Xanthan production on wastewaters from wine industry

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Abstract

Wine industry generates large volumes of wastewaters resulting from numerous cleaning operations that occur during the different stages of winemaking. Disposal of these effluents in the environment causes huge problems due to their high organic and inorganic load and seasonal variability. The bioconversion of winery wastewaters in valuable product, such as xanthan, is an important alternative to overcome environmental problems. In this research, the possibility of xanthan production using *Xanthomonas campestris* on blended wastewaters from different stages of white and rose wine production with initial sugar content of 50 g/L was investigated. In addition to the media parameters (content of sugars, total and assimilable nitrogen, phosphorus, total dissolved salts and apparent viscosity), raw xanthan yield and degree of sugar conversion into product were determined in order to examine the success of xanthan biosynthesis. In applied experimental conditions, xanthan yield of 20.92 and 30.64 g/L and sugar conversion into product of 40.23 and 60.73% were achieved on wastewaters from white and rose wine production, respectively. The results of these experiments suggest that winery wastewaters, after additional optimization of the process in terms of the substrate composition and the cultivation conditions, may be a suitable raw material for industrial xanthan production.

Keywords: xanthan, *Xanthomonas campestris*, wastewaters, wine industry.

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Wine production by grape must fermentation plays a big role in the agricultural industry. The total annual production of grapes in the Republic of Serbia is about 170 thousand tons while the total area of vineyards is about 22 thousand hectares covering 11% of the total permanent plantations. The amount of produced wine is variable from year to year, and the average value is about 220000 hL. Considering that the total volume of winery wastewaters varies in the range from 0.5 up to 14.0 L per L of wine produced, as well as they contain various organic and inorganic contaminants [1], it is evident that there are large amounts of waste effluents generated in our country with a notable environmental impact.

Winery wastewaters are generated by numerous activities during the wine production that mainly include cleaning of tanks and barrels, washing of floors and equipment, rinsing of transfer lines, bottling facilities, filtration units, product losses and rainwaters diverted into the wastewaters management system. Both the volumes and the composition of these waste effluents vary daily and throughout the year depending on activities within the winery. Also, characteristics of wastewaters may vary from one winery to another, depending on the size of the winery, the kind of the wine produced (white, rose, red or special wines) and the unique style of winemaking [1,2].

Overall the wastewaters from wine industry are characterized by an acidic pH values, high concentration of biodegradable organic matters, variable content of suspended solids and notable content of macronutrients, micronutrients and heavy metals, as well as cleaning agents. Winery wastewaters are mainly composed of sugars (glucose and fructose), followed by organic acids (acetic, tartaric, propionic, lactic), ethanol, glycerol, esters and polyphenolic compounds which represents an organic load to the environment [3, 4]. The analyses of winery wastewaters indicate that the organic acids play a more prominent role in the acidity of the wastewaters, whereas the high concentration of sugars contributes largely to high values of chemical oxygen demand (*COD*) [5].

Rapid expansion of the wine industry around the world during the last few decades has created huge problems caused by disposal of winery wastewaters in the environment. The environmental impacts of winery wastewaters include pollution of ground and surface water, soil degradation, damage to vegetation and the occurrence of odors [6]. In order to minimize the negative environmental impact and recycle the wastes generated by the wine industry, different treatment methods have been proposed. A promising alternative

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is bioconversion of these waste effluents into value-added products.

In previous research, xanthan production was suggested as a possible solution for utilization of wastewaters from wine industry [7,8]. Xanthan is a natural polysaccharide produced by the bacterium *Xanthomonas campestris*. Due to its excellent rheological properties xanthan has become one of the most important industrial biopolymers employed in many processes. This microbial polysaccharide is an attractive alternative for the replacement of traditional gums obtained from plants and marine algae by chemical extraction process. Xanthan is used as a thickening agent, stabilizer, or emulsifier, and combined with other polymers as a gelling agent in the food and non--food industries [9,10]. The demand for xanthan has increased steadily every year and, according to a study conducted by Grand View Research Inc., global xanthan gum market is expected to reach USD 987.7 million in 2020.

