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EXAMINATION OF XANTHAN PRODUCTION ON BIODIESEL INDUSTRY EFFLUENT-BASED MEDIUM IN LAB-SCALE BIOREACTOR

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ABSTRACT

Xanthan is microbial polysaccharide with outstanding rheological properties, non-toxic nature, biodegradability, and biocompatibility. This biopolymer is widely used in food, biomedical, pharmaceutical, petrochemical, chemical and textile industry. Industrial xanthan production is generally conducted by aerobic submerged cultivation of *Xanthomonas campestris* strains on the media with glucose or sucrose under optimal conditions. Results from previous research indicate that xanthan can be successfully produced on media containing crude glycerol from biodiesel industry by different *Xanthomonas* species. The aim of this study was to examine the course of xanthan biosynthesis by the reference strain *X. campestris* ATCC 13951 in lab-scale bioreactor on medium containing crude glycerol generated in domestic biodiesel factory. The bioprocess was monitored by the analysis of cultivation medium samples taken in predetermined time intervals, and its success was estimated based on the xanthan concentration in the medium, separated biopolymer average molecular weight and degree of nutrients conversion. At the end of bioprocess, cultivation medium contained 12.34 g/L of xanthan with the average molecular weight of $3.04 \cdot 10^5$ g/mol. Within this study, the achieved degree of glycerol, total nitrogen and total phosphorous conversion were 75.91%, 53.27% and 38.96%, respectively.

Keywords: xanthan, lab-scale bioreactor, biodiesel industry effluent, crude glycerol

1. INTRODUCTION

Xanthan is known as one of the most widely examined polysaccharides of microbial origin [1]. This bacterial-derived biopolymer is extensively used in food, cosmetics, pharmaceutical, paper, textile and other industries owing to its exceptional rheological properties, non-toxic nature, biodegradability, and biocompatibility [2, 3]. Besides the industry, xanthan is also widely used in medicine, biomedical engineering, agriculture, and wastewater treatment. Market demand for xanthan has been increasing progressively, with an annual rate of 5–10%. The estimated production of xanthan is believed to be 30000 tons per year and since 2005, China has become one of the largest xanthan producers [4].
The selection of the producing strains, cultivation medium composition, and bioprocess parameters highly affect the success of xanthan production [5]. Although various *Xanthomonas* species, such as *X. malvacearum*, *X. phaseoli*, *X. axonopodis* and *X. euvesicatoria*, are able to biosynthesise xanthan, *X. campestris* is most commonly used producing strain in industry [6, 7]. Commercial production of xanthan is generally conducted as aerobic submerged batch cultivation of the reference strain *X. campestris* ATCC 13951 on appropriately formulated media under optimal conditions [8]. Cultivation medium for xanthan production has a clearly defined composition, in favour of providing the necessary macronutrients, of which the most important are carbon and nitrogen [9]. Generally used carbon sources in the media for xanthan production are glucose and sucrose [10], while yeast extract, casein hydrolysates, peptone, soy flour, ammonium and nitrate salts are mostly used as nitrogen sources [11]. Based on the literature data, the highest xanthan yield is achieved when yeast extract is used as a nitrogen source in cultivation medium [12]. Taking into account that the cost of substrate is crucial factor for commercial xanthan production and the rising prices of the aforementioned nutrients, it is of great importance to find more economical carbon and nitrogen source in order to reduce the overall production costs [13]. Since carbon source is the major

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component of xanthan production medium, there is a need to first explore suitable alternative for this nutrient. Waste streams and by-products from different industries are heterogeneous in their composition and that is why they have a great potential as alternative substrate for microbial production of various products, including xanthan. According to the results from previous studies xanthan can be successfully obtained on media containing carbon sources from kitchen and agro-industrial waste and by-products [14-16]. However, usage of aforementioned alternative substrates in xanthan production is limited due to their intensive utilization for therapeutic application and production of other high value-added products [17-19] and thus there is a need for exploitation of other alternative substrates of lower market value.

