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Xanthan Production Using Wastewaters from Rose Wine Industry: Screening of *Xanthomonas euvesicatoria* Isolates

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Abstract

Wastewaters, as the major waste stream of the wine industry, are usually disposed in crude form due to the lack of sustainable treatments, which poses rising environmental threat. Considering biodegradability, nutrients content and other specific characteristics, winery wastewaters are suitable for utilization in xanthan production. In this study, the screening of local wild-type *Xanthomonas euvesicatoria* strains, isolated from pepper leaves, for xanthan production on medium containing wastewaters from rose wine industry, with initial sugar content of 25 g/L, was performed. Bioprocess success was estimated based on the quantity and quality of separated biopolymer. Additionally, composition of collected wastewaters was determined, and the obtained data indicate the importance of their proper management. The results of screening experiments suggest that applied *X. euvesicatoria* isolates have a statistically significant effect on xanthan concentration in cultivation medium, its molecular weight, as well as on apparent viscosity of xanthan aqueous solution. According to the obtained results, xanthan concentration varied from 4.0 g/L to 10.0 g/L, while the values of average molecular weight of xanthan and apparent viscosity of its solution ranged from $2.5 \cdot 10^5$ g/mol to $8.5 \cdot 10^5$ g/mol and from 40 mPa · s to 60 mPa · s, respectively. The results from this study suggest that *X. euvesicatoria* PL2 isolate showed the greatest potential for xanthan production on medium containing wastewaters from rose wine industry because of determined quantity of good-quality biopolymer. Further research is necessary in order to improve proposed bioprocess as sustainable biotechnological solution for winery wastewaters utilization.

Keywords

waste valorization, winery wastewater, biotechnological production, xanthan, *Xanthomonas euvesicatoria*

1 Introduction

Wine production is one of the oldest agro-industrial technologies and a significant sector in the world economy that is highly relevant to the environment and society. Moreover, wine is beverage with numerous health benefits because it contains compounds with cardioprotective, anticarcinogenic, antimicrobial, and anti-inflammatory properties [1, 2]. Despite the fact that winemaking is considered as an environmentally friendly process, it generates between 1.3 kg and 1.5 kg of waste per liter of produced wine, and approximately 75% of this waste are different fractions of wastewaters [3]. Winery wastewaters contain ethanol, glucose, fructose, organic acids, glycerol, esters, pigments, polyphenolic compounds, and other compounds from grapes and wines, which may pose a threat to the environment [4]. The constant increase in global wine production over the last few decades has created various ecological problems caused by disposal of untreated winery wastewaters [5]. To reduce the negative environmental impacts of liquid waste generated by the wine industry, different treatment methods and processes for utilization have been proposed. The approaches that are developed for treatment of winery wastewaters can be classified into five groups, i.e., physicochemical treatment (precipitation, sedimentation, coagulation/flocculation, etc.), biological treatment (aerobic and anaerobic microbial processes), membrane filtration and separation technologies (nanofiltration and reverse osmosis), advanced oxidation processes (ozonation, photo-Fenton oxidation, etc.), and combined

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biological and advanced oxidation processes [3]. Among them, anaerobic digestion is the most suitable for treatment of effluents characterized by large seasonal variations in concentration and type of contaminants, such as winery wastewaters [6]. However, in order to increase sustainability of the wine industry, utilization of generated wastewaters for obtaining value-added products is much preferred solution [7]. Consequently, valorization of winery wastewaters as raw materials for production of fungal biomass protein [8], microalgal biomass [9, 10] and biocontrol agents [11] was recommended. Still, biotechnological xanthan production on winery wastewater-based medium has been the most studied in recent years [12–16].

Xanthan is an anionic extracellular heteropolysaccharide produced by the metabolic activity of the bacteria from the genus *Xanthomonas*. Molecular formula of xanthan is $(C_{35}H_{49}O_{29})_n$, but its molecular weight distribution depends on the association between structural chains and usually varies from around 2×10^5 g/mol to 2×10^7 g/mol [17–19]. The molecular weight of xanthan contributes to its high viscosity and shear-thinning behavior, and thus, it has been widely used in a range of applications in the food and cosmetics industry, as well as in pharmaceutical and medical applications, including drug delivery and tissue engineering [20–22].

