BIOLOGICAL TREATMENT OF DIFFERENT FOOD INDUSTRIAL WASTEWATER BY *Xanthomonas campestris*

Jelena Dodić, Jovana Grahovac, Aleksandar Jokić, Bojana Bajić, Siniša Dodić, Damjan Vučurović, Stevan Popov
Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
e-mail: johana@uns.ac.rs

ABSTRACT

Biological wastewater treatment is certainly one of the most important biotechnological processes, which have been used for over a century to treat municipal and industrial wastewaters. Industrial wastewater causes large-scale environmental problems mainly because of its extremely high organic content. Xanthan biosynthesis is usually performed on substrates containing different carbohydrates and nitrogen sources. These nutrients are often obtained from different raw materials that are intermediate or by-products of various food technologies. One of the greatest factors limiting the use of xanthan in large-scale fermentation processes is the cost of production when compared with similar polymers from algae or plants. The cost of feedstock used for polymer production is area where savings could be made in this respect. The present study examines xanthan production by *Xanthomonas campestris* under aerobic conditions on different food industrial wastewaters. Cultivation media were prepared to contain same amounts of carbon and nitrogen sources and all experiments were performed simultaneously, so that all stages of the biotechnological process would be carried out under identical conditions. In order to determine success of the performed biosynthesis, yield of xanthan and sugar conversion were determined. From this point of view, similar results were obtained. Also, different viscosities of cultivation media at the end of process were compared. Determined significant differences of viscosities and at the same time similar yield of xanthan suggest that biosynthesized polymers have different quality. High values of sugar and nitrogen conversions advocate that significant decrease of organic content in applied industrial wastewaters.

1. INTRODUCTION

Food and beverage industrial wastewater represents a large environmental pollutant. Industry in Vojvodina produces 80% of the total amount of wastewater, or 88% of organic pollution, expressed as COD, and within industry, the share of food industry is 31%, with the COD load as much as 82%, while the production of alcoholic beverages produce 2,9% of industrial wastewaters and 6,1% of the COD load. A significant part of food and beverage industrial wastewater pollution comes from high content of organic matter (1). A genetic characteristic of *Xanthomonas campestris* is xanthan biosynthesis when cultivated on media with an appropriate composition. In terms of carbon source, the mentioned producing microorganism is not too demanding. Glucose, sucrose and hydrolyzed starches are usually used as carbon sources and xanthan yield is affected by concentration of carbon source and carbon to nitrogen ratio. Xanthan gum has unique physical properties with number of industrial applications (food industries, cosmetics and petroleum industries) and may be used as stabilizer, emulsifier and thickening agent, lubricant, mobility control agent and inhibitor of crystallization. This polyanionic, hydrophilic biopolymer is a product of secondary metabolism (2). Among the microbial gums, xanthan occupies a prominent place in the market by having rheological properties that are quite different and unusual, such as a high degree of pseudoplasticity, a high viscosity even at low concentrations, 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 (3).

Commercial production of xanthan gum uses glucose as the carbon substrate; consequently the price of xanthan production is high. For this reason, recent research in the field has particularly focused on the search for cheaper natural alternatives for the currently used substrates, namely glucose or sucrose, so as to control the cost of the production process as well as of the final product. One of the ways to decrease xanthan price, is using cheaper substrate like agricultural wastes (4).

Effluents from food processing industries are significant environmental pollutants and it is necessary to purify them before discharging into recipients. This process is often expensive and therefore the final product price increases. The aim of this study is to examine possibility of biological purification of food and beverage industrial wastewaters, from several different factories on the territory of Vojvodina, through conversion of organic compounds by microorganism *Xanthomonas campestris*, obtaining high value product – xanthan, simultaneously.