Several research studies have confirmed that crude glycerol can be successfully used in biotechnological production of xanthan [5, 20]. According to the literature, some *Xanthomonas* isolates are able to metabolize glycerol in higher degree than glucose [7]. Considering that research attributed to the xanthan biosynthesis on glycerol-based media is still in initial stages and there is a need for improvement, and thereupon, it is necessary to monitor the course of the cultivation of the selected strain on a crude glycerol-based medium in order to observe the critical points of the bioprocess and define the next steps in the research.

The aim of this study is to examine xanthan biosynthesis by cultivation of the reference strain *X. campestris* ATCC 13951 in a lab-scale bioreactor on medium containing crude glycerol from biodiesel production in domestic factory. The bioprocess was monitored by the analysis of cultivation medium samples taken in predetermined time intervals, in terms of rheological behaviour, biomass concentration, as well as content of essential nutrients for biotechnological production of xanthan. Bioprocess efficiency was estimated based on the quantity and quality of separated xanthan and conversion rate of the most important nutrients at the end of biosynthesis.

2. MATERIALS AND METHODS

2.1 Producing microorganism and inoculum preparation

The reference strain *X. campestris* ATCC 13951 was used as the producing microorganism in this research. The applied strain was stored at 4ºC on agar slant (Yeast Maltose Agar, HiMedia, India) and subcultured every four weeks within the Microbial Culture Collection of the Faculty of Technology Novi Sad, Serbia.

Commercial liquid medium (Yeast Maltose Broth, HiMedia, India) was used for its incubation during inoculum preparation. Prepared media were sterilized by autoclaving (121°C, 2.1 bar, 20 min).

Within this study, inoculum was prepared in two steps: Inoculum I and Inoculum II. Producing microorganism was subcultured on agar slant and incubated at 25°C for 48 h. Inoculum I preparation procedure was included suspending of producing microorganism cells in commercial liquid medium. The prepared suspension was then incubated in aerobic conditions at 25°C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany) for 48h. Inoculum II preparation was performed by adding 10% (v/v) of Inoculum I in commercial liquid medium followed by incubation in identical conditions as for inoculum I preparation.

2.2 Xanthan production

Biotechnological production of xanthan was conducted on medium containing crude glycerol from biodiesel production in factory located in the Republic of Serbia. Glycerol content in crude glycerol was around 50% (w/v) and its content in cultivation medium was adjusted to around 17.00 g/L, based on the results from the previous study [21, 22]. The cultivation medium also contained yeast extract (3.0 g/L), $(NH_4)_2SO_4$ (1.5 g/L), K_2HPO_4 (3.0 g/L) and $MgSO_4 \cdot 7H_2O$ (0.3 g/L). The pH value of production medium was adjusted to 7.0±0.2 and then sterilized by autoclaving (121°C, 2.1 bar, 20 min).

The xanthan production was carried out in 3 L lab-scale bioreactor (Biostat® A plus, Sartorius AG, Germany) with 2 L of crude glycerol-based cultivation medium. Inoculation was performed by adding 10%

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(v/v) of inoculum prepared as previously described. The xanthan biosynthesis was carried out under aerobic conditions for 168 h. In the first 48 h, the biosynthesis was performed at temperature of 25°C, air flow rate of 1 vvm and agitation rate of 200 rpm, and afterwards, temperature and air flow rate were increased to 30°C and 2 vvm, respectively, while agitation rate was corrected as needed and according to the dissolved oxygen concentration which was maintained at values higher than 30% during the biosynthesis.

At the end of biosynthesis, xanthan was separated from the supernatant of cultivation medium (10,000 rpm, 10 min, ultracentrifuge Hettich Rotina 380 R,Germany) by precipitation with cold 96% (v/v) ethanol in the presence of potassium chloride. Ethanol was gradually added to the supernatant at constant stirring until the alcohol content in mixture was 60% (v/v). 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% (v/v). After precipitation, the mixture was kept on 4°C, for 24 h and then centrifuged (4,000 rpm, 15 min). The precipitate was dried to a constant mass at 60°C and this data was used to calculate the xanthan concentration in medium.

