest that the cultivation of a microbial producer on optimal nutrient media leads to a change in the structural-dynamic nature of the formation of xanthan. In this case, the proportion of more regular and compactly packed (higher molecular weight) structures in the composition of EPS increases. The obtained data on NMR relaxometry correlate with the results of determining the kinematic viscosity of the studied hydrocolloid solutions.

4 Conclusion

The composition of the nutrient medium for cultivating the xanthan producer *Xanthomonas campestris* is essential for both biomass growth and EPS synthesis. No correlation was found between the accumulation of biomass and the release of xanthan when changing the substrates in the composition of the nutrient medium. The use of substrates that allow increasing the concentration of biomass does not affect or affects to a small extent the change in the kinematic viscosity of the culture liquid, due to the accumulation of xanthan. The composition of the regulated standard nutrient medium for cultivating the xanthan producer is not optimal. To increase the viscosity of post-fermentation solutions, it is advisable to prepare a nutrient medium based not on citric, but on fumaric acid, and to replace glucose with sucrose as part of the feed.

Optimization of the composition of the nutrient medium leads to a change in the structural-dynamic construction of microbial polymer molecules due to an increase in the proportion of high-molecular more densely packed structures, which leads to an increase in the viscosity of EPS solutions.

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