2. EXPERIMENTAL

2.1. Production microorganism

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

2.2. Cultivation media

The cultivation media for the production of xanthan was wastewater from six different food and beverage proceeding factories: wastewater from oil industry (marked as OW), wastewater from brewery (marked as BW), alcohol industry wastewater (marked as AW), dairy industry wastewater (marked as DW), confectionery industry wastewater (marked as CW), mill industry wastewater (marked as MW), mill industry wastewater with the addition of enzyme-hydrolyzed starch (marked as MHW). Also, eight cultivation media was synthetic media containing glucose (marked as GCM), were used as control. These wastewaters were first analyzed to determine initial content of carbon and nitrogen. On the basis of obtained results all cultivation medias were enriched by addition of appropriate sugar (glucose for OW, AW, CW and GCM; maltose for BW; lactose for DW and starch for MW and MHW), so that the quantity of carbon source in medias is 1,5%. As a nitrogen source, yeast extract and $(NH_4)_2SO_4$ (in 2:1 ratio) were added, so that total nitrogen content is 0,02%. Each cultivation media was enriched with mineral salts in a quantity of 0,05% $MgSO_4 \cdot 7H_2O$ and 0,25 % K_2HPO_4. The pH value of the cultivation media was then set to 7,0 and sterilized in an autoclave at 121°C and overpressure of 1,1 bar during 20 minutes.

2.3. Cultivation

Inoculum was 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 media (marked as GCM) for 36h, at 28°C. Samples were spontaneously aerated and externally mixed (laboratory shaker, 150 rpm). The inoculation was performed by adding 10% of prepared inoculums. The biotechnological process of xanthan production was carried out under same experimental conditions in Woulff bottles, each containing 1,500 mL of the cultivation medium for biosynthesis with the appropriate composition (OW, BW, AW, DW, CW, MW, MHW and GCM). Cultivation was carried out under aerobic conditions (with air which was

conditioned in terms of moisture content and temperature, at a flow rate of 0.01 L/L·min in the first 48 h, and after that 0.02 L/L·min) 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. This regulation of process parameters was done according to the literature data (5). From the moment of inoculation, every 24 h, samples were taken for the analysis.

2.4. Product separation

After 120 h, biosynthesis was stopped and the cultivation broth was centrifuged at 10.000·G for 10 minutes (Eppendorf Centrifuge 5804) and the supernatant was cooled in an ice bath. While in the ice bath, ethanol (minimum 96%) was added in small portions (1 drop per second) till the content of 60% while constantly being mixed with a laboratory stirrer (UM-15, Tehtnica, Železniki). A saturated solution of KCl was added when half of the needed ethanol amount was poured into the cooled supernatant, in a quantity to reach a final content of 1%. The temperature of the mixture did not exceed 15°C. Afterward precipitation, with the aim of dehydration of the precipitated xanthan, the mixture was kept at 4°C for 24h. The final step of xanthan separation was carried out by centrifuging the mixture (3500 rpm for 15 minutes) on a laboratory centrifuge (LC-320, Tehtnica, Železniki). The precipitate was dried to constant mass on 60°C. This information was used to calculate the xanthan yield.

2.5. Analitical methods

The course of biosynthesis was monitored, every 24 h, by analyzing the samples taken from the cultivation broth. Depending on the analytical method, the sample was or was not processed before measuring. The separation of the solid from the liquid phase in the cultivation broth was carried out by centrifuging it at 10.000·G for 10 minutes (Eppendorf Centrifuge 5804). The reducing sugars content was monitored indirectly based on the glucose content in the supernatant of the cultivation broth by the method according to Miller (6). Total nitrogen content, in the cultivation broth supernatant, was determined by the Kjeldahl method (7). Rheological properties of cultivation broth samples were determined using rotational viscometer (REOTEST 2 VEB MLV Prüfgeräte-Verk, Mendingen, SitzFreitel) with double gap coaxial cylinder sensor system, spindle N. Volume of samples was 10ml. Based on deflection of measuring instrument, α (Skt) and using the equation: $\tau=0,1\cdot z\cdot\alpha$, shear stress, τ (Pa) was calculated, under defined values of shear rates. Value of constant z (dyn/cm^2·Skt) is 3,08. According to Ostwald de Vaele equation, which describes viscosity of pseudoplastic fluids, and calculated values of shear stress, rheological parameters were calculated.

3. RESULTS AND DISCUSSION

Eight cultivation media with enriched food industrial wastewater, from different food processing industries, were examined for xanthan productivity, quality of produced biopolymer and nitrogen and sugar conversion.