In this context, there is demand for improvement of xanthan production process in order to increase product yield. Therefore, the aim of this research paper was to examine the possibility of xanthan production using *Xanthomonas campestris* on blended wastewaters from different stages of white and rose wine production with high initial sugar content. Process efficacy was estimated based on the raw xanthan yield and degree of sugar conversion into product.

EXPERIMENTAL

Producing microorganism

Xanthomonas campestris ATCC 13951 was used in these experiments for xanthan biosynthesis. The strain of producing microorganism was stored at 4 °C on yeast maltose (YM) agar medium (containing: 15.0 g/L glucose, 3.0 g/L yeast extract, 3.0 g/L malt extract, 5.0 g/L peptone and 20.0 g/L agar) and subcultured at monthly intervals.

Substrates

Wastewaters, obtained from medium sized wineries located in vineyards of Fruška Gora, Vojvodina, Serbia, were used as substrates for xanthan production. Waste effluents from different stages of white (W) and rose (R) wine production were collected during the washing of the crusher (I), press (II) and tanks after clarification of must by flotation (III) or cold static decantation assisted with the addition of pectolytic enzymes (IV), filtered and then analyzed in terms of chemical composition. Based on obtained results (Table 1), wastewaters from white wine production were mixed and adjusted to total sugars content of 50 g/L, while wastewaters from rose wine production were mixed and enriched with glucose and fructose to achieve the same sugar content. $CaCO_3$ in concentration of 2 g/L was added to the substrates and pH value was adjusted to 7.0 before use.

Xanthan production

The xanthan production was carried out in 3 L laboratory bioreactor (Biostat® A plus, Sartorius AG, Germany) with 2 L of cultivation media. The bioreactor with the appropriate substrate was sterilized by autoclaving at 121 °C and pressure of 2.1 bar for 20 min. The sterile media was inoculated by adding 10 vol.% of inoculum prepared by double passaging in aerobic conditions, on YM broth (containing 15.0 g/L glucose, 3.0 g/L yeast extract, 3.0 g/L malt extract and 5.0 g/L peptone), at 25 °C on a laboratory shaker at 150 rpm for 36 h. The xanthan biosynthesis was carried out in batch mode under aerobic conditions (air flow rate of 1 vvm in the first 36 h, and 2 vvm afterwards) for 96 h. In the first 36 h, the biosynthesis temperature and agitation rate were 25 °C and 200 rpm, after which they were increased to 30 °C and 500 rpm, respectively.

Chemical analysis of substrates and cultivation broth

The samples of substrates and cultivation broth taken after inoculation and every 12 h were analyzed in terms of chemical composition. Depending on the analytical method, the samples were or were not processed before measuring. The separation of the *Xanthomonas campestris* cells from the cultivation broth was carried out by centrifugation at 10000 rpm for 10 min (Hettich Rotina 380 R, Germany), and after the change of conditions the preparation of samples for determination nitrogen and phosphorus content was included the dilution prior to centrifugation.

Reducing substances content in substrates and supernatants of cultivation broth was determined by spectrophotometric method [11]. Sugar content (glucose and fructose) in substrates was determined by high pressure liquid chromatography (HPLC). The samples were filtered through a 0.45 μm nylon membrane (Agilent Technologies Inc, Germany) and then analysed. The HPLC instrument (Thermo Scientific Dionex UltiMate 3000 series) was equipped with a pump HPG-3200SD/RS, autosampler WPS-3000(T)SL (10 μL injection loop), column Zorbax NH2 (250 mm×4.6 mm, 5 μm) and detector RefractoMax520. 75 vol.% acetonitrile was used as eluent with a flow rate of 1.2 mL/min and elution time of 20 min at column temperature of 25 °C. The contents of total nitrogen and phosphorus were determined from the samples of substrates and supernatants using method proposed by Kjeldahl [12] and spectrophotometric method [13], respectively.

The cultivation broth samples were also analyzed in terms of assimilable nitrogen and total dissolved salts

(TDS) content. The assimilable nitrogen, expressed as amino and ammonia nitrogen, was determined in the supernatants by the Formol titration method [14], while TDS was measured directly in the cultivation broth by the laboratory multiparameter analyzer Consort C863 (Consort, Belgium) with the conductivity electrode.