2.3 Analysis of cultivation media

The biomass concentration was expressed as viable cell count per millilitre of cultivation medium and is determined by counting of colony forming units (CFU). The samples of cultivation medium, taken under aseptic conditions, were serially diluted in sterile saline solution and plated on agar plates (Yeast Maltose Agar, HiMedia, India) which were incubated at 25°C for 48 h. Living bacterial cell on the plate was grown into a colony, and the viable cell count in the cultivation medium was then calculated by multiplying the final number of colonies by the dilution factor.

The samples of cell-free cultivation media taken in previously defined time intervals, obtained by centrifugation at 10,000 rpm for 15 min (Rotina 380 R, Hettich Lab Technology, Germany), were analysed for glycerol, total nitrogen and total phosphorus contents.

Glycerol content was determined by high performance 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 HPG-3200SD/RS pump, WPS-3000(T)SL autosampler (10 μL injection loop), Zorbax NH2 column (250 mm × 4.6 mm, 5 μm) and RefractoMax520 detector. 70% (v/v) acetonitrile was used as eluent with a flow rate of 1 mL/min and elution time of 10 min at a column temperature of 30°C. The contents of total nitrogen and phosphorus were determined using volumetric method proposed by Kjeldahl [23] and spectrophotometric method [24], respectively.

The nutrient content results were used to calculate degree of crude glycerol, total nitrogen and total phosphorus conversion (K, %) using Equation (1):

$$K_Y = \frac{(Y_0 - Y)}{Y_0} \cdot 100 \quad (1)$$

where Y_0 is initial nutrient content (g/L), while Y is residual nutrient content (g/L).

The results for glycerol content after inoculation and xanthan concentration in medium were used to calculate the degree of initial glycerol conversion into xanthan ($K_{P/S}$, %) using Equation (2):

$$K_{P/S} = \frac{P}{S_0} \cdot 100 \quad (2)$$

where S_0 is the initial glycerol content (g/L) and P is the xanthan concentration in medium at the end of bioprocess (g/L).

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The initial and residual glycerol content results as well as xanthan concentration in medium were used to calculate the degree of metabolized glycerol conversion into xanthan ($K_{P/\Delta S}$, %) using Equation (3):

$$K_{P/\Delta S}=\frac{P}{S_0 - S}\cdot 100 \quad (3)$$

where S_0 is the initial glycerol content (g/L), S is the residual glycerol content (g/L), and P is the xanthan concentration in medium at the end of bioprocess (g/L).
The rheological behaviour of cultivation medium samples taken in previously defined time intervals were evaluated 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 Equation (4):

$$\tau = 0.1 \cdot z \cdot \alpha \quad (4)$$

where z is the constant with the value 3.08 dyn/cm^2·Skt.
The pseudoplastic behaviour of the cultivation medium was confirmed by fitting the experimental data to the Ostwald-de-Waele model using the power regression. The values of the consistency factor (K, Pa·s^n), flow behaviour index (n) and determination coefficient (R^2) were determined by Excel software 2013 and used for calculation of medium apparent viscosity (η_a, mPa·s) from Equation (5):

$$\eta_a = K \cdot D^{n-1} \quad (5)$$

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

2.4 Analysis of xanthan

The average molecular weight of the separated xanthan was estimated based on the intrinsic viscosity of its 1% (w/v) solution in 0.1 M sodium chloride using the Mark-Houwink type equation [25].