Xanthan production is performed by aerobic submerged batch cultivation of producing microorganism on appropriate medium under optimal conditions in stirred tank bioreactor with adequate constructional characteristics [15]. Although *Xanthomonas campestris* is the most widely used in the industry and many scientific studies, xanthan also can be produced by various *Xanthomonas* species such as *X. malvacearum*, *X. phaseoli*, *X. axonopodis* and *X. euvesicatoria* [23, 24]. Data from the available literature suggest that reference strain *X. campestris* ATCC 13951 possesses a great ability to biosynthesize xanthan on media with wastewaters from different stages of white [12], rose [13] and red wine production [16], as well as their mixtures [14]. Considering the fact that quantity and quality of xanthan are greatly affected by the strain used for its production [25], isolation and selection of novel *Xanthomonas* strain, which has the ability to produce xanthan on medium with winery wastewaters, are very important steps in this bioprocess improvement.

The aim of this study was screening of local wild-type *X. euvesicatoria* strains for xanthan production on wastewaters from different stages of rose wine production. Bioprocess success was estimated based on the quantity and quality of separated xanthan.

2 Material and methods

Isolation of phytopathogens from pepper leaves with bacteriosis symptoms was performed in laboratories at the Department of Phytomedicine and Environmental Protection (Faculty of Agriculture, University of Novi Sad), while xanthan production and characterization were carried out in laboratories at the Department of Biotechnology (Faculty of Technology Novi Sad, University of Novi Sad).

2.1 Producing microorganisms

Five *Xanthomonas* strains, locally isolated from infected pepper leaves (PL1, PL2, PL3, PL4, and PL5) and identified as *X. euvesicatoria* [26], were used as xanthan-producing microorganisms. All strains were stored at 4 °C on agar slant (Yeast Maltose Agar, HiMedia, India) in the Microbial Culture Collection of the Faculty of Agriculture in Novi Sad, Serbia.

2.2 Cultivation media

Agar slant (Yeast Maltose Agar, HiMedia, India) was used for refreshing cultures, while liquid medium (Yeast Maltose Broth, HiMedia, India) was used for cells multiplication in the inoculum preparation procedure.

Xanthan production was performed on medium containing wastewaters obtained from medium-sized winery located in Fruška Gora vineyards (Vojvodina, Serbia). Liquid effluents were collected in different stages of rose wine production, i.e., during washing of the crusher, press, and tanks after clarification of the grape must (unfermented grape juice) by flotation, filtered and then analyzed. Based on the obtained results (Table 1), wastewater from clarification of grape must by flotation was centrifuged (4000 rpm, 10 min; Rotina 380 R, Hettich Lab Technology, Germany), and then supernatant was diluted with equal volumes of the other two effluents to achieve the sugar content of 25 g/L. Calcium carbonate was added as buffering agent to the medium in the concentration of 2 g/L.

The pH value of all media was adjusted to 7.0±0.2, and then sterilized by autoclaving (121 °C, 2.1 bar, 20 min).

2.3 Inoculum preparation

Xanthomonas isolates were subcultured on agar slant and incubated at 25 °C for 48 h. Further, inoculum preparation by double passaging procedure was included suspending of producing microorganism cells in commercial liquid medium into two steps. Second passage was inoculated with 10% (v/v) of broth obtained in the first. The incubation of each passage was carried out in aerobic conditions at 25 °C and 150 rpm (laboratory shaker KS 4000i

Table 1 Characterization of wastewaters from different stages of rose wine production

Parameter	Stage of rose wine production		
	Crushing	Pressing	Flotation
pH (1)	6.35	6.88	3.49
Total suspended solids (mg/L)	692.00	308.00	57480.00
Reducing substances (g/L)	7.79	3.06	194.49
Sugar (g/L)	5.07	2.38	180.78
Fructose (g/L)	ND	1.09	88.64
Glucose (g/L)	5.07	1.29	92.14
Total nitrogen (mg/L)	224.00	203.00	1610.00
Assimilable nitrogen (mg/L)	11.20	4.20	355.60
Total phosphorus (mg/L)	10.75	1.71	217.43
Total dissolved salts (mg/L)	495.00	403.00	1620.00

ND: not detected

control, Ika®Werke, Germany) for 36 h. Inoculation of production medium was performed by adding 10% (v/v) of prepared inoculum.