In the samples of substrates, sodium and potassium content, as well as calcium and magnesium content were determined by atomic absorption spectrophotometry [15].

Evaluation of rheological behavior

The rheological properties of the cultivation broth samples taken in the predetermined time intervals was determined using rotational viscometer (Reotest 2 VEB MLV Prufgerate-Verk, Mendingen, Sitzfreitel) with double gap coaxial cylinder sensor system, spindle N. Based on deflection of measuring instrument (α, Skt), shear stress (τ, Pa) was calculated under defined values of shear rates (D, 1/s) using the Eq. (1):

$$\tau = 0.1z\alpha \tag{1}$$

where z is the constant with the value 3.08 dyn/cm^2·Skt. The pseudoplastic behavior of the cultivation broth was confirmed by fitting the Ostwald-de-Waele model to the experimental data evaluated by regression of the power law. The values of the consistency factor (K, Pa·s^n), flow behavior index (n) and regression coefficient (R^2) were determined by Excel software 2010 and used for calculation of apparent viscosity (η_a, mPa·s) from Eq. (2):

$$\eta_a = 1000KD^{n-1} \tag{2}$$

Product separation

The xanthan was recovered from the supernatant of cultivation broth by precipitation with cold 96 vol.% ethanol in the presence of the electrolyte. Ethanol was gradually added to the supernatant at constant stirring until the alcohol content in mixture was 60 vol.%. A saturated solution of potassium chloride was added when half of the necessary ethanol amount was poured into the supernatant in a quantity obtain a final content of 1 vol.%. Obtained mixture was kept at 4 °C for 24 h in order to dehydrate the precipitated xanthan, and then centrifuged at 4000 rpm for 15 min (Tehtnica LC--321, Slovenia). The precipitated polymer was dried to constant weight at 60 °C in order to determine raw xanthan yield. Ethanol used for xanthan precipitation was recycled by distillation.

RESULTS AND DISCUSSION

Winery wastewaters are effluents with high organic and inorganic load and very complex composition. In previous study, wastewaters from different stages of white and rose wine production were analyzed in terms of parameters that are commonly used for characterization of waste streams [16]. For the purposes of this research, chemical composition of these waste effluents was determined. Obtained results are presented in Table 1.

Analyses of raw materials showed that the compositions of the most important nutrients for xanthan biosynthesis in these effluents were typical for winery wastewaters [3]. The observed differences are the consequences of the stage of vinification, type of wine produced and used winemaking technology.

Given that wastewaters with their composition proved to be an excellent substrates for both xanthan production and biomass growth [7,8], in this research xanthan biosynthesis on blended wastewaters from different stages of white and rose wine production was examined in order to improve production process. Wastewaters collected during the washing of the crusher, press and tanks after clarification of must were used for substrate preparation since these effluents are generated in the time period up to 24 h. Waste streams from other stages of wine production are made in the long intervals. Therefore, the collection of all waste-

Table 1. Composition of wastewaters from different stages of wine production

Parameter	Winery wastewater					
	W I	W II	W III	R I	R II	R IV
Reducing substances, g/L	4.02	4.64	137.80	25.47	13.23	43.00
Sugars, g/L	3.92	3.79	144.42	26.81	14.08	47.11
Fructose, g/L	2.08	1.93	70.77	13.13	7.24	15.92
Glucose, g/L	1.84	1.86	73.65	13.68	6.84	31.19
Total nitrogen, mg/L	91.00	105.00	2310.00	336.00	203.00	252.00
Phosphorous, mg/L	9.20	8.80	157.00	34.00	26.00	33.50
Sodium, mg/L	8.60	9.70	7.00	10.00	10.50	8.50
Potassium, mg/L	47.00	109.00	924.00	409.00	382.00	754.00
Calcium, mg/L	62.00	105.00	118.00	56.00	42.00	55.00
Magnesium, mg/L	29.00	41.00	101.00	21.00	20.00	31.00

waters from wine industry with the aim of application as substrates in biotechnological production would be very difficult in terms of providing appropriate storage conditions.

