

Waste-to-Resource Strategies for Environmental Sustainability: Application of Biopolymers Produced from Winery Effluents

Ida Zahović^{1*}, Jelena Dodić¹, Andrijana Bilić², Maria M. Savanović², Sanja J. Armaković², Zorana Trivunović¹

¹ Department of Biotechnology, Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21102 Novi Sad, Serbia

² Department of Chemistry, Faculty of Sciences, University of Novi Sad, Biochemistry and Environmental Protection, 21000 Novi Sad, Serbia

* Corresponding author, e-mail: ida.zahovic@uns.ac.rs

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Abstract

Biopolymers represent biomaterials with important industrial applications. Among them, bacterial-derived biopolymers have shown potential to become promising materials for environmental remediation in the near future, as evidenced by several environmental applications that have recently been thoroughly studied. In this study, the emulsifying properties and the influence of xanthan produced by cultivation of the strain *Xanthomonas campestris* ATCC 13951 and a *Xanthomonas* strain isolated from crucifers on a medium containing winery effluents on pindolol removal were analyzed. The results indicate that xanthan produced on the winery effluents-based medium by both producing strains can be used as an emulsion forming and stabilizing agent between aqueous solutions and hydrocarbons or natural oils, and that this biopolymer enhances the efficiency of the removal of pindolol under UV irradiation. The results obtained provide additional information on the environmental applications of xanthan produced from winery effluents.

Keywords

environmental protection, xanthan, emulsification, pindolol removal, pharmaceuticals

1 Introduction

The rising prevalence of organic and pharmaceutical pollutants in water, driven by the widespread use of medications, personal-care products, and hormones for health and cosmetic purposes, represents a class of emerging pollutants that substantially threaten the environment and human health, becoming a significant global concern [1, 2]. β -blockers constitute a frequently used group of pharmaceuticals employed in treating cardiovascular diseases, as well as other conditions such as hyperthyroidism, glaucoma, migraine prophylaxis, and anxiety management [3]. After decades of widespread and excessive use, the concentration of β -blockers in surface water may reach several ng/dm³ or μ g/dm³ [4]. Noteworthy among these β -blockers is pindolol, widely used in pregnancy to address hypertension due to its recognized harmlessness to the fetus [5]. Pindolol has been detected in concentrations of 22 ng/dm³ in river waters [6], 3 ng/dm³ in groundwater [7], and 0.03 ng/dm³ in wastewater [8]. Conventional

treatment methods have been widely used in the removal of organic contaminants. However, pollutants are not completely degraded and pass through treatment, only partially removed or transformed into by-products that are more persistent or even more toxic than the parent compound. These findings suggest that conventional treatment methods are not completely effective in controlling pharmaceutical pollution, raising concerns for public health and the ecosystem [9]. Compared to conventional treatment methods, photodegradation-based processes have shown considerable potential for the removal of organic pollutants from water. Under appropriate conditions, these processes may lead to extensive degradation and mineralization of contaminants, with the possibility of using sunlight as an energy source [10, 11].

The water pollution caused by hydrocarbons and oils has also increased alarmingly in recent years. The main sources of pollution are anthropogenic activities associated

with rapid industrialization that result in the deposition of hydrocarbon pollutants in the environment. Hydrocarbon-based pollutants usually originate from petroleum products, heavy hydrocarbons, halogenated solvents, and other [12]. Several methods, including physical, chemical, and biological treatments, are proposed as a suitable solution for the removal of the aforementioned pollutants. However, there is a lack of accelerated application of these methods due to low purification efficiency, high energy consumption, operating costs, and equipment complexity [13]. Hence, there is a need for another efficient, eco-friendly, and cost-effective method for the removal of hydrocarbons and oils from water resources.