2.4 Xanthan production process

Experiments were carried out in 300 mL Erlenmeyer flasks with 100 mL of the production medium. Xanthan production process was performed under aerobic conditions at 30 °C and 150 rpm (laboratory shaker KS 4000i control, Ika Werke, Germany) for 72 h.

2.5 Xanthan separation

At the end of the bioprocess, the cultivation media samples were centrifuged at 10,000 rpm for 30 min (Rotina 380 R, Hettich Lab Technology, Germany) in order to remove the producing microorganism cells. Xanthan was separated from the obtained supernatant by precipitation with cold 96% (v/v) ethanol in the presence of the electrolyte, as reported previously [15].

2.6 Analytical methods

2.6.1 Analysis of winery wastewaters

The pH value was measured by the multi-parameter analyzer Consort C863 (Consort, Belgium) with a glass pH electrode. The samples of wastewaters were then analyzed in terms of dry matter and total suspended solids using Standard Methods for the Examination of Water and Wastewater [27].

Reducing substances content was detected using method proposed by Miller [28]. Sugar content, expressed as the sum of glucose and fructose, was determined by high pressure liquid chromatography as previously described [14]. The content of total and assimilable (amino and ammonia) nitrogen was determined using method proposed by Helrich [29] and the Formol titration method [30], respectively. Total phosphorus content was estimated by method presented by Gales et al. [31]. The content of total dissolved salts was measured by the multi-parameter analyzer Consort C863 (Consort, Belgium) with a conductivity electrode.

2.6.2 Analysis of cultivation medium

Rheological behavior of cultivation medium samples was evaluated using a RheoStress 600HP rheometer (Thermo HAAKE,Germany) with a cone-plate system and C60/1Ti sensor. All rheological measurements were carried out at 25 °C and shear rates from 0 to 1000 1/s. The data obtained for each sample was fitted to the Ostwald-de-Waelle (power law) model in Microsoft® Excel 2010 software (MS Office, Microsoft Corporaton, USA) in order to determine the consistency factor (K, Pa · s^n) and flow behavior index (n). The goodness of fit was verified by the determination coefficient. The values of rheological parameters were used for calculation of apparent viscosity (η_a, mPa · s) from Eq. (1):

$$\eta_a = K \cdot D^{n-1}, \tag{1}$$

where D is the shear rate with the value of 100 s^{-1}.

2.6.3 Analysis of separated xanthan

The average molecular weight of separated xanthan was estimated based on the intrinsic viscosity of its solution in 0.1 M sodium chloride using the Mark-Houwink type equation [32].

The apparent viscosity of 1% (w/v) aqueous xanthan solution was calculated based on the results of rheological measurements, as described in Section 2.6.2.

2.7 Data analysis

All experiments were carried out in triplicate and the results were averaged. The experimental results were analyzed using One-Way ANOVA, followed by Duncan's multiple range test. Statistical analysis was carried out at significance level of $\alpha = 0.05$ using Statistica™ 14.0.0 software [33] (TIBCO Software Inc., USA).

3 Results and discussion

3.1 Characterization of wastewaters from rose wine industry

Wastewaters from different stages of rose wine production, collected during washing of equipment after crushing and pressing of grapes, as well as clarification of grape must

by flotation, were analyzed in terms of parameters that are used for the characterization of raw materials in xanthan production. The obtained results are presented in Table 1 and indicate that the analyzed wastewaters had a high organic and inorganic load, which is typical for effluents generated in domestic [14–16] and worldwide wineries [4].

The noticed differences in the values of the observed parameters (Table 1) are the result of the winemaking stage. It is evident that the wastewater generated during flotation tank washing is characterized by the lowest pH value and the highest concentration of suspended solids and nutrients. However, all the analyzed effluents are not suitable for environmentally safe disposal and must be adequately treated. Nevertheless, the values of nutrients content suggest that wastewaters from rose wine industry can be a suitable raw material for the preparation of xanthan production medium.