Based on chemical composition of selected winery wastewaters cultivation media were prepared as previously described. The xanthan production was carried out in the laboratory bioreactor under controlled conditions using *Xanthomonas campestris* ATCC 13951. The consumption of the most important nutrients for xanthan biosynthesis and the product formation were examined during the cultivation.

Reducing sugars content

The xanthan production depends on the type and concentration of carbon sources in the cultivation media. It was found that the xanthan yield increases with increasing the initial sugar content in the media [17]. In order to achieve maximum xanthan production, the strain of producing microorganism was cultivated on the wastewaters from wine industry with initial sugar content of about 50 g/L (48.64 g/L for white and 51.06 g/L for rose wine production's wastewaters). The reducing substances content, expressed as the content of reducing sugars, was determined in all samples of cultivation broth and obtained results are presented in Figure 1.

Results shown in Figure 1 indicate that sugars content significantly decreased during the 96 h of cultivation. At the end of the process, residual sugar content in winery wastewaters based media were 17.74 g/L for white wine and 10.77 g/L for rose wine, thus achieving the sugars conversion of 63.53 and 78.91%, respectively. A greater amount of metabolized sugar in media based on wastewaters from rose wine production may be due to the enrichment with commercial glucose and fructose which were added in order to achieve the desired initial sugars content and to maintain the ratio of fermentable sugars as in wastewaters. However, both substrates contained enough sugars for prolongation of the process with the aim to increase the quantity of the biopolymer.

Total and assimilable nitrogen content

Nitrogen is an essential nutrient that affects the growth of *Xanthomonas campestris* cells. A number of nutritional studies showed that lower concentration of nitrogen favors the xanthan production [18]. However, within the framework of this research, correction of initial nitrogen content in substrates was not performed. According to the obtained results (Figure 2), media based on wastewaters from white wine production contained more total as well as assimilable nitrogen than media based on wastewaters from rose wine production. Initial content of total and assimilable nitrogen in media prepared with white wine production's effluents were 413.00 and 142.80 mg/L, and these values in media prepared with rose wine production's effluents were 329.00 and 96.60 mg/L, respectively. It is evident that content of total nitrogen in both media was significantly higher than the assimilable nitrogen. Of the total nitrogen in the substrates only about 30% was assimilable, *i.e.*, amino and ammonia nitrogen.

Also, from the results presented in Figure 2 it can be seen that during the first 48 h of cultivation total and assimilable nitrogen content in both substrates significantly decreased. During this time, total and assimilable nitrogen content were reduced for 147.00 and 106.40 mg/L in wastewaters from white wine production and 105.00 and 63.00 mg/L in wastewaters from rose wine production, respectively. These results have shown that both media contained some other nitrogen compounds which *Xanthomonas campestris* cells can metabolize in applied experimental conditions. With further prolongation of cultivation time, the changes in the content of this nutrient were insignificant. After 96 h of cultivation values of total nitrogen conversion were 36.32% for white and 33.13% for rose wine production's waste-

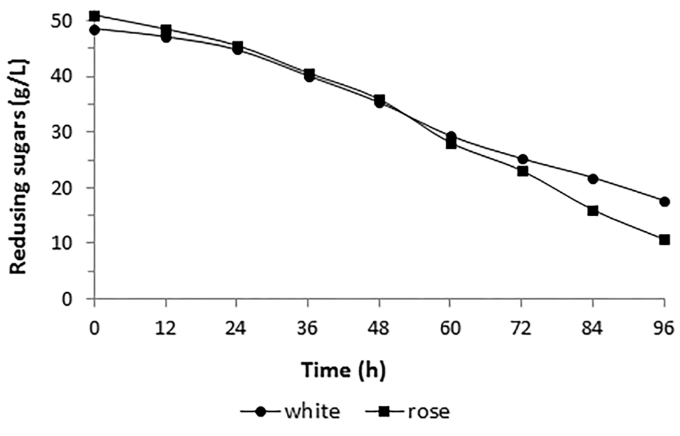

Figure 1. Reducing sugars content in media based on winery wastewaters.