3. RESULTS AND DISCUSSION

In accordance with the defined aim of this research, xanthan was produced by reference strain *X. campestris* ATCC 13951 on medium prepared with crude glycerol generated in domestic biodiesel facility. The bioprocess course in applied experimental conditions was monitored by the analysis of cultivation medium samples, taken in previously defined time intervals, in terms of rheological behaviour, biomass concentration, as well as content of essential nutrients for biotechnological production of xanthan. The obtained results are graphically represented in Fig. 1. The bioprocess success was assessed based on the xanthan concentration in medium, separated biopolymer average molecular weight, and degree of conversion of total glycerol, initial glycerol and metabolized glycerol into xanthan, nitrogen and phosphorus and results of these analyses are summarized in Tab. 1.
Xanthan production was conducted under controlled conditions, and pH, temperature and dissolved oxygen concentration of cultivation media were measured and regulated by adding acid or base and by adjustment of mixing speed and aeration intensity, respectively. During xanthan production, the pH of cultivation medium decreases from neutral to values close to 5 due to the production of organic acids and xanthan which contains acid groups [9]. Considering the fact that the optimum pH for the bacterial growth range is between 6 and 7.5 and the optimum pH range for the xanthan production is between 7 and 8 [26], this parameter was regulated during the bioprocess, i.e. it was maintained above 6.0 by adding 5.0 M KOH.

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The dissolved oxygen concentration was maintained at values greater than 30% of saturation during the entire bioprocess by regulating the aeration intensity and mixing speed as recommended in literature [27].

3.1 Monitoring of the xanthan production

Cell concentration in the medium at the beginning of cultivation was $4.40 \cdot 10^8$ CFU/mL. Based on the graphically presented results in Fig. 1, it can be seen that during the first 48 h of cultivation the producing microorganism multiplied intensively and the cell concentration in the medium increased to $1.02 \cdot 10^{10}$ CFU/mL. This denotes that exponential growth phase of producing microorganism occurred since the beginning of bioprocess and lasted until 48 h. Between 48 h and 72 h, a slower increase of biomass concentration was achieved, getting to a maximum value of $1.16 \cdot 10^{10}$ CFU/mL in 72 h. There was no significant change in the biomass concentration after 72 h, and hence it can be believed that the stationary phase of producing microorganism growth has occurred. After 168 h of biosynthesis, the biomass concentration was $1.14 \cdot 10^{10}$ CFU/mL. The behaviour of applied producing strain is in agreement with the behaviour of another *Xanthomonas* strain which was cultivated in similar conditions, indicating that achieved biomass concentration is appropriate for successful xanthan production on crude glycerol-based medium [22].

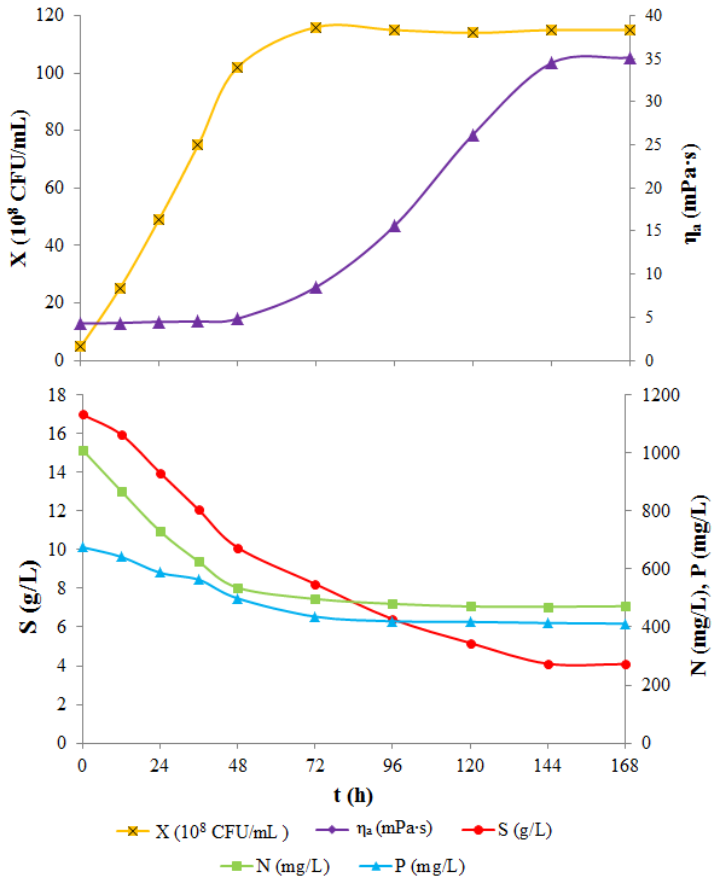

Figure 1. The course of xanthan production in 3 L lab-scale bioreactor on crude glycerol-based medium in terms of biomass concentration (X), medium apparent viscosity (η_a), glycerol content (S), total (N) content, and total phosphorus content (P)

