BIOSYNTHESIS OF XANTHAN GUM ON WASTEWATER FROM CONFECTIONARY INDUSTRY

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ABSTRACT

Xanthan gum is one of the major commercial biopolymers employed in many industrial processes owing to its unique physical properties such as a high degree of pseudoplasticity and high viscosity even at low concentrations. Commercially available xanthan gum is relatively expensive due to glucose or sucrose being used as the sole carbon source for its production and cost reduction could be achieved by using less expensive substrates, such as food industrial wastewaters. Effluents from the confectionery industry, because of its high organic content, are significant environmental pollutants and before their release into environment it is necessary to purify them. The present study examines xanthan production by *Xanthomonas campestris* under aerobic conditions on wastewaters from five different factories of the confectionery industry. Xanthan yield was obtained as a quantitative characteristic of the process and was in the range between 4.28 g/L and 10.03 g/L and its quality is determined by following rheological characteristics of obtained cultivation media. The results obtained in this study indicate that wastewater from confectionary industry can be used as the basis of media for the production of this highly valuable product.

Keywords: xanthan, wastewater treatment, biosynthesis, sucrose

1. INTRODUCTION

Xanthan gum is an extracellular heteropolysaccharide discovered in the late 1950s and is the first biopolymer produced industrially. It is synthesized by gram-negative phytopathogen bacterium X*anthomonas. campestris* and represent an attractive alternative for the replacement of traditional gums obtained from plants and marine algae (Jeeva et al., 2011). Xanthan solutions are highly viscous even at low concentrations and display a pseudoplastic, or shear thinning behavior, stability and compatibility with most metallic salts, excellent solubility and stability in acidic and alkaline solutions and resistance to degradation at elevated temperatures and various pH levels (García-Ochoa et al., 2000; Faria et al., 2011). It is a widely used biopolymer in the food and pharmaceutical industries, as well as petroleum production, pipeline cleaning, enhanced oil recovery etc. (Sutherland, 2001).The cost involvement with the fermentation media represents a critical aspect for the commercial production of xanthan due to glucose or sucrose being used as the sole carbon source (Mudoi et al, 2013).

Confectionery industry generates high amounts of wastewater which contains high concentrations of readily biodegradable organic materials characterized with high COD and BOD (Ersahin et al., 2011). The main characteristic of xanthan biosynthesis is non-specificity of carbohydrate substrate, which is why wastewaters from the food processing industry can be used as the basis of media for this bioprocess. In this way, environmental pollution is reduced, as well as cost of xanthan production.

The aim of this study was to examine the possibility of xanthan production with *Xanthomonas campestris* by conversion of organic compounds from confectionery industry wastewaters obtained from different parts of the production processes of five different factories on the territory of Vojvodina.

2. MATERIALS AND METHODS

2.1. Materials

Production microorganism
As a producing microorganism the reisolate of a referent culture Xanthomonas campestris ATCC 13951, labeled as A-1, was used for experiments.

Cultivation media
Wastewaters from five different factories of confectionery industry located in Vojvodina (marked as CW1 to CW5) were used as cultivation media for the production of xanthan. All wastewaters were first analyzed

to determine initial carbon and nitrogen content and on the basis of obtained results all cultivation media were enriched by addition of sucrose, so that the initial concentration of the carbon source is 1.5%. As a nitrogen source, yeast extract and (NH4)2SO4 (in 2:1 ratio) were added, so that the total nitrogen content is 0.02%. Also, 0.05% MgSO4·7H2O and 0.25 % K2HPO4 were added and the pH value of the cultivation media was set to 7.0 and sterilized in an autoclave at 121°C and overpressure of 1.1 bar during 20 min.

Cultivation

The inoculation of cultivation media was performed by adding 10% of inoculums prepared in two steps - first, by refreshing the culture by incubation for 24h at 28°C and second, by double passage of microorganism on the synthetic YMB media (containing: 1.5% of glucose, 0.3% of malt extract, 0.3% of yeast extract and 0.5% of peptone) for 36h, at 28°C. Samples were spontaneously aerated and externally mixed (laboratory shaker, 150 rpm). The biotechnological process of xanthan production was carried out under same experimental conditions in five Woulff bottles, containing 1,500 mL of media. Cultivation was carried out under aerobic conditions (air flow rate of 0.01 L/L·min in the first 48 h, and 0.02 L/L·min afterwards) and with external mixing at conditions mentioned above. In the first 48 h, the cultivation temperature was 28°C, after which it was increased to 30°C. The total time of cultivation was 120h. Regulation of process parameters was done in accordance with the literature data (Rosalam and England, 2006).