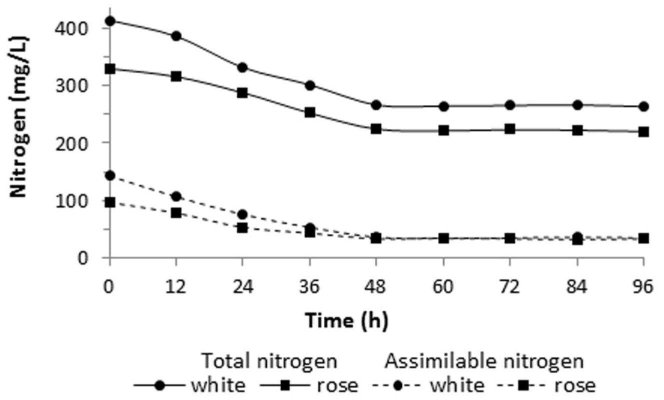

Figure 2. Total and assimilable nitrogen content in media based on winery wastewaters.

waters, while values of assimilable nitrogen conversion were 75.49 and 65.22%, respectively.

Total phosphorus content

In many researches it was found that the high phosphorus content in the media stimulates growth of producing microorganism cells, which may have negative impact on the biosynthesis of secondary metabolites, such as xanthan [18]. Nevertheless, during the preparation of the media based on winery wastewaters, correction of initial phosphorus content was not performed. The total phosphorus content determined during the cultivation is presented in Figure 3.

The results shown in Figure 3 indicate that media based on wastewaters from rose wine production contained more phosphorus (31.02 mg/L) than the media based on wastewaters from white wine production (20.84 mg/L). During the initial stages of cultivation total phosphorus content significantly decreased in both media, whereby decreasing trend was more pronounced in wastewaters from white wine production. After 36 h of cultivation total phosphorus content in media based on wastewaters from white wine production was 6.00 mg/L, while this nutrient content in media based on wastewaters from rose wine production after 48 h was 20.68 mg/L. With further prolongation of the cultivation, the total phosphorus content slowly decreased in both media and this decrease was not significant as in the first stages. At the end of the process, achieved values of phosphorus conversion were 79.16 and 36.67% for wastewater from white and rose wine production, respectively. Considerably higher value of phosphorus conversion indicates that media based on wastewater from white wine production contained more assimilable phosphorus. Although values of residual phosphorus content in both media were not in accordance with the limit value (2.00 mg/L) that wastewaters from production of alcoholic beverages must contain before being discharged into the environment [19], by application of this process a significant reduction of phosphorus content in wastewaters from wine industry was achieved.

Total dissolved salts content

To provide proper growth and metabolism of *Xanthomonas campestris* cells, the media must contain several micronutrients, such as potassium, iron, calcium, etc. These substances are added to the media in

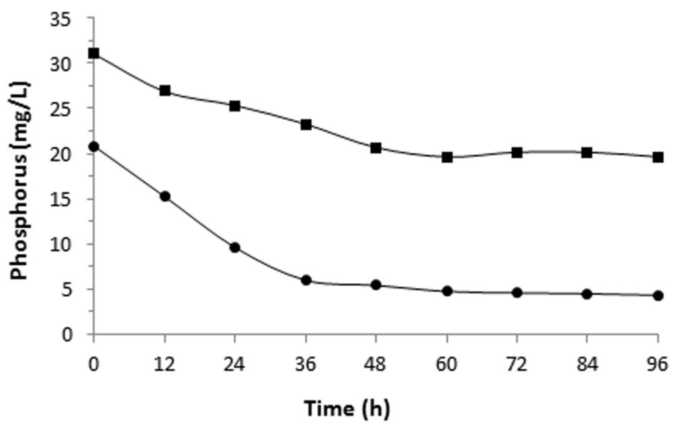

Figure 3. Total phosphorus content in media based on winery wastewaters.

the form of solid salts, and their solubility is dependent on the temperature and the pH value of cultivation media [18]. Considering that pH value is one of the factors that greatly affect the success of xanthan biosynthesis, the value of this parameter was monitored during the cultivation and maintained in the range 6.5–7.5 which is optimal for xanthan production [20]. Initial pH value of about 6.8 for both media in the first few hours of cultivation increased to about 7.5 and then remained unchanged or slowly decreased. After 60 h of cultivation the pH value of both substrates intensively decreased due to intensive production of organic acids and xanthan.