Application of biobased materials in the removal of organic compounds such as pharmaceutical pollutants has attracted increasing attention during the past few years [14–16]. Scientists worldwide are focused on the development of new bio-inspired and biocompatible materials with outstanding properties, and based on the literature data, one of the economically and environmentally acceptable solutions is the application of the bacterial-derived polymer, xanthan, in the removal of previously mentioned environmental pollutants [14, 17, 18]. Exceptional rheological characteristics, together with its biocompatibility, biodegradability, and non-toxicity, make this biopolymer extensively used in food, cosmetics, pharmaceutical, paper, textile, and other industries [19]. The most common applications of xanthan in the food industry are as a stabilizer, thickener, and emulsifier, owing to its high viscosity at low concentrations and excellent solubility in both hot and cold water. Nevertheless, the application of xanthan is not only limited to the industry, but also to medicine, biomedical engineering, agriculture, and wastewater treatment, as mentioned earlier [20]. The industrial-scale production of xanthan is generally conducted by aerobic submerged batch cultivation of the reference strain *Xanthomonas campestris* ATCC 13951 on the medium containing glucose or sucrose, and under optimal conditions [19]. Due to the increasing demand and rising costs of these carbon sources, xanthan is increasingly being produced on media containing alternative, low-value substrates such as industrial waste streams and by-products [21, 22]. Nevertheless, relatively few studies have investigated its properties and potential applications. The aim of this study was to investigate the potential of xanthan, produced on a winery effluents-based medium by different *Xanthomonas* strains isolated from infected crucifers, for environmental applications through evaluation of its emulsifying properties and its influence on the

removal of pindolol in water. The novelty of the present study lies in the first-time evaluation of xanthan produced from winery effluents by local isolates of *Xanthomonas* strains in terms of its prolonged emulsification performance and its potential in pindolol removal under UV irradiation, revealing different removal efficiencies. Although winery effluents have previously been investigated as substrates for xanthan production, to the best of our knowledge, neither the prolonged emulsification performance of the resulting biopolymer nor its application in pindolol removal has been previously reported.

2 Materials and methods

Xanthan production and characterization were carried out in laboratories at the Department of Biotechnology (Faculty of Technology Novi Sad, University of Novi Sad), while UV irradiation experiments were performed in laboratories at the Department of Chemistry, Biochemistry and Environmental Protection (Faculty of Sciences, University of Novi Sad).

2.1 Xanthan production

The reference strain *X. campestris* ATCC 13951 and *Xanthomonas* strain isolated from infected crucifers were used as the xanthan-producing microorganisms. Both strains were stored in the Microbial Culture Collection of the Faculty of Technology Novi Sad, Serbia, at 4 °C on agar slant (Yeast Maltose Agar, HiMedia, India) and subcultured every four weeks.

Xanthan production was carried out on cultivation medium containing effluents from different stages of white wine production, obtained from medium sized wineries located in Fruška Gora vineyards (Vojvodina, Serbia). Cultivation medium preparation (sugar content 25 g/L), inoculum preparation, xanthan biosynthesis, and its separation from the cultivation medium were performed as described in a previous study [23].

2.2 Determination of xanthan properties

Physical-chemical properties of xanthan samples OV and OV-II, obtained by cultivation of the reference strain *X. campestris* ATCC 13951 and *Xanthomonas* strain isolated from crucifers, respectively, on cultivation media containing winery effluents, were examined. Detailed procedures for determination of dry matter content, ash content, total nitrogen content, the average molecular mass and solubility of xanthan, as well as the pH value, electrical conductivity and rheological properties of 1% (w/v) xanthan solutions are provided in previous study [14].

2.3 Examination of xanthan application

2.3.1 Emulsification activity

The emulsification activity of OV, OV-II, and commercial xanthan K (Shandong Fufeng Fermentation Co., Ltd., China) in hydrocarbons (n-hexane, toluene, liquid paraffin, and chloroform) and oils (sunflower, soybean, and olive oil) was tested by measuring the emulsification index after 24 h and 240 h using a method described in a previous study [14].

These experiments were carried out in triplicate (three independent experimental replicates), and the results were averaged. Mean values and standard deviations for repetitions were calculated using Microsoft Excel [24]. The experimental data were processed using analysis of variance (two-way ANOVA) to evaluate the effects of the xanthan sample and hydrocarbon/oil used on the emulsification index after 24 h and 240 h of rest, including the interaction effect between the two factors. Duncan's multiple range test determined significant differences between the means of the emulsification index of samples obtained when using different hydrocarbons/oils and generated homogeneous groups of analyzed results. Statistical analysis was performed using TIBCO Statistica™ (14.0.0) software [25]. The significance level (α) was set at 0.05, representing the predefined acceptable risk of a Type I error, while the p-value (p) indicates the probability of obtaining results at least as extreme as those observed, assuming the null hypothesis is true. Results with $p < \alpha$ (0.05) were considered statistically significant.