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The xanthan production under the experimental conditions was evaluated according to the rheological behaviour of the crude glycerol-based medium during the cultivation of the applied producing strain. Rheological measurements of cultivation medium were performed and the obtained values were fitted with a power law equation of Ostwald-de Waele model in order to define the values of the flow behaviour index and the consistency factor, on the basis of which the apparent viscosity of cultivation medium was calculated. Accordingly, the change in the apparent viscosity of cultivation medium during the xanthan production by the reference strain *X. campestris* ATCC 13951 in lab-scale bioreactor on medium containing crude glycerol is also represented in Fig. 1. As it can be seen from graphically presented results, the value of apparent viscosity of the medium during biosynthesis did not change significantly up to 48 h. Initial value of apparent viscosity of the medium was 4.23 mPa·s and in 48 h the value of this parameter was 4.79 mPa·s. After 48 h of cultivation a serious increase in the apparent viscosity of the cultivation medium was noticed up to 144 h, when its value amounted 34.78 mPa·s. Based on the results shown in Fig. 1 it can be noticed that the value of the apparent viscosity of the cultivation medium increased with lower intensity after 144 h of cultivation, and at the end of biosynthesis, the value of this parameter was 35.12 mPa·s. Moreover, the rheological measurement showed that all samples have pseudoplastic properties, a known characteristic of xanthan solutions [9]. This finding is supported by the values of flow behavior index which decreased from 0.5834 to 0.4767 indicating that during the cultivation in applied experimental conditions pseudoplastic behavior of crude glycerol-based medium became more pronounced. The Ostwald-de-Waele model showed a good agreement with the experimental data, since the regression coefficients were higher than 0.92 for all tested samples.

Considering that the concentration of carbon source in cultivation media affects the xanthan yield, cultivation medium samples were analysed in terms of glycerol content. The results shown in Fig. 1 indicate that the glycerol content in cultivation medium decreased during the xanthan production in applied experimental conditions. According to the results presented in Fig. 1, it can be noticed that during the first 48 h of cultivation there was an intense decrease in the glycerol content from initial 16.98 g/L to a value of 10.11 g/L. After 48 h, the glycerol content continued to decrease with a lower intensity until 144 h. In 144 h the content of this nutrient was 4.11 g/L. After 144 h of cultivation, there was no significant change in the glycerol content in the medium and its value in 168 h was 4.09 g/L. The obtained results indicate that if there is no carbon source consumption, xanthan biosynthesis does not occur in the stationary phase of producing microorganism growth [11] and the bioprocess can be shorten for 24 h. The obtained results demonstrate that intensive metabolic activity of reference strain *X. campestris* ATCC 13951 appeared in the first 48 h of cultivation in 3 L lab-scale bioreactor, confirming that the used producing strain has the ability to metabolize crude glycerol from biodiesel industry in applied experimental conditions. This finding is in agreement with the results from previous studies when performing the bioprocess by the different producing strains [22, 28].

Nitrogen source also represent an essential nutrient in xanthan production media [9]. The obtained results given in Fig. 1 show that initial content of total nitrogen in medium decreased intensively from the very beginning of the cultivation. Therefore, the total nitrogen content was reduced from the initial 1010 mg/L to 536 mg/L in the first 48 h. As it can be seen in the Fig. 1, the period of intensive consumption of nitrogen components is in accordance with the exponential growth phase of the producing microorganism. After 48 h of cultivation there was no significant change in the total nitrogen content, which is a result of the onset of the stationary growth phase of the microorganism. At the end of bioprocess, the residual concentration of overall nitrogen components was 472 mg/L.