3.2 Screening of xanthan-producing strains

In accordance with the defined aim, xanthan production was performed by five different wild-type *X. euvesicatoria* strains, isolated from infected pepper leaves, on medium containing wastewaters from different stages of rose wine production. Considering that pseudoplastic behavior is expected for water solutions of microbial polysaccharides [34], xanthan production under applied experimental conditions was evaluated based on the rheological behavior of media obtained after cultivation of *X. euvesicatoria* isolates. The pseudoplastic properties of the cultivation media were confirmed by the values of flow behavior index (n) that represents the level of deviation from Newtonian flow behavior. The value of this parameter is equal to 1 for Newtonian fluids, greater than 1 for dilatants, and less than 1 for pseudoplastic fluids [35]. The flow behavior index values, determined for the medium samples analyzed in this study, ranged from 0.3513 to 0.8163. Hence, the obtained values of the flow behavior index suggest pseudoplastic behavior, which is characteristic of xanthan solutions [36]. As consistency factor is proportional to the viscosity, this parameter was also observed. Based on the obtained results, the values of consistency factor in this study varied from 0.0344 Pa · s^n to 0.7665 Pa · s^n, which indicates the difference in the quantity and quality of xanthan produced by cultivation of examined *X. euvesicatoria* isolates in applied experimental conditions.

3.2.1 Statistical analysis of experimental results

Bioprocess success was estimated based on the xanthan concentration in medium at the end of cultivation of *X. euvesicatoria* isolates and separated biopolymer quality. The xanthan quality depends on various factors, including the applied producing strain, and can be evaluated based on several parameters, such as the viscosity of its solutions, composition of macromolecules, and molecular weight [37]. In this study, the quality of produced xanthan was estimated based on the average molecular weight and apparent viscosity of its aqueous solution.

In order to examine the effect of varying *X. euvesicatoria* isolates on xanthan quantity and quality, statistical analysis of experimental data was carried out. The results of One-Way ANOVA, as well as the results of post hoc analysis by Duncan's multiple range test for xanthan concentration in medium, its average molecular weight, and apparent viscosity of xanthan aqueous solution are further discussed. The summary results of One-Way ANOVA for the aforementioned parameters are presented in Table 2.

The results presented in Table 2 show that the *p*-values for all analyzed parameters are much lower than 0.05, which indicates that applied *X. euvesicatoria* isolates have a statistically significant effect on the xanthan concentration in cultivation medium, its molecular weight, as well as on apparent viscosity of the xanthan aqueous solution. These results are in accordance with previous research findings where xanthan production was performed by *Xanthomonas* strains isolated from pepper leaves on the media with different carbon sources (glucose, lactose and starch), and it is confirmed that selection of producing microorganism have statistically significant effect on

Table 2 One-Way ANOVA results for the effect of varying *X. euvesicatoria* isolates on xanthan quantity and quality

Parameter	Variability	SS	DF	MS	*F*-value	*p*-value
Xanthan concentration in medium	Strain	79.4894	4	19.8723	86.9340	< 0.000001
	Error	2.2859	10	0.2286	–	–
Molecular weight of xanthan	Strain	$6.6320 \cdot 10^{11}$	4	$1.6580 \cdot 10^{11}$	127.4240	< 0.000001
	Error	$6.7622 \cdot 10^{11}$	10	$1.3012 \cdot 10^{9}$	–	–
Apparent viscosity of 1% xanthan solution	Strain	1097.15	4	274.29	37.1480	0.000006
	Error	73.84	10	7.38	–	–

SS: sum of squares; DF: degrees of freedom; MS: mean square

xanthan concentration in media and apparent viscosity of its aqueous solution [38]. Moreover, findings from this study are in agreement with results from another previous research where *Xanthomonas* strains isolated from pepper leaves were cultivated on the medium containing glucose, commercial glycerol and crude glycerol from biodiesel industry, and it is also confirmed that variation of *Xanthomonas* strains have statistically significant effect on xanthan quantity and its molecular weight [39, 40]. In addition, results obtained in similar researches, where the xanthan production was performed by *Xanthomonas* strains isolated from different plants (cabbage, kale, cauliflower, walnut, pepper, begonia, anthurium lemon, and peach tree), confirmed the influence of variation of producing microorganisms on produced biopolymer quantity and quality [19, 37, 41, 42].

