DEFINITION OF INITIAL PHOSPHORUS SOURCE CONCENTRATION IN WASTE GLYCEROL-BASED MEDIUM FOR XANTHAN BIOSYNTHESIS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

Phosphorus nutrition appears to be very important in the xanthan production, next to carbon and nitrogen. Various studies indicated that xanthan production is controlled by phosphorus concentration in cultivation medium and that phosphorus is generally added in the form of phosphate salts. This paper presents an analysis of the effect of various K_2HPO_4 concentrations (0-3.00 g/L) in waste glycerol-based medium on xanthan production using *Xanthomonas* strain PL 4 isolated from pepper leaves. The bioprocess success was assessed based on the values of xanthan concentration in media at the end of biosynthesis, average molecular weight of the polymer, degree of glycerol conversion into xanthan and degree of total phosphorus conversion. The obtained results show that the increase of K_2HPO_4 concentration in medium from 0 g/L to 2.50 g/L significantly contributes to the rise in xanthan amount and its molecular weight, while further increase of phosphorus source concentration has a statistically insignificant effect on the values of these parameters. The greatest bioprocess success is achieved when the concentration of K_2HPO_4 in medium was 2.50 g/L. Cultivation of producing strain on medium with optimal K_2HPO_4 concentration resulted in high production of xanthan (13.26±0.20 g/L) of good quality (3.38±0.14·10^5 g/moL) along with a relatively high degree of phosphorus conversion (73.62±0.02%).

KEYWORDS: biotechnological production; xanthan; *Xanthomonas* isolate; cultivation media; waste glycerol, phosphorus source

INTRODUCTION

Xanthan is a microbial extracellular heteropolysaccharide that is produced by bacteria of the genus *Xanthomonas* [1]. Due to its outstanding rheological characteristics and its ability to modify the flow behavior of solutions, it is widely used in the food, pharmaceutical, petrochemical, chemical and textile industries [2]. Although many types of bacteria from the genus *Xanthomonas* biosynthesizes xanthan, it is industrially most widely produced by aerobic submerged batch cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on the appropriate medium under optimal conditions [3]. According to Allied Market Research it is estimated that xanthan market value in 2023 will reach amount to 1184 billion US dollars. Commercially available xanthan is relatively expensive due to usage of glucose or sucrose as carbon sources in the xanthan production medium. However, the results of several studies show that waste glycerol, which is generated in huge amounts during the production of biodiesel, is a cheap and available alternative substrate, suitable for use in biotechnological production of xanthan [4-6]. Research related to the xanthan biosynthesis on waste glycerol-based media is still in initial stages and there

is a need for further research and optimization of biotechnological production of xanthan on this complex cultivation medium in order to achieve high efficiency.

Cultivation medium composition, producing strains and bioprocess parameters have significant effect on the yield and the properties of xanthan [7]. Besides carbon and nitrogen sources, cultivation medium for xanthan production contains other important nutrients such as phosphorus, sulfur, potassium, calcium and magnesium. These nutrients are most commonly added to cultivation media in the form of phosphoric and/or sulfuric acid salts [8]. According to the data from available literature, phosphorus is usually added to cultivation medium in the form of K_2HPO_4, in an amount from 1.00 g/L to 3.00 g/L [4, 7]. Although recent studies have been focused on optimization of waste glycerol-based medium in order to ensure maximal biosynthesis of xanthan of great quality, there are no available data from scientific literature related to the examination of the effect of phosphorus concentration and phosphorus-containing compounds in cultivation medium on the success of xanthan biosynthesis.

The aim of this study is to examine the effect of the different K_2HPO_4 concentrations in waste glycerol-based cultivation medium on xanthan production using strain *Xanthomonas* PL 4 isolated from pepper leave. Process efficacy was estimated based on the quantity and quality of biosynthesized xanthan and conversion of phosphorus.

EXPERIMENTAL

PRODUCING MICROORGANISMS

The strain *Xanthomonas* PL 4 isolated from infected pepper leave was used as the producing microorganism in these experiments. Producing strain was stored at 4°C on agar slant (YMA®, HiMedia, India) and subcultured every four weeks.

CULTIVATION MEDIA

The commercial medium (YMB®, HiMedia, India) and glycerol-based growth medium (2 g/L glucose, 3 g/L glycerol from waste glycerol and 5 g/L malt extract) were used for inoculum preparation (inoculum I and inoculum II, respectively), while xanthan production was performed on medium with waste glycerol in a quantity of 16.0 g/L. Cultivation medium for xanthan production also contained $(NH_4)_2SO_4$ in a quantity of 3.0 g/L. The pH value of both glycerol-based media was adjusted to 7.0±0.2. All media were sterilized by autoclaving (121°C, 2.1 bar, 20 min) and stored at 4°C until use.