According to the literature, phosphorous have a great effect on the bacterial growth and the production of xanthan [9], and therefore, cultivation medium samples were also analysed in terms of phosphorous content. The results shown in Fig. 1 indicate that an intensive consumption of phosphorus is evident. Considering all previously discussed results, it can be noted that the change in the total phosphorus content is similar to the change in the content of total nitrogen during the xanthan production on crude glycerol-based medium. The initial phosphorus content of 675.10 mg/L decreased intensively in the first 48 h, to the

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value of 499.20 mg/L. The change in the total phosphorus content in cultivation medium was insignificant after 48 h and at the end of cultivation phosphorus content was 412.02 mg/L.
Taking into account the results for the change in the content of the most important nutrients in crude glycerol-based medium during this bioprocess, it can be concluded that the metabolic activity of used producing strain in applied experimental conditions was easily carried out, i.e. that the biomass concentration has increased, and that all conditions for the successful xanthan biosynthesis were provided. Additionally, the discussed changes in the value of apparent viscosity of the medium during the cultivation of used producing strain are a reliable confirmation that xanthan was produced in the applied experimental conditions.

3.2 Efficiency of xanthan production

With the purpose of observing performance of xanthan production on crude glycerol-based medium, selected indicators of bioprocess success were determined and the obtained results are given in Tab. 1.

Table 1. Indicators of the success of xanthan production by reference strain X. campestris ATCC 13951 in 3 L lab-scale bioreactor on crude glycerol-based medium

Indicator	P (g/l)	$K_{P/S}$ (%)	$K_{P/\Delta S}$ (%)	K_S (%)	K_N (%)	K_P (%)	M_w (10^5 g/mol)
Value	12.34	72.67	95.73	75.91	53.26	38.96	3.04

P - xanthan concentration in medium; $K_{P/S}$ - degree of initial glycerol conversion into xanthan; $K_{P/\Delta S}$ - degree of metabolized glycerol conversion into xanthan; K_S - degree of glycerol conversion; K_N - degree of total nitrogen conversion; K_P - degree of total phosphorus conversion; M_w - average molecular weight of xanthan.

At the end of the cultivation, xanthan concentration in the medium was 12.34 g/L (Tab. 1). This value is higher comparing to the values obtained in previous researches, when xanthan was produced by *Xanthomonas* strains, isolated from pepper leaves and crucifers, on crude glycerol-based medium in smaller volumes (300 mL Erlenmeyer flasks and 2.0 L Woulff bottles) and in the similar lab-scale bioreactor (3.0 L) where xanthan concentration in media varied from around 5.00 g/L to 11.00 g/L [22, 28, 29]. Xanthan concentration obtained in this study is also higher in comparison with the results obtained when reference strain *X. campestris* ATCC 13951 was cultivated on medium containing crude glycerol (15.00 g/L). In this research, xanthan concentration in media varied from 6.77 g/L to 7.22 g/L [30]. Considering the aforementioned results and results obtained in research where xanthan content of 5.59 g/L was achieved during the *X. campestris mangiferaeindicae* 2103 cultivation on crude glycerol-based medium (20 g/L) in a 4.5 L bioreactor [20], it is clear that xanthan biosynthesis conducted within this research was very successful if the concentration of produced biopolymer is considered as an indicator. Findings from this study also indicate that producing strain, medium composition, process parameters, as well as the geometry of the vessel in which the bioprocess is carried out has a great effect on xanthan biosynthesis.
In addition to xanthan production, the conversion of important nutrients presents a very important indicator of the bioprocess success. Previously discussed results from Fig. 1 indicate that the glycerol, total nitrogen and total phosphorus content in the medium decreased during the xanthan biosynthesis in applied experimental conditions. At the end of bioprocess, degree of glycerol conversion was very high, amounting 75.91%. Initial and metabolized glycerol conversions into xanthan were also high, amounting 72.67% and 95.73%, respectively (Tab. 1). Conversion of glycerol achieved in this study is higher comparing to the value of the degree of glycerol conversion of 63.14% obtained in previous study when the same strain was cultivated on crude glycerol-based medium but in smaller volume and less intensive bioprocess conditions [28]. The value of the degree of glycerol conversion achieved in present study is also higher comparing to the value of this parameter of 62.82%, achieved when xanthan was produced by *Xanthomonas* PL 3 strain in crude glycerol-based medium (glycerol content of 20 g/L) in a 3 L lab-scale bioreactor [22]. The values of metabolized glycerol conversion into xanthan and initial glycerol conversion into xanthan in