According to the results obtained in this study, selection of the producing strain is of significant importance for the bioprocess success in applied experimental conditions. This is probably due the fact that different *Xanthomonas* species possess different metabolic pathways and cycles [44].

In addition to the Table 2, the results of the statistical analysis for xanthan concentration in medium, its average molecular weight and apparent viscosity of its aqueous solution are also presented graphically by Box & Whisker Plots in Figs. 1–3 in Sections 3.2.2, 3.2.3 and 3.2.4, respectively. Values in the same figure marked with the same letter are not significantly different in a 95% confidence interval.

3.2.2 Effect of strain selection on xanthan concentration in medium

The results of statistical analysis of the effect of different *X. euvesicatoria* isolates on xanthan concentration in medium containing wastewaters from rose wine industry at the end of cultivation are presented in Fig. 1.

The results presented in Fig. 1 suggest that xanthan production on medium containing wastewaters from rose wine industry, in applied experimental conditions, is possible by all examined *X. euvesicatoria* isolates, and that there is a statistically significant difference in the xanthan concentration in media when using different strains. Data presented in Fig. 1 indicate that the xanthan concentration in media at the end of the bioprocess varied from approximately 4.0 g/L to 10.0 g/L. When compared to the results from a previous study, where xanthan concentration, after cultivation of the reference strain *X. campestris* ATCC 13951 on media prepared with wastewaters from selected stages of rose wine production (sugar content: 25 g/L), varied from 8.03 g/L

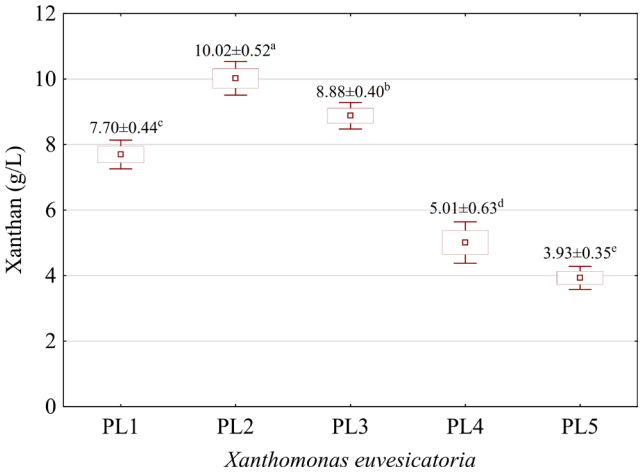

Fig. 1 Effect of different *X. euvesicatoria* isolates on xanthan concentration in medium
Values (mean ± standard deviation) marked with the same letter are not statistically significant different (95% confidence interval)

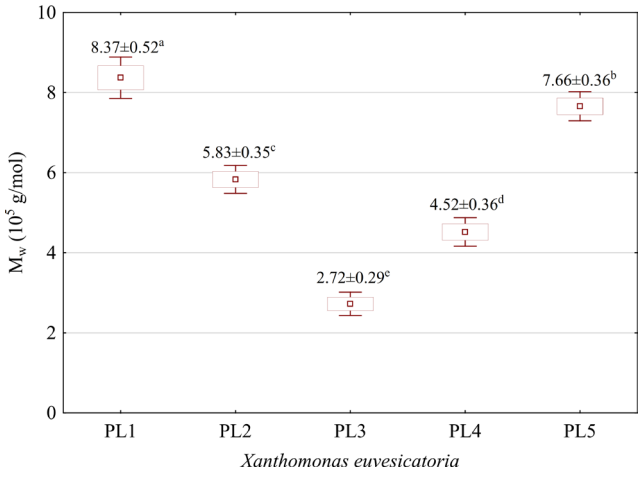

Fig. 2 Effect of different *X. euvesicatoria* isolates on xanthan average molecular weight (M_W)
Values (mean ± standard deviation) marked with the same letter are not statistically significant different (95% confidence interval)

and 17.52 g/L [13], it can be noticed that applied *X. euvesicatoria* isolates show good potential for xanthan production on wastewaters from rose winemaking. In addition, values of xanthan concentration in medium obtained in this study are in a similar range with results reported in research where reference strain *X. campestris* ATCC 13951 was cultivated on media containing wastewaters from certain stages of white wine production process (sugar content: 25 g/L) and where the xanthan concentration was in the range of 4.0 g/L to 10.67 g/L [12].