2.3.2 UV irradiation experiments

Pindolol ($M = 309.4$ g/mol, $\geq 99\%$, Sigma-Aldrich, Hamburg, Germany) was used as received. The standard solution (0.05 mmol/dm³) was made with ultrapure water ($\kappa = 0.055$ μ S/cm, pH 6.6) and stored in a dark place.

Experiments were conducted using a pindolol solution exposed to UV irradiation in the presence of xanthan samples (1.0 mg/cm³). Prior to the UV irradiation experiments, dark controls containing pindolol and the examined xanthan samples were performed to assess possible adsorption. No measurable decrease in pindolol concentration was observed under these conditions. In the direct photolysis study, the solution underwent UV irradiation without the addition of xanthan. A 20 cm³ solution was placed in a Pyrex glass cell equipped with a plain window onto which the light beam was focused. Before irradiation, the suspension was sonicated for 15 min. Subsequently, a magnetic stirring bar was added to the cell, and the suspension was continuously stirred in the stream of O₂ (3.0 cm³/min rate), thermostated

at 25 ± 0.5 °C. The distance between the cell wall and the lamp was 10 cm [14]. As the UV radiation source, a 6 W UV-C lamp was utilized (Philips, type: TUV 6W G6T5, Bulb Lamp Germicidal Tube, Netherlands, with an intensity of 1.190 W/m² in the visible region and $187.6 \cdot 10^{-3}$ W/m² in the UV region). The radiation energy fluxes were measured using Delta Ohm HD 2102.2 (Padova, Italy) fitted with the LP 471 UV (spectral range 315–400 nm) and LP 471 RAD (spectral range 400–1050 nm) sensors.

To monitor the kinetics of pindolol removal, an ultra-fast liquid chromatography with diode array detection (UFLC-DAD, Shimadzu, Japan) system was used. Details on chromatographic conditions can be found in our previous study [10]. A glass electrode (SenTix 20, WTW, Xylem Analytics, Germany) connected to a pH meter (pH/Cond 340i, WTW, Xylem Analytics, Germany) was used to measure the pH value.

3 Results and discussion

3.1 Properties of xanthan samples

In addition to nontoxicity, biocompatibility, and biodegradability, the generally recognized as safe (GRAS) status is another very important reason why xanthan is widely used in various industries. Different types of xanthan are commercially available, exhibiting different properties and potential applications [26]. To assess the potential xanthan samples OV and OV-II in the removal of environmental pollutants, their characterization was conducted. The obtained results for relevant parameters are given in Table 1.

The data shown in Table 1 suggest that both OV and OV-II xanthan samples occur in regular form as free flowing powder of cream color. The values of dry matter, ash, and total nitrogen content for the OV sample are 85.44%, 18.99%, and 1.58%, respectively, while the

Table 1 Properties of xanthan samples

Parameters	Xanthan samples	
	OV	OV-II
Appearance	free flowing powder	free flowing powder
Color	dark cream	cream
Dry matter (%)	85.44	85.47
Ash (%)	18.99	9.69
Total nitrogen (%) (w/v)	1.58	1.27
Molecular mass (10^6 g/mol)	1.09	1.33
Apparent viscosity of 1% aqueous solution (mPa·s)	68.51	110.92
Solubility in water (4 °C to 60 °C)	complete	complete
Electrical conductivity (mS/cm)	2.11	1.21
pH of 1% aqueous solution	8.02	7.14

values of these parameters for sample OV-II are 85.47%, 9.69%, and 1.27%, respectively. Variations in dry matter, ash, and total nitrogen content among samples are probably caused by differences in purity.