3.1. Xanthan yield and sugar conversion

According to the plan of experiment, xanthan was precipitated in order to determine the success of the performed biosynthesis in terms of xanthan yield and sugar conversion. The results of gravimetrical measurement are presented in Table 1. Based on the results in Table

1, the highest yield of xanthan was obtained on the wastewater from brewery (15,56 g/L) initially containing 15,97g/L of maltose. High yield was also achieved in wastewaters, enriched with glucose, from oil (14,18 g/L), confectionery (10,92 g/L) and alcohol industry (8,27 g/L). In these mediums the sugar conversion values were high as well as conversion of sugar into final product. In case of OW, conversion was 104,04% and explanation for this may be that applied strain of *X. campestris* used some components of medium that were not reducing sugars, for biosynthesis of xanthan. This corresponds with results obtained from the literature, that high degree of conversion (90%), calculated on digestible sugars, is achieved when sugar concentration in media is less than 2% (8).

Table 1. Xanthan yield and sugar conversion in enriched food industrial wastewaters after 120 h of xanthan biosynthesis

Medium	Content of reducing sugars[g/L]		Sugar conversion(3) [%]	Xanthan yield [g/L]	Conversion(4) [%]
	S_0(1)	S(2)			
BW	15,97	2,08	86,98	15,56	97,43
OW	13,63	0,36	97,36	14,18	104,04
CW	14,29	0,68	95,24	10,92	76,42
AW	12,49	0,16	98,72	8,27	66,21
DW	14,29	13,46	5,81	0	0
MHW	13,77	3,97	71,17	5,78	41,98
GCM	15,27	2,58	83,10	7,52	49,25

(1) sugar content in the inoculated media
(2) sugar content after 120 h of cultivation
(3) sugar conversion [%] = $(S_0-S)/S_0 \cdot 100$
(4) conversion [%] = $P/S_0 \cdot 100$

On the wastewater from dairy industry biosynthesis of xanthan did not occur. Based on that result it can be concluded that applied strain of *X. campestris* is not able to grow and produce xanthan in a medium containing lactose as carbon source. Results obtained in this work are similar to literature data, where for initial amount of carbon source of 2%, yields of xanthan for sucrose, maltose and lactose were 13,234g/L, 12,321g/L and 1,008g/L, respectively (9). In wastewater from mill industry, 15g/L of starch were added and yield of xanthan was 5,45g/L with the conversion of 36,33%. Conversion of sugar could not be calculated because applied analytical method (6) is suitable only for reducing sugars.

Yield of xanthan on wastewater from mill industry enriched with enzyme-hydrolyzed starch is 5,78 g/L, sugar conversion is 71,17 % and 41,98 % of sugar was converted into product. According to similar results obtained for MW and MHW, applied strain of *X. campestris* has amylolitic activity. Yield of synthetic cultivation media containing glucose (7,52 g/L) is notably lower then glucose enriched wastewaters (OW, CW and AW). The explanation for that could be that wastewaters contain some unidentified substances that microorganism used as sources of carbon for growth and production of xanthan.

Nitrogen conversion value (data not shown) in OW, AW, BW and CW medias, could not be accurately determined, because applied separation technique is not appropriate for such viscous cultivation broths. During the first 48h of cultivation, decrease of nitrogen content was significant, but increasing viscosity of cultivation broths prevented further measurements. Value of nitrogen conversion in GCM was 70%, and in MW, MHW and DW were between 40% and 50%.

3.2. Rheological behavior of cultivation broth

Flow curves, relationship between shear rate and shear stress, of all but DW cultivation broth after 120h of biosynthesis, shown in Figure 1, represent pseudoplastic type of flow. That is also confirmed by values of flow behavior index and coefficient of correlation presented in Table 2.

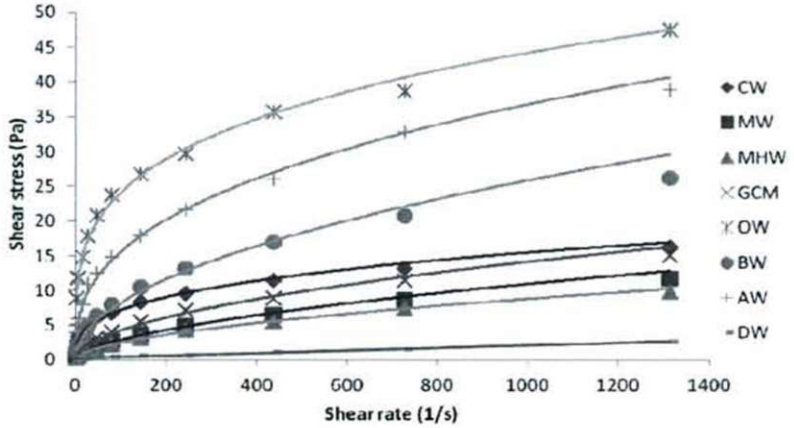

Figure 1. Shear stress as a function of shear rate in cultivation broths after 120 h of biosynthesis