Graphically represented results (Fig. 1) indicate that the highest xanthan concentration in medium was achieved when cultivation was performed by *X. euvesicatoria* PL2 isolate (10.02 ± 0.52 g/L) in applied experimental conditions.

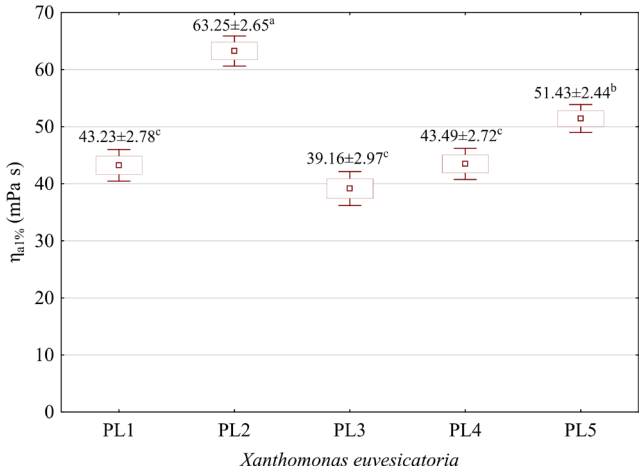

Fig. 3 Effect of different *X. euvesicatoria* isolates on apparent viscosity of 1% (w/v) xanthan aqueous solution (η_a)
Values (mean ± standard deviation) marked with the same letter are not statistically significant different (95% confidence interval)

A slightly lower xanthan concentration was obtained when using *X. euvesicatoria* PL3 (8.88 ± 0.40 g/L) and *X. euvesicatoria* PL1 (7.70 ± 0.44 g/L) isolates, while a considerably lower quantity of this biopolymer was obtained after cultivation of *X. euvesicatoria* PL4 isolate (5.01 ± 0.63 g/L). The lowest xanthan concentration was obtained with the *X. euvesicatoria* PL5 isolate (3.93 ± 0.35 g/L). Differences in the productivity among wild-type *X. euvesicatoria* strains used in this study are probably due to nutrient needs and therefore biopolymer yield may be different among various strains, as reported previously when the same isolates were cultivated on semi-synthetic media with glucose, lactose, starch and glycerol [38, 39], as well as complex medium with crude glycerol from biodiesel industry [40]. Since the industrial xanthan production is performed by cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on semi-synthetic media with glucose or sucrose [36], it would be interesting to compare results obtained in this study with the results achieved in standard conditions. Hence, it is noticed that the productivity of *X. euvesicatoria* PL2 isolate on medium containing wastewaters from rose wine industry (sugar content: 25 g/L) is lower compared to the productivity of *X. campestris* ATCC 13951 on glucose and sucrose containing media (sugar content: 20 g/L) [45] for about 32% and 24%, respectively. These differences are not excessive and can be eliminated by additional improvement of production process (for example, in terms of production medium composition, operational conditions, separation procedure…).

3.2.3 Effect of strain selection on xanthan molecular weight

The results of statistical analysis of the effect of different *X. euvesicatoria* isolates on average molecular weight of xanthan obtained after cultivation on medium containing wastewaters from different stages of rose wine producton are shown in Fig. 2.

The results represented in Fig. 2 suggest that there is a statistically significant difference in the average molecular weight of xanthan produced by different *X. euvesicatoria* isolates in applied experimental conditions. The average molecular weight of xanthan obtained in this study ranged from approximately $2.5 \cdot 10^5$ g/mol to 8.5×10^5 g/mol. This is in agreement with the results from the previous study where *Xanthomonas* strains isolated from pepper leaves were cultivated on media containing glucose or glycerol (20 g/L) and molecular weight values of produced xanthan were in the range from $1.0 \cdot 10^5$ g/mol to $8.0 \cdot 10^5$ g/mol [39]. On the other hand, values of molecular weight of xanthan obtained in this study are higher compared to the results obtained in previous research where *Xanthomonas* strains isolated from pepper leaves were cultivated on medium containing crude glycerol from biodiesel production (glycerol content: 20 g/L). In that case, the values of this quality indicator ranged from $0.5 \cdot 10^5$ g/mol to $2.3 \cdot 10^5$ g/mol [40].