The molecular mass of the OV-II sample $1.33 \cdot 10^6$ g/mol was comparably higher than the value of this parameter for sample OV which was $1.09 \cdot 10^6$ g/mol. This indicates that the selection of producing strain has a great effect on the structure of the produced biopolymer [27, 28]. These and the values of apparent viscosity of 1% aqueous solutions of OV and OV-II samples, which were 68.51 mPa·s and 110.92 mPa·s, respectively, support the findings from a previous study that indicate that xanthan samples of different structures may exhibit different solution properties [14]. Results in Table 1 show that 1% aqueous solutions of the analyzed xanthan samples are soluble in both, cold and hot water, indicating a great potential of both samples for environmental applications at different temperatures. The polyelectrolyte nature of the xanthan molecule responsible for its interactions with other materials is confirmed by the values of electrical conductivity of 2.11 mS/cm for a 1% aqueous solution of OV sample and 1.21 mS/cm for a 1% aqueous solution of OV-II sample. Finally, the pH value of a 1% aqueous solution of both samples was around neutral, which is important for the preservation of environmental quality. Distinctive properties of OV and OV-II samples compared to the commercial xanthan characterized in a previous study [14] are probably caused by variation in the production process and different degrees of purity.

3.2 Emulsification-based environmental application

Environmental pollution of water is a major concern that must be tackled on the highest priority to sustain the environment and future generations. In this study, the emulsification activity of OV, OV-II, and K samples was examined with widely used food oils and organic solvents such as n-hexane, toluene, chloroform, and liquid paraffin, as common water pollutants [29]. Statistical analysis of experimental data was carried out to examine the effect of different hydrocarbons/oils and sample type on the emulsification index of xanthan.

The two-way ANOVA revealed statistically significant effects of hydrocarbon/oil type, xanthan sample, and their interaction on the emulsification index (EI) after both 24 h and 240 h of rest ($p < 0.000001$). Hydrocarbon/oil type exhibited the highest F-values at both time points (2227.47 and 3593.04, respectively), indicating the strongest statistical effect among the factors examined. The interaction between hydrocarbon/oil type and xanthan sample was

also statistically significant at both time points, demonstrating that the emulsification performance of the xanthan samples depended on the hydrocarbon/oil used.

The results of Duncan's multiple range test [25] and summary results of the statistical analysis for the effect of different hydrocarbons/oils and xanthan samples on the emulsification index after 24 h and 240 h of rest are presented graphically with Box & Whisker Plots in Fig. 1.

The results given in Fig. 1 indicate that the emulsification activity of examined samples was different when using natural oils and hydrocarbons after 24 h and 240 h of rest. Emulsification index of xanthan samples after 24 h of rest (EI_{24}) was in the range from $4.92 \pm 0.08\%$ to $67.58 \pm 0.84\%$ (Fig. 1(a)). The highest value of EI_{24} was obtained when the emulsifying activity of OV-II sample in soybean oil was examined. In a previous study, the emulsifying activity of xanthan biosynthesized by reference strain *X. campestris* ATCC 13951 on medium containing crude glycerol from biodiesel production was examined in the same oils, and the highest value of EI_{24} of around 67%