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aforementioned research conducted by the authors was far lower comparing to the value achieved in the present study. In industrial conditions, the degree of carbon sources conversion into xanthan ranges from 50-85% [31], so it can be concluded that in this research, very high efficiency of bioprocess has been achieved.

As mentioned earlier, nitrogen and phosphorus sources are also essential nutrients in cultivation media for xanthan production. Based on the data presented in Tab. 1 total nitrogen conversion achieved within this study was 53.26%, and this value is greater comparing to the value of 33.07% achieved in previous study when the cultivation of the same producing strain on crude glycerol-based medium was performed in 2.0 L Woulff bottle [29] for 168 h with inoculum prepared for 72 h and value of 41.51 achieved when xanthan production was conducted by *Xanthomonas* PL 3 strain on medium containing crude glycerol [22]. The value of total phosphorus conversion of 38.96% obtained in the present research is higher comparing to the result obtained in aforementioned research when degree of total phosphorus conversion during the cultivation of the *Xanthomonas* PL 3 strain on crude glycerol-based medium was 24.80% [22]. This finding indicates that among other parameters, the success of xanthan production is highly influenced by the selection of producing strain, as reported previously [5, 7].

The quality of xanthan is also an important indicator of xanthan production efficiency and it can be estimated based on several parameters, such as the viscosity of its solutions, composition, molecular weight, etc. [32]. In the present study, the average molecular weight of separated xanthan was used as biopolymer quality indicator. From the results given in Tab. 1 it can be seen that average molecular weight of xanthan produced on medium containing crude glycerol from biodiesel production is $3.04 \cdot 10^5$ g/moL. This indicates that the findings from this study are in accordance with the results obtained in previous study where xanthan biosynthesis was performed on crude glycerol-based medium by different *Xanthomonas* strains, isolated from crucifers and pepper leaves. Average molecular weight of separated biopolymers in this research was in the range from $5 \cdot 10^4$ g/moL to $3.0 \cdot 10^5$ g/moL [22]. The obtained results also indicate that xanthan produced within present study is of greater quality, if the average molecular weight of separated xanthan was used as biopolymer quality indicator, than xanthan produced by *Xanthomonas* PL 3 strain in similar conditions [22].

Taking all the results from Fig. 1 and Tab. 1 into consideration, it can be concluded that xanthan production by cultivation of reference strain *X. campestris* ATCC 13951 in 3 L lab-scale bioreactor on medium containing crude glycerol from biodiesel production was successful. Biotechnological production of xanthan on crude glycerol-based medium represents a promising solution for sustainable valorisation of this effluent demonstrating a promising potential for purpose of minimizing the negative impact of crude glycerol from biodiesel production on the environment.

4. CONCLUSIONS

The obtained results have confirmed that crude glycerol generated by the domestic biodiesel industry can be used as a sole carbon source in cultivation medium for a successful xanthan production by reference strain *X. campestris* ATCC 13951. Besides the good quality and quantity of the produced biopolymer, acceptable conversion of essential nutrients was also achieved within this research. The results of this study have a great importance from an ecological point of view, considering the fact that the biotechnological production of xanthan on a cultivation medium containing crude glycerol from the biodiesel industry represents a promising solution for the sustainable valorization of this effluent.

Moreover, the results obtained in this study represent valuable information that can be used in future investigations related to the optimization of the bioprocess in terms of increasing the xanthan yield and quality, the bioprocess scale-up, as well as the estimation of possible applications of this biopolymer.

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