In this research, in the media based on wastewaters from wine production insoluble $CaCO_3$ was added to maintain proper pH value. Therefore, the content of total dissolved salts (TDS) was measured in all samples of cultivation broth and obtained results are shown in Figure 4.

The results presented in Figure 4 indicated that TDS content in media based on winery wastewaters had not changed significantly in the first 60 h of cultivation. During further cultivation time, TDS content increased more intensively which was a result of lower pH values of cultivation media. Since during the process a correction of the pH value of both media was performed, further research should include the optimization of $CaCO_3$ content in media based on winery wastewaters.

Rheological behavior of cultivation media

The amount of xanthan produced in applied experimental conditions was evaluated based on the rheological behavior of cultivation broth samples taken in the predetermined time intervals (36, 48, 60, 72, 84 and 96 h from the moment of inoculation). The rheological parameters were determined from relationship between shear rate and shear stress. The obtained values of consistency factor (K, Pa·s^n), flow behavior index (n) and regression coefficient (R^2) are presented in Table 2.

Flow behavior index represents a level of deviation from Newtonian flow behavior. The rheological measurement showed that all cultivation broth samples had pseudoplastic properties, a known characteristic of xanthan solutions [18], because the values of flow behavior index were $0 < n < 1$. The values of this parameter decreased from 0.5955 to 0.4480 for media based on wastewaters from white wine production and from 0.5829 to 0.2794 for media based on wastewaters from rose wine production which indicated that during the cultivation pseudoplastic characteristics of media became more pronounced. Also, the Ostwald-de-Waele model showed a good agreement with the experimental data, since the regression coefficients were higher than 0.97 in all tests.

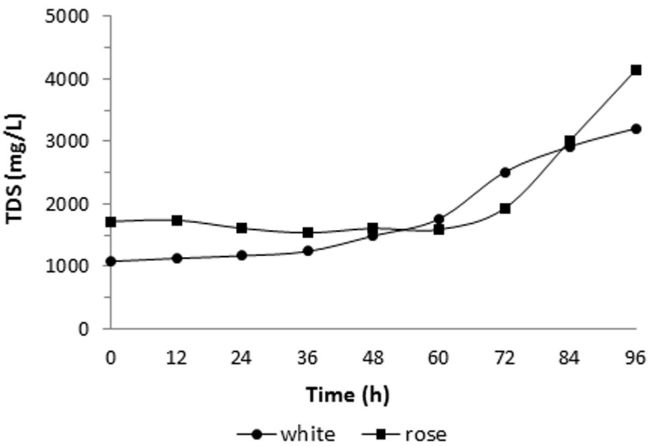

Figure 4. Total dissolved salts content in media based on winery wastewaters.

Table 2. Rheological parameters of media based on winery wastewaters

Time, h	White wine			Rose wine		
	K / Pa·s^n	n	R^2	K / Pa·s^n	n	R^2
36	0.1197	0.5955	0.978	0.1942	0.5829	0.987
48	0.1887	0.5440	0.997	0.4569	0.4883	0.994
60	0.2471	0.5175	0.997	1.1803	0.4143	0.995
72	0.3330	0.4965	0.993	2.7132	0.3347	0.999
84	0.5166	0.4572	0.994	4.3264	0.2975	0.999
96	0.9876	0.4480	0.996	6.1391	0.2794	0.998

The viscosity of xanthan solutions strongly depends on the biopolymer content and also on the chains length which is characteristic of applied producing microorganism, media composition as well as the molecular crosslinking by divalent ions from the media (Ca^{2+}) due to pyruvate and acetate groups in the molecule. Therefore, xanthan biosynthesis during the process was monitored by determination of apparent viscosity of the cultivation broth samples and the obtained results are shown on Figure 5. This method does not provide the data on the amount of xanthan in cultivation broth, but it is simple because it does not demand expensive equipment and has a quick response, and as such is excellent for monitoring the xanthan production process.