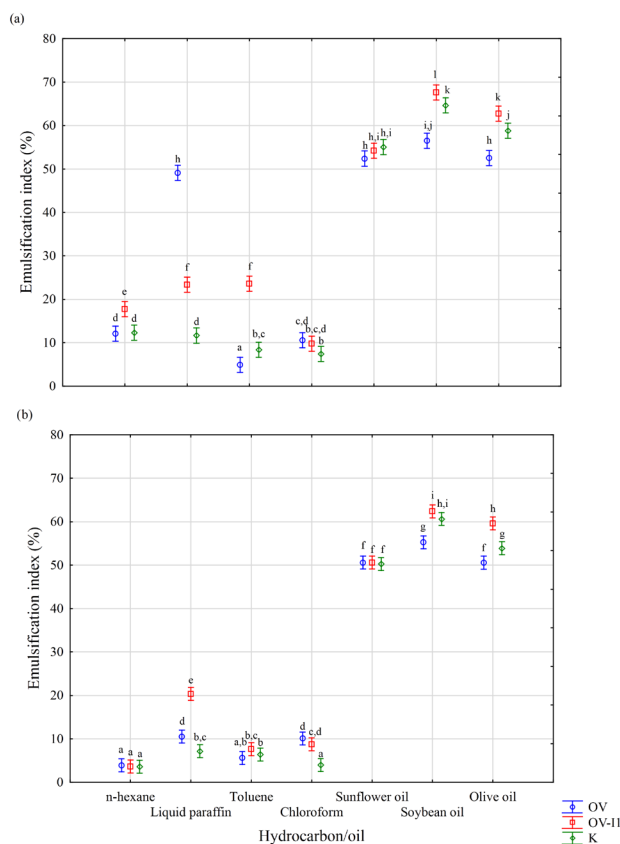


Fig. 1 Emulsification index of xanthan samples OV, OV-II, and K in different hydrocarbons/oils after (a) 24 h of rest and (b) 240 h of rest. Values for the same group of results marked with the same letter are not significantly different at the predefined significance level (α) = 0.05. The predefined significance level, usually set at 0.05, representing the acceptable risk of a Type I error.

was reported in soybean oil, too [14]. The results given in Fig. 1(a) clearly indicate the difference in the emulsifying activity of the examined xanthan samples in hydrocarbons and oils used. According to the obtained results, the emulsifying activity of xanthan samples in hydrocarbons after 24 h of rest was lower than 50%. The OV sample exhibited the highest emulsification activity in liquid paraffin among other hydrocarbons, and the value of EI_{24} in this case was $49.11 \pm 0.89\%$. This finding is consistent with a previous study, in which xanthan exhibited the highest emulsification index after 24 h of rest when liquid paraffin was used, compared with other hydrocarbons [30]. The lowest value of EI_{24} of xanthan samples in different hydrocarbons/oils was $4.92 \pm 0.08\%$ and it was obtained when the emulsifying activity of the OV sample was examined in toluene. The values of EI_{24} in natural oils obtained in this study are comparably higher than the values reported in earlier research, where the value of EI_{24} in different oils for xanthan biosynthesized on commercial medium by *X. campestris* was in the range from 8.75% to 33.75% [31]. Based on the results presented in Fig. 1 (a), sample OV-II exhibited higher emulsifying activity in the tested hydrocarbons/oils after 24 h of rest than sample K, except in sunflower oil, whereas sample OV showed lower emulsifying ability than sample K in the tested natural oils. This suggests that different strains have a great effect on the biopolymer structure, and therefore on its properties, as confirmed in a previous study where different pathovars of *Xanthomonas* isolates were used for xanthan production [32].

Research regarding the emulsifying activity of xanthan is mainly based on the determination of the emulsification index of this biopolymer in different hydrocarbons and oils after 24 h of rest, and there is no available data on the emulsification activity for a longer period of time [14, 31, 33]. Thus, in this study, the emulsifying activity after 240 h was also examined.

The results of the statistical analysis for the effect of different hydrocarbons and natural oils, and xanthan samples on the emulsification index after 240 h of rest are shown in Fig. 1(b). The values of emulsification index of xanthan samples after 240 h of rest (EI_{240}) were in the range from $3.57 \pm 0.13\%$ to $62.37 \pm 0.50\%$. Compared to the previously discussed results presented in Fig. 1(a), it can be seen that in this case, the highest value of emulsification index was also obtained when the emulsifying activity of the OV-II sample in soybean oil was examined. This value was $62.37 \pm 0.50\%$ and there was no significant difference between this and the value of the EI_{240} obtained when emulsifying activity of K sample was examined in

soybean oil which amounted $60.62 \pm 0.50\%$ ($p = 0.100383$). According to the obtained results OV-II sample exhibited the highest emulsification activity in liquid paraffin among other hydrocarbons and the value of EI_{240} for this case was $20.34 \pm 0.34\%$. On the other side, the values of EI_{240} for sample OV were $10.51 \pm 1.57\%$ in liquid paraffin, $10.10 \pm 1.60\%$ in chloroform, and less than 10% in other hydrocarbons. The obtained results indicate that the lowest value of EI_{240} was obtained when the emulsifying activity of OV, OV-II, and commercial xanthan was examined in n-hexane, and that there is no significant difference between their values, which is confirmed by p -values of 0.788316, 0.773376, and 0.968902, respectively.