INOCULUM PREPARATION

Producing strain was subcultured on agar slant and incubated at 25°C for 48 h. Inoculum preparation procedure was included the incubation of refreshed producing microorganism in liquid media in two steps: preparation of inoculum I and preparation of inoculum II. First step (inoculum I preparation) considered the suspending of producing microorganism cells in YMB® and incubation for 48 h. Inoculum II preparation considered addition of 10% (v/v) of inoculum I to glycerol-based growth medium and incubation for 36 h. Both suspensions were incubated in aerobic conditions at 25°C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany).

XANTHAN PRODUCTION

The xanthan production was carried out in 300 mL Erlenmeyer flasks with 100 mL of the waste glycerol-based medium. Inoculation was performed by adding 5% (v/v) of inoculum II prepared as previously described.

The biosynthesis was performed under aerobic conditions at 30°C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany) for 168 h.

XANTHAN SEPARATION

At the end of biosynthesis, the xanthan was separated from the supernatant of cultivation medium obtained using an ultracentrifuge (Hettich Rotina 380 R, Germany) at 10,000 rpm for 10 min, by precipitation with cold 96% (v/v) ethanol in the presence of the potassium-chloride as electrolyte as described in previous research [9]. The concentration of the produced biopolymer was evaluated by determining the weight of the dry product per litre of cultivation medium.

DETERMINATION OF CULTIVATION MEDIUM RHEOLOGICAL PROPERTIES

The rheological behavior of cultivation medium samples taken at the end of the bioprocess 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 Eq. (1):

$$\tau = 0.1 \cdot z \cdot \alpha \quad\text{.. (1)}$$

where z is the constant with the value 3.08 $dyn/cm^2 \cdot Skt$. The pseudoplastic behavior 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 behavior 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 Eq. (2):

$$\eta_a = K \cdot D^{n-1} \quad\text{... (2)}$$

where D is shear rate with the value of 100 1/s.

DETERMINATION OF XANTHAN MOLECULAR WEIGHT

The average molecular weight of the separated xanthan was estimated based on the intrinsic viscosity of its solution in 0.1 M sodium chloride using the Mark-Houwink type equation [10].

DETERMINATION OF GLYCEROL AND PHOSPHOROUS CONTENT

The samples of cell-free cultivation media taken after inoculation and 168 h of cultivation, obtained by centrifugation at 10,000 rpm for 15 min (Rotina 380 R, Hettich Lab Technology, Germany), were analyzed for glycerol 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 glycerol content results after inoculation and xanthan concentration in media at the end of biosyntheis were used to calculate the degree of conversion of glycerol to xanthan ($K_{S/P}$, %) using equation:

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

where S_0 is the initial glycerol content (g/L), whereas P is xanthan concentration in media at the end of biosynthesis (g/L).

The content of total phosphorus was determined using the spectrophotometric method proposed by Gales et al., 1966 [11]. The phosphorus content results after inoculation and 168 h of cultivation were used to calculate the degree of total phosphorus conversion (K_{Pt}, %) using equation:

$$K_{Pt} = \frac{P_{t0} - P_t}{P_{t0}} \cdot 100 \qquad (4)$$

where P_{t0} is the initial phosphorus content (g/L) and P_t is the residual phosphorus content (g/L).

DATA ANALYSIS

All experiments were carried out in triplicate and the results were averaged. The experimental data were processed by one-way analysis of variance (One-Way ANOVA). Significant differences between the means were determined by Duncan's multiple range test at the significance level of α=0.05 using Statistica 13.2 software (Dell Inc., USA).

RESULTS AND DISCUSSION

In accordance with the defined aim of this research, xanthan biosynthesis was performed by the strain *Xanthomonas* PL 4 isolated from pepper leaves on waste glycerol-based cultivation media containing K_2HPO_4 in concentrations from 0 g/L to 3.00 g/L.

Considering the fact that the xanthan solutions and xanthan cultivation broths at the end of biosynthesis are highly viscous and show pseudoplastic behavior [8], the possibility of xanthan production on waste glycerol-based cultivation media with addition of K_2HPO_4 in different concentrations were evaluated comparing the rheological properties of cultivation media after biosynthesis. The rheological parameters were determined from the relationship between shear rate and shear stress, and obtained values are presented in Table 1.