According to the results presented on Figure 5, apparent viscosity of media based on wastewaters from white wine production did not change drastically in the first 84 h of cultivation. On the other hand, apparent viscosity of media based on wastewaters from rose wine production increased significantly after 48 h of cultivation. Maximal values of apparent viscosity for both media was reached in 96 h of cultivation and amounted 63.12 mPa·s for white wine and 169.40 mPa·s for rose wine. Considerably lower value of this parameter for wastewaters from white wine production indicates that in media based on wastewaters from rose wine production greater amount of sugars was converted into xanthan.

Efficacy of xanthan production process

In order to determine efficacy of xanthan production on blended winery wastewaters with initial sugar content of 50 g/L, the raw xanthan yield and degree of sugar conversion into product were determined. In applied experimental conditions, xanthan yield of 20.92 and 30.64 g/L were achieved on media based on wastewaters from white and rose wine production, respectively. Although both media contained the same initial sugar content, obtained xanthan yield on wastewaters from rose wine production was about 32% higher than that obtained on wastewaters from white wine production. However, according to the literature data [17], the xanthan yield on glucose based media with initial sugar content of 50 g/L is not significantly different from that obtained on both winery wastewaters. The observed differences are the consequences of the different initial content of total and assimilable nitrogen, phosphorus and other compounds.

Value of sugar conversion into product in media based on wastewaters from rose wine production was 60.73%, which is in accordance with the literature data [21], while this value was lower in media based on wastewaters from white wine production and amounted 40.23%. These values suggest that, in applied experimental conditions, metabolized sugars in wastewaters from white wine production were mostly utilized for biomass growth and energy requirements, while in wastewaters from rose wine production sugars were used for xanthan biosynthesis as confirmed by values of apparent viscosity of the media and raw xanthan yield.

CONCLUSIONS

In accordance with the defined aim, in this research the possibility of xanthan production using *Xanthomonas campestris* ATCC 13951 on blended wastewaters from different stages of white and rose wine production with initial sugar content of 50 g/L was examined. For the substrates preparation wastewaters collected during the washing of the crusher, press and tanks after clarification of must were used. During the cultivation rheological behavior of the media was significantly changed, from the Newtonian at the beginning to very pseudoplastic in 96 h (consistency factor of 0.9876 and 6.1391 Pa·s^n and flow behavior index of 0.4480 and 0.2794 for media based on wastewaters from white and rose wine production, respectively).

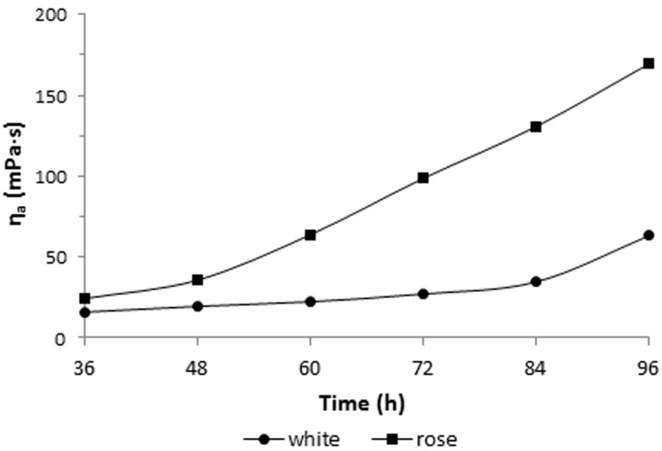

Figure 5. Apparent viscosity of media based on winery wastewaters.

Process efficacy was estimated based on the raw xanthan yield and degree of sugar conversion into product. In applied experimental conditions, xanthan yield of 20.92 and 30.64 g/L and sugar conversion into product of 40.23 and 60.73% were achieved on wastewaters from white and rose wine production, respectively. These results as well as residual nutrient content in media based on wastewaters from white and rose wine production (sugars of 17.74 and 10.77 g/L, nitrogen of 263.00 and 220.00 mg/L, assimilable nitrogen of 35.00 and 33.60 mg/L, phosphorus of 4.34 and 19.65 mg/L, respectively) obtained in this research suggest that blended winery wastewaters, after additional optimization of the process in terms of the substrate composition, especially in sugars content, and the cultivation conditions, may be a suitable raw material for industrial xanthan production. The use of these raw materials for xanthan biosynthesis would reduce the production costs and environmental problems caused by disposal of winery wastewaters which is confirmed by values of nitrogen and phosphorus conversion (36.32 and 79.16% for white wine and 33.13 and 36.67% for rose wine, respectively).