Given that the viscosity and consistency factor are proportional, values of consistency factor (Table 2) indicate different quality and quantity of synthesized biopolymer. Values of flow behavior index, high values of consistency factor as well as results of xanthan yield point that glucose enriched (OW, AW and CW) and maltose enriched (BW) mediums contained high amount of xanthan with the good quality. According to the literature data (9), yield of xanthan in wastewaters enriched with starch (MW and MHW) and in glucose synthetic media, was significantly lower than expected and obtained rheological parameters indicate that biosynthesized xanthan have lower quality. Based on rheological parameters as well as the value of yield, on wastewater containing lactose, biosynthesis of xanthan has not occurred.

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

Media	*K*	*n*	R^2
OW	*7,1274*	*0,2641*	*0,99*
AW	*2,7847*	*0,3735*	*0,99*
CW	*1,5373*	*0,334*	*0,99*
BW	*0,8358*	*0,4967*	*0,99*
GCM	*0,3789*	*0,5235*	*0,99*
MW	*0,1968*	*0,5815*	*0,99*
MHW	*0,1954*	*0,5509*	*0,99*
DW	/	/	/

4. CONCLUSION

Production of xanthan on different, enriched wastewaters was examined in this study. From the obtained results it can be seen that the highest yield was obtained from the maltose enriched wastewater (15,56g/L) followed by glucose enriched wastewaters (14,18g/L for OW,

10,92g/L for CW and 8,27g/L for AW). These yields are much higher comparing to yield of xanthan on glucose synthetic media (7,52g/L). Also the significant decrease of sugar content is obtained in cultivation medias containing glucose (98,72% for AW, 97,36% for OW, 95,24% for CW and 83,10% for GCM) and maltose (86,98%). Applied strain of *Xanthomonas campestris* do not have the ability to synthesize xanthan from lactose but it have amylolitical activity which can be seen according to similar yields of cultivation mediums containing starch (5,45g/L) and hydrolyzed starch (5,78g/L). Based on these results, enzyme hydrolysis of starch under these experimental conditions is not justified. This suggests the further optimization of the wastewaters containing starch under applied experimental conditions.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of the Ministry of Education and Science, Republic of Serbia, project number: TR-31002.

REFERENCES

1. Klašnja, M., Šćiban, M. (2000): Elements of anaerobic process for treatment of wastewaters from food and beverage industries, Acta Periodica Technologica, 2000:31, 3-22 p.
2. Dodić, J.M., Vučurović, D.G., Grahovac, J.A., Dodić, S.N., Popov, S.D., Jokić, A.I. (2011): Utilization of maltose enriched spend grains liquor for xanthan production, Acta Periodica Technologica, 2011:42, 211-221 p.
3. Faria, S., Petkowicz, C.L.O., Morais, S.A.L.,Terrones, M.G.H., Resende, M.M., Franca, F.P., Cardoso, V.L. (2011): Characterization of xanthan gum produced from sugar cane broth, Carbohydrate Polymers, 2011:86, 469– 476 p.
4. Moshaf, S., Hamidi-Esfahani, Z., Azizi, M.H. (2011): Optimization of conditions for xanthan gum production from waste date in submerged fermentation, World Academy of Science, Engineering and Technology 2011:81.
5. Rosalam, S., England, R. (2006): Review of xanthan gum production from unmodified starches by *Xanthomonas campestris sp.*, Enzyme Microb. Tech., 2006:39, 197- 207 p.
6. Miller, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., 1959:31, 426-428 p.
7. Cohen, J.B. (1926): Practical Organic Chemistry, MacMillan and Co., London.
8. Moraine, R.A., Rogovin, P. (1971): Xanthan biopolymer production at increased concentration by pH control, Biotechnol. Bioeng. 1971:13, 381-391 p.
9. Sharma, G., Leela, J.K. (2000): Studies on xanthan production from *Xanthomonas campestris*, Bioprocess Engineering, 2000:23, 687-689 p.