Table 1. Rheological properties of waste glycerol-based cultivation media containing K_2HPO_4 in different concentrations

K_2HPO_4 (g/l)	K [Pa·s^n]	n [1]	R^2	η_a [mPa·s]
0.0	0.2998	0.5238	0.916	29.98±4.02
0.5	0.2421	0.5653	0.954	31.04±2.47
1.0	0.3366	0.5120	0.943	34.36±0.38
1.5	0.3275	0.5383	0.928	36.77±0.77
2.0	0.4286	0.4870	0.9487	37.52±2.62
2.5	0.4614	0.4838	0.944	38.77±3.53
3.0	0.4612	0.5027	0.947	40.93±4.52

K -consistency factor;n - flow behavior index;
R^2 - determination coefficient;η_a - apparent viscosity.

Flow behavior index (n) shows a level of deviation from Newtonian flow behavior. For a Newtonian fluid, flow behavior index is equal to 1, greater than 1 for a dilatant and less than 1 for a pseudoplastic fluid [12]. According to the results presented in Table 1, values of flow behavior index for cultivation media obtained after biosynthesis in applied experimental conditions were in the range from 0.4838 to 0.5653. The obtained values of flow behaviour index confirm the pseudoplastic characteristics of the cultivation media and this indicates that xanthan biosynthesis is confirmed in all media.

Values of the coefficient of determination (R^2) higher than 0.910 indicate a very good corresponding of the measured values with the Ostwald-de-Waele model, which further confirms pseudoplastic behavior of all tested samples.

The viscosity of xanthan solution is greatly effected with the concentration of the biopolymer, but also on its structure [8]. Taking into account that the consistency factor (K) is proportional to the viscosity,

the different values of this parameter shown in Table 1 indicate the difference in the quantity and quality of xanthan produced by the strain *Xanthomonas* PL 4 on waste glycerol-based cultivation media containing K_2HPO_4 in concentrations from 0 g/L to 3.0 g/L. The obtained results presented in Table 1 show that values of the apparent viscosity (η_a) of cultivation media at the end of bioprocess were in the range from 29.98±4.02 mPa·s to 40.93±4.52 mPa·s.

The values of apparent viscosity of cultivation medium obtained in this study are in accordance with result from previous research when different *Xanthomonas campestris* strains were cultivated on waste glycerol-based medium containing 3.00 g/L K_2HPO_4 [5]. The highest value of viscosity of cultivation medium (40.93±4.52 mPa·s) was obtained when strain *Xanthomonas* PL 4 was cultivated on waste glycerol-based cultivation medium containing 3.00 g/L K_2HPO_4 and the lowest value of this

parameter (29.98±4.02 mPa·s) was achieved by cultivation of the same producing strain on waste glycerol-based medium without any addition of K_2HPO_4.

In order to examine the effect of the different K_2HPO_4 concentrations (0-3.00 g/L) in waste glycerol-based medium on xanthan production using strain *Xanthomonas* PL 4, statistical analysis of experimental data was carried out. The results of One-Way ANOVA analysis, as well as results of post hoc analysis by Duncan's multiple range test for xanthan concentration in media, its average molecular weight and degree of total phosphorus conversion are further discussed.

The results of statistical analysis of the effect of the different K_2HPO_4 concentrations in cultivation medium on xanthan concentration (P, g/L), its average molecular weight (M_W, 10^5 g/moL) and degree of total phosphorus conversion (K_{Pu}, %) are shown in Table 2.

Table 2. Analysis of variance (One-Way ANOVA) of the different K_2HPO_4 concentrations in cultivation medium on xanthan concentration, its average molecular weight and degree of phosphorus conversion

Parameters	Variability	SS	DF	MS	F-odnos	*p*-vrednost
P (g/L)	K_2HPO_4 (g/l)	27.155	6	4.526	39.280	< 0.000001
	Error	1.613	14	0.115	-	-
M_W (10^5 g/moL)	K_2HPO_4 (g/l)	11.509	6	1.918	61.632	< 0.000001
	Error	0.436	14	0.031	-	-
(K_{Pt}, %)	K_2HPO_4 (g/l)	1600.500	5	320.100	79453.000	< 0.000001
	Error	0	12	0		

SS – sum of squares; DF – degrees of freedom; MS – mean square; P-xanthan concentration in media; M_w-average molecular weight of xanthan; K_{Pt}-degree of total phosphorus conversion.

From the results presented in Table 2 it can be noticed that the *p*-values are much lower than 0.05 for all three observed parameters, which indicates that K_2HPO_4 concentration has a statistically significant effect on xanthan concentration in cultivation media, its average molecular weight and degree of total phosphorus conversion.

The obtained results of statistical analysis of the effect of the different K_2HPO_4 concentrations in cultivation medium on xanthan concentration, its average molecular weight and degree of total phosphorus conversion are presented graphically by Box & Whisker Plots in Figure 1-3.