Product separation

Biosynthesis was stopped after 120 h and the cultivation broth was centrifuged at 10.000·G for 10 minutes (Eppendorf Centrifuge 5804). Ethanol (minimum 96%) was gradually added to the obtained supernatant until it had a content of 60%, while constantly being cooled in an ice bath and mixed with a laboratory stirrer (UM-15, Tehtnica, Železniki). A saturated solution of KCl was added when half of the ethanol was poured into the cooled supernatant, until it had content of 1%. The temperature of the mixture did not exceed 15°C. After precipitation the mixture was kept at 4°C for 24h in order to dehydrate the precipitated xanthan. The final step of xanthan separation was to centrifuge the mixture (3500 rpm for 15 minutes) on a centrifuge (LC-320, Tehtnica, Železniki). The precipitate was dried to a constant mass on 60°C and this data was used to calculate the xanthan yield.

2.2. Methods

The course of biosynthesis was monitored every 24 h by analyzing the samples taken from the cultivation broth. The separation of solid and liquid phases in the cultivation broth samples were carried out by a centrifuge at 10.000·G for 10 minutes (Eppendorf Centrifuge 5804).

Reducing sugars content was determined indirectly based on the content of sucrose hydrolyzed with the addition of ccHCl (100°C, 5min) and neutralizated with 5N KOH, in the supernatant of the cultivation broth by the method according to Miller (1959). Total nitrogen content was determined by the Kjeldahl method (Herlich, 1990). A rotational viscometer (REOTEST 2 VEB MLV Prüfgeräte-Verk, Mendingen, SitzFreitel), with a double gap coaxial cylinder sensor system, spindle N, was used for determination of rheological properties of the cultivation media samples. Volume of samples was 10 ml. Based on deflection of measuring instrument, α (Skt) under defined values of shear rates shear stress, τ (Pa) was calculated using the equation:

$$\tau = 0.1 \cdot z \cdot \alpha \quad (1)$$

Value of constant z (dyn/cm^2·Skt) is 3.08. According to the Ostwald de Vaele equation, which describes viscosity of pseudoplastic fluids, and calculated values of shear stress, rheological parameters were calculated.

3. RESULTS AND DISCUSSION

After determination of initial carbon and nitrogen contents in obtained wastewaters (Tab. 3) cultivation media were prepared to contain same amounts of these nutrients and all experiments were performed simultaneously, so that all stages of the biotechnological process would be carried out under identical conditions. Five cultivation media were examined for xanthan yield and sugar conversion in order

to determine the success of performed biosynthesis and obtained results are presented in Tab. 1. Based on the results in Tab. 1, xanthan yield, as a quantitative characteristic of the process, was highest in the CW1 media (10.03 g/L) and lowest in CW5 media (4.28 g/L). CW2 and CW4 media had similar values of xanthan yield (9.5 g/L), as well as sugar conversion (about 60%) and value of conversion of sugar into product (about 80%). Obtained results of xanthan yield were lower than the results obtained from literature for sucrose used as a basic sugar for biosynthesis (Leela and Sharma, 2000). Values of sugar conversion were in the range between 51.60 and 66.85%, which is in accordance with literature data (García-Ochoa et al., 2000) and only in the CW5 media this value is significantly lower and amounted 28.59%.

Table 1. Xanthan yield, sugar conversion and conversion of sugar into xanthan in enriched confectionery industry wastewaters after 120 h of biosynthesis

Media	Xanthan yield, P [g/L]	Sugar conversion(1) [%]	Conversion(2) [%]
CW1	10.03	66.85	79.87
CW2	9.43	60.84	83.55
CW3	7.74	51.60	76.20
CW4	9.60	61.94	79.81
CW5	4,28	28.59	54.67

(1) sugar conversion [%] = $(S_0\text{-}S)/S_0 \cdot 100$
(2) conversion [%] = $P/S_0 \cdot 100$

Even though used amount of sugars did not exceed 2%, the degree of conversion was lower than expected (Moraine and Rogovin, 1971) and this could be explained by the fact that cultivation media contained some substances that have an inhibitory effect on xanthan production. Also, increased viscosity of cultivation media leads to diffusional limitations due to reduced oxygen solubility and finally to lower xanthan production.

In applied experimental conditions quality of produced biopolymer was evaluated based on rheological behavior of obtained cultivation media. Graphical representation between shear rate and shear stress, flow curves, of all cultivation broths after 120h of biosynthesis are shown in Fig. 1. All samples represent a pseudoplastic type of flow, according to Fig. 1, as well as values of the flow behavior index (Tab. 2).