Observing the mean values of the average molecular weight of separated xanthan (Fig. 2), it can be noted that the *X. euvesicatoria* PL1 isolate produced the biopolymer with the highest molecular weight ($8.37 \pm 0.52 \cdot 10^5$ g/mol) that is with better quality and potentially wider possibilities of application. Good-quality xanthan was also obtained when *X. euvesicatoria* PL5 ($7.66 \pm 0.36 \cdot 10^5$ g/mol) and *X. euvesicatoria* PL2 ($5.83 \pm 0.35 \cdot 10^5$ g/mol) isolates were cultivated in applied experimental conditions. Xanthan with a somewhat lower average molecular weight was obtained when *X. euvesicatoria* PL4 isolate ($4.52 \pm 0.36 \cdot 10^5$ g/mol) was used, whereas *X. euvesicatoria* PL3 isolate produced xanthan with the lowest average molecular weight ($2.72 \pm 0.29 \cdot 10^5$ g/mol). Differences in xanthan quality are probably present due the fact that the length of macromolecules depends mainly on biosynthesis pathways, genetic variability of the producing strains [44].

3.2.4 Effect of strain selection on apparent viscosity of xanthan aqueous solution

The results of statistical analysis of the effect of different *X. euvesicatoria* isolates on apparent viscosity of aqueous

solution of xanthan, produced in applied experimental conditions, are shown in Fig. 3.

The graphically represented results (Fig. 3) indicate that there is a statistically significant difference in the apparent viscosity of xanthan aqueous solution when different *X. euvesicatoria* isolated were used. The apparent viscosity of 1% (w/v) xanthan aqueous solution achieved in this study ranged from approximately 40 mPa · s to 60 mPa · s.

If the apparent viscosity of xanthan aqueous solution is considered as biopolymer quality indicator, the mean values presented in Fig. 3 suggest that *X. euvesicatoria* PL2 isolate seems to be the best strain for good-quality xanthan production (63.25 ± 2.65 mPa · s). The obtained result is in accordance with findings from previous research, where the same *X. euvesicatoria* isolate proved to be the best strain for the production of xanthan with the highest apparent viscosity i.e., highest quality, on media containing glucose, lactose, or starch as a carbon source (content: 20 g/L) [38]. Based on the results presented in Fig. 3, xanthan of lower quality was biosynthesized by *X. euvesicatoria* PL5 isolate (51.43 ± 2.44 mPa · s), whereas there was no significant difference in biopolymer quality when *X. euvesicatoria* PL1 (43.23 ± 2.78 mPa · s), *X. euvesicatoria* PL3 (39.16 ± 2.97 mPa · s), and *X. vesicatoria* PL4 isolates (43.49 ± 2.72 mPa · s) were used, which was confirmed by *p*-values of 0.096558, 0.908995, and 0.091636, respectively. The results obtained in this study are in accordance with findings from earlier research [41], which proved that the viscosities of the produced xanthan solutions depend on the *Xanthomonas* strains used.

4 Conclusions

The results of this study have confirmed the significant potential of *X. euvesicatoria* strains, locally isolated from infected pepper leaves, to metabolize sugars from medium containing wastewaters generated in rose wine industry, simultaneously producing xanthan. According to the obtained values of analyzed parameters, *X. euvesicatoria* PL2 isolate showed the greatest potential for good-quality xanthan production in applied experimental conditions. Findings from this study indicate that the utilization of winery wastewaters in proposed bioprocess represent a promising and sustainable biotechnological solution for reduction of environmental problems caused by improper disposal of these effluents, as well as total costs of xanthan production since no water for preparation of cultivation media is required and consequently, considerable amount of water can be saved. Finally, the results obtained in this study represent reliable information for bioprocess modeling and optimization in order to increase xanthan yield and quality, bioprocess monitoring and kinetic analysis that is prerequisite for scaling up, as well as the examination of possible applications of xanthan produced on wastewaters from rose wine industry.

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