The results of Duncan's multiple range test and summary results of the statistical analysis for the effect of different hydrocarbons/oils and xanthan samples on the emulsification index after 24 h and 240 h of rest obtained in this study suggest that the selection of hydrocarbon/oil and xanthan samples is of great importance for their emulsifying activity. All samples examined in this study showed better results when using natural oils. Further, sample OV-II showed greater emulsifying activity for all hydrocarbons and oils, except for liquid paraffin, after 24 h of rest. Even though sample OV showed a better ability to form and stabilize emulsion with liquid paraffin after 24 h of rest, OV-II proved to have better potential after 240 h of rest. The reason why liquid paraffin stands out among other hydrocarbons used in this study, when emulsifying activity for 24 h and 240 h of rest is observed may be attributed, at least in part, to its physicochemical properties. The dielectric constants of n-hexane, toluene, liquid paraffin, and chloroform are 1.90, 2.38, 2.25, and 4.81, respectively. The relatively low dielectric constant of liquid paraffin indicates its low polarity, which, together with high viscosity, interfacial properties, phase compatibility may influence its interactions with the emulsifying components and consequently affect emulsion formation and stability [34, 35]. The main reason for the better emulsifying activity of OV-II comparing to K and OV samples could be attributed to its viscosity originated from its molecular mass, considering that this property is a key factor in emulsifying property of a polymer [36], as well as to its electrical conductivity which is responsible for the ability to interact with other compounds [37].

Taking into account all that has been discussed above, it can be noticed that the selection of producing strain for xanthan production has a significant effect on the structure of this biopolymer and its properties, and that the OV-II sample has a great potential for application in emulsification-based

environmental application of natural oils from wastewaters and the environment. In order to improve the emulsifying activity of biopolymers in hydrocarbons, the effect of aqueous phase composition (salinity and pH) and temperature on emulsification index should be examined. Furthermore, integrating sustainable agro-industrial residues as substrates with advanced machine learning optimization could significantly enhance the techno-economic viability of producing specialized variants like the OV-II sample, bridging the gap between circular economy goals and high-performance environmental remediation technologies [38].

3.3 Application in pindolol removal

In this investigation, pindolol direct photolysis was evaluated and compared with experiments performed in the presence of xanthan samples. After 180 min, direct photolysis under UV irradiation proved inefficient in pindolol removal (Fig. 2). In Fig. 2 c represents the pindolol concentration at a given irradiation time, while c_0 represents its initial concentration. Successful direct photolysis requires an overlap between the absorption spectrum of the investigated compound and the emission spectra of the lamp. Considering the absorption maximum of pindolol (217 nm) and the used lamp (approximately 250 nm), the obtained results were anticipated.

The presence of OV did not have an effect on the removal of pindolol from the water solution (Fig. 2). The presence of K and OV-II resulted in increased pindolol removal. In the presence of K, 15% of pindolol was removed after 180 min of irradiation (Fig. 2), whereas the highest removal efficiency was for OV-II (43%).

Results obtained by this study are focused on the removal of the pindolol molecule from the solution and therefore do

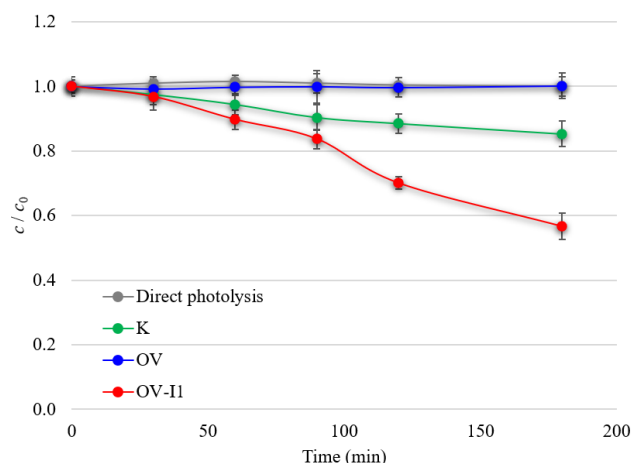


Fig. 2 Removal of pindolol in the presence of xanthan samples OV, OV-II, and K under UV irradiation

not provide information on its degradation mechanism and mineralization. Future studies could focus on the identification of degradation intermediates and the assessment of mineralization, in order to clarify the degradation pathway and evaluate the environmental safety of this process.