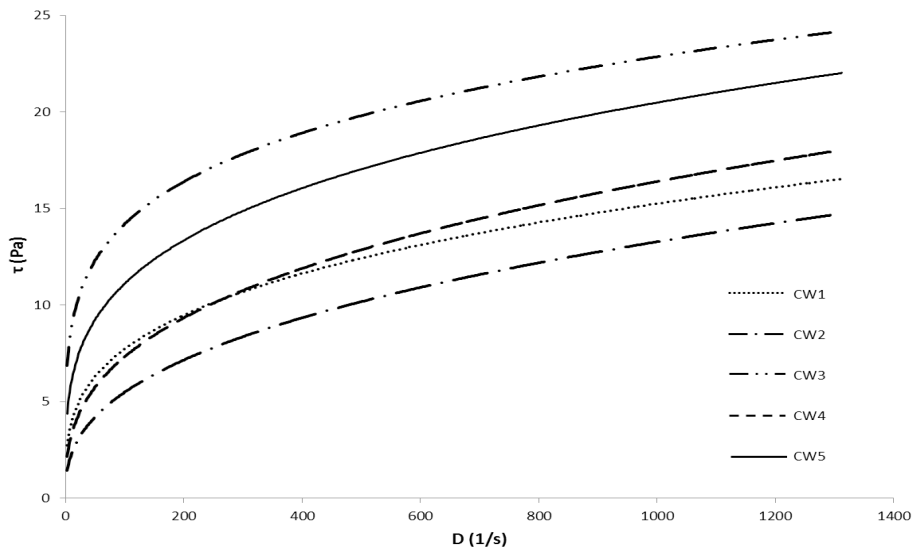

Figure 1. Shear stress as a function of shear rate in cultivation broths after 120 h of biosynthesis

Table 2. Rheological parameters and coefficient of correlation for cultivation broths after 120 h of biosynthesis

Media	K	n	R^2
CW1	1.9763	0.2958	0.99
CW2	0.9328	0.3844	0.98
CW3	5.4696	0.2070	0.99
CW4	1.4572	0.3504	0.99
CW5	3.2609	0.2660	0.98

Values of the consistency factor K (Tab. 2) indicate a different quality and quantity of synthesized biopolymer, given that viscosity and the consistency factor are proportional. Based on the values of the consistency factor it can be concluded that xanthan with the best quality is synthesized in the CW3 media while the low value of the consistency factor as well as the result of xanthan yield indicate that the CW5 media contained the lowest amount of xanthan with the lowest quality. Values of the flow behavior index, high value of the consistency factor and the results of xanthan yield suggest that the CW1 media contained the highest amount of xanthan with good quality.

In addition to producing a high value product, the aim of this paper was to examine the possibility of biological purification of confectionery industry wastewaters obtained from different parts of production processes, which was done by determining and comparing the contents of carbon and nitrogen before and after the performed biosynthesis. Considering the results shown in the Tab. 3 relating to the initial content of carbon in the wastewaters it can be seen that obtained values were significantly different. Initial values of carbon in the CW1 and CW2 media was less than 0.05 % while in the CW3, CW4 and CW5 media this value was significantly higher and amounts to about 0.8%. This can be explained by the fact that used wastewaters were obtained from the different factories as well as from different parts of the production processes before or after a certain purification treatment. After biosynthesis, values of carbon content decreased in all wastewaters by about 55% except in the CW5 media where this value was lower (about 11%) which is in accordance with results of xanthan yield, sugar conversion as well as conversion of sugar to xanthan in this media. Decrease in nitrogen content in the CW1 and CW3 media after biosynthesis of xanthan was about 15% while in the CW2, CW4 and CW5 media was around 50%.

Table 3. Comparison of carbon and nitrogen content in wastewaters before and after xanthan biosynthesis

Medium	$C^{(1)}$ [%]	$N^{(1)}$ [%]	$C^{(2)}$ [%]	$N^{(2)}$ [%]
CW1	0.0460	0.0035	0.0210	0.0030
CW2	0.0410	0.0042	0.0099	0.0021
CW3	0.9520	0.0112	0.3570	0.0098
CW4	0.7240	0.0070	0.313	0.0042
CW5	0.7710	0.0084	0.680	0.0050

(1) Content before biosynthesis
(2) Content after biosynthesis

4. CONCLUSION

In this study, the production of xanthan on five different confectionery industry wastewaters enriched with sucrose was examined. From the obtained results it can be seen that in the four out of five used wastewaters, the obtained yield is in the range between 7.74 g/L and 10.03 g/L while the conversion of xanthan was about 80%. In only one examined media these values were lower and were 4.28 g/L for xanthan yield and 54.67% for conversion of xanthan. Also, carbon and nitrogen content decreased in all wastewaters but not to a significant extent. Based on these results, wastewaters from the confectionery industry can be used as a basis of media for xanthan production, but further optimization of process is necessary in order to achieve higher yields and better purification of used wastewaters.

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