Observing the results presented in Figure 1 it can be noticed that there is statistically significant difference in xanthan concentration in media when K_2HPO_4 was added to waste glycerol-based cultivation medium in different concentrations.
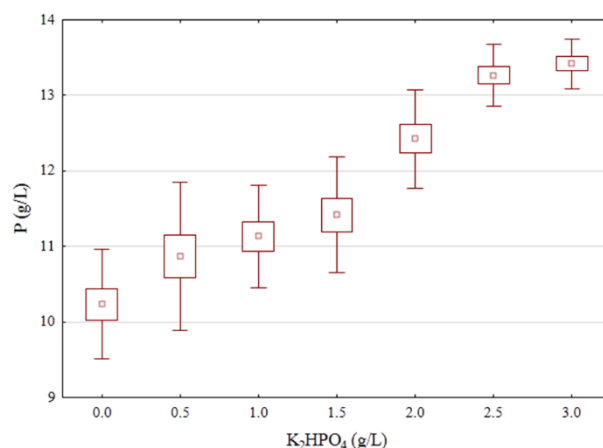

Figure 1. Effect of the different K_2HPO_4 concentrations in waste glycerol-based cultivation medium on xanthan concentration (P)

According to the results presented in Figure 1, concentration of xanthan produced in applied experimental conditions was in the range from around 9.50 g/L to 14.00 g/L. It is evident that the highest xanthan concentration was obtained when K_2HPO_4 concentration in cultivation medium was 3.00 g/L.

Results presented in Figure 1 suggest that there is no statistically significant difference in the xanthan concentration in media when K_2HPO_4 concentration in cultivation medium was 2.50 g/L and 3.00 g/L. The lowest xanthan concentration was obtained when biosynthesis was performed on medium without adittion of K_2HPO_4.

The graphically presented results in Figure 2 show that there is statistically significant difference in average molecular weight of xanthan obtained when K_2HPO_4 was added to cultivation medium in different concentrations. Considering the results presented in Figure 2 it can be noticed that avreage molecular weight of xanthan obtained on media with different concentration of K_2HPO_4 ranged from about $1 \cdot 10^5$ g/moL to $4 \cdot 10^5$ g/moL. The obtained results show that xanthan with the highest average molecular weight was achieved when K_2HPO_4 was added to cultivation medium in maximal concentration (3.00 g/L). As it can be seen from the Figure 2, there is no statistically significant difference in the average molecular weight of xanthan when K_2HPO_4 concentration in cultivation medium was 2.50 g/L and 3.00 g/L. Xanthan with the lowest average molecular weight was obtained when biosynthesis was performed on medium without adittion of K_2HPO_4.

From graphically presented results (Figure 3) it can be observed that there is statistically significant difference in degree of total phosphorus conversion obtained when K_2HPO_4 was added to waste glycerol-based cultivation medium in different concentrations. The results presented in Figure 3 show that a value of degree of total phosphorus conversion achieved after cultivation of producing strain on media with different concentration of K_2HPO_4 ranged from about 60% to 90%. The highest degree of total phosphorus conversion was achieved when K_2HPO_4 was added to cultivation medium in minimal concentration (0.50 g/L). High value of degree of total phosphorus conversion was also achieved when K_2HPO_4 concentration in cultivation medium was 1.00 g/L, 1.50 g/L and 2.00 g/L. The obtained results show that

the lowest conversion of total phosphorus was achieved when K_2HPO_4 was added to cultivation medium in maximal concentration (3.00 g/L).
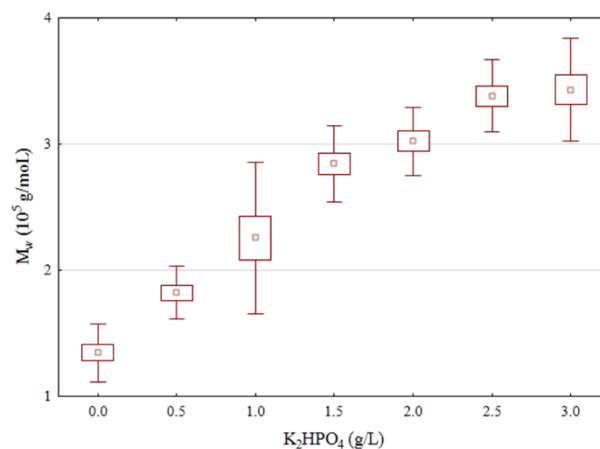

Figure 2. Effect of the different K_2HPO_4 concentrations in waste glycerol-based cultivation medium on xanthan average molecular weight (M_w