The pH value of the solutions was monitored during the UV irradiation experiments (Fig. 3). During direct photolysis, a consistent pH value of around 10.0 was recorded. The addition of xanthan samples slightly decreased the initial pH of the standard solution from 10.0 to 9.7 for K and OV-II, and to 9.4 for OV. Throughout the experiments, the pH value did not change significantly.

The enhanced pindolol removal observed in the presence of OV-II (Fig. 2) may be related to differences in its physicochemical properties (Table 1). Compared to OV, this sample had lower ash content and higher molecular mass and apparent viscosity. These differences indicate that the choice of producing strain affected the structure and properties of the obtained xanthan. Although OV showed higher electrical conductivity, OV-II achieved higher pindolol removal. Therefore, the observed differences in pindolol removal cannot be explained by electrical conductivity alone. However, a direct causal relationship between individual physicochemical properties of xanthan and the enhanced pindolol removal should be further examined, since different factors can contribute to the obtained results. The results suggest that several physicochemical properties of xanthan may influence its performance during pindolol removal. The precise mechanism responsible for the enhanced pindolol removal in the presence of OV-II remains to be established.

4 Conclusion

The results of this study showed that xanthan produced on cultivation medium containing winery effluents can form emulsions with different hydrocarbons and oils. These

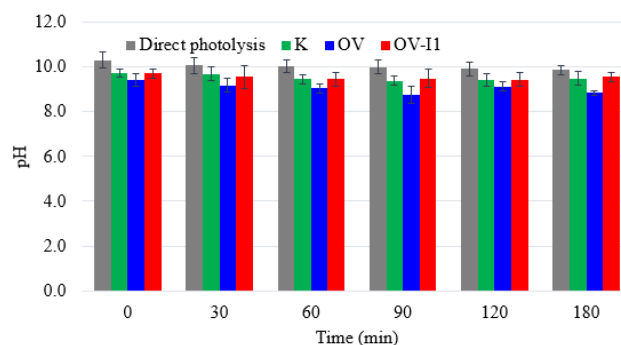


Fig. 3 Change in pH during examination of the influence of xanthan samples OV, OV-II, and K on the efficiency of pindolol removal under UV irradiation

findings indicate that winery effluents may be used as a suitable substrate for xanthan production, while the obtained biopolymer may find application in processes involving hydrocarbons and oils. Moreover, the presence of xanthan samples K and OV-II increased the efficiency of pindolol removal under UV irradiation, resulting in 15% and 43% removal, respectively. On the other hand, OV did not significantly affect pindolol removal. The obtained results suggest that differences in the physicochemical properties of xanthan may influence its performance during pindolol removal.

Overall, the results obtained in this study support the feasibility of producing xanthan from winery effluents and highlight its potential for environmental applications. However, several challenges should be considered for the integration of xanthan production into a winery-wastewater biorefinery. The variable composition of winery effluents, particularly in terms of organic load and nutrient availability, requires appropriate substrate characterization and may necessitate adjustments to the medium preparation procedure, while also affecting bioprocess performance and xanthan productivity. In addition, at larger scales, sterilization requirements and downstream recovery and purification may increase water and energy consumption and affect the overall process efficiency. Further optimization of bioprocess conditions and recovery strategies is needed to improve performance and feasibility of biosynthesis at larger scales. An integrated approach in which xanthan production is coupled

with existing winery wastewater-treatment infrastructure could potentially reduce resource consumption while simultaneously contributing to wastewater valorization and the recovery of value-added bioproducts. Although the use of winery effluent as a substrate offers a promising strategy for improving resource efficiency and supporting circular bioeconomy approaches, its overall environmental benefit depends on the resources required throughout the production process. In particular, energy and water consumption associated with cultivation, sterilization, downstream processing, and xanthan recovery should be considered when evaluating the sustainability of the proposed approach. Therefore, the substitution of conventional substrates with winery effluent does not inherently guarantee a lower environmental footprint. Future studies should address these aspects through bioprocess optimization and comprehensive environmental assessment, including life-cycle assessment, to determine the overall sustainability and environmental benefits of xanthan production using winery effluent.

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