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IZVOD

PROIZVODNJA KSANTANA NA OTPADNIM VODAMA INDUSTRIJE VINA

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Proizvodnja vina je značajna grana industrije koja tradicionalno nosi veliku ekonomsku vrednost u poljoprivredno-prehrambenom sektoru. U Republici Srbiji se u proseku godišnje proizvede oko 220000 hl vina. Imajući u vidu podatke da količina otpadnih voda vinarija varira u intervalu od 0,5 do 14,0 l/l proizvedenog vina, kao i da one nose značajno organsko i neorgansko opterećenje, očigledno je da se u našoj zemlji generišu velike količine efluenata koji narušavaju kvalitet životne sredine. Otpadne vode vinarija uglavnom potiču iz operacija pranja sprovedenih tokom različitih faza proizvodnje vina, ali i iz rashladnih sistema. Podaci o zapremini i sastavu ovih otpadnih tokova su različiti među vinarijama, ali i u okviru iste vinarije od godine do godine, a zavise od proizvodne aktivnosti, veličine vinarije, vrste vina koje se proizvodi i primenjenog tehnološkog postupka. Predloženi su brojni tretmani za degradaciju zagađujućih materija prisutnih u otpadnim vodama vinarije među kojima je proizvodnja visokovrednih proizvoda mikrobiološkim putem jedna od najperspektivnijih alternativa. Tokom ranijih istraživanja potvrđena je mogućnost biosinteze ksantana na pojedinačnim frakcijama otpadnih voda koje se generišu u različitim fazama proizvodnje vina. U okviru ovih istraživanja ispitana je mogućnost biosinteze ksantana na zbirnim otpadnim vodama iz proizvodnje belog i roze vina. Prilikom pripreme podloge za biosintezu namešavane su otpadne vode skupljene tokom pranja muljače, prese i tanka nakon bistrenja šire tako da početni sadržaj šećera bude 50 g/l. Ovi efluenti su odabrani jer se generišu u kratkom vremenskom periodu (do 24 h) dok otpadne vode iz drugih faza proizvodnje vina nastaju u većim vremenskim razmacima, te bi skupljanje svih otpadnih tokova industrije vina sa ciljem primene kao sirovine u biotehnološkoj proizvodnji bilo veoma teško prvenstveno u pogledu obezbeđivanja uslova skladištenja u kojima ne bi došlo do promene njihovog sastava. Proizvodnja ksantana izvedena je u laboratorijskom bioreaktoru primenom soja *Xanthomonas campestris* ATCC 13951. Tok biosinteze praćen je analizom uzoraka kultivacione tečnosti u pogledu sadržaja najznačajnijih nutrijenata za biotehnološku proizvodnju i reoloških karakteristika kultivacione tečnosti. Tokom kultivacije došlo je do značajne promene reoogije medijuma koji je od Njutnovskog fluida na početku postao izrazito pseudoplastičan u 96 h što potvrđuju vrednosti faktora konzistencije i indeksa toka (0,9876 Pa·s^n i 0,4480 za belo vino, odnosno 6,1391 Pa·s^n i 0,2794 za roze vino). Efikasnost bioprocesa procenjena je na osnovu prinosa sirovog ksantana i stepena konverzije supstrata u proizvod. U primenjenim eksperimentalnim uslovima, prinosi ksantana na otpadnim vodama iz proizvodnje belog i roze vina iznosili su 20,92 i 30,64 g/l, pri čemu je ostvarena konverzija šećera u proizvod od 40,23, odnosno 60,73%, redosledom. Rezultati dobijeni u okviru ovih istraživanja predstavljaju osnovu za optimizaciju proizvodnje ksantana na otpadnim vodama vinarija sa ciljem povećanja prinosa i kvaliteta biopolimera, kao i smanjenja negativnog uticaja na životnu sredinu koje ima ispuštanje ovih otpadnih tokova.

Ključne reči: Ksantan • *Xanthomonas campestris* • Otpadne vode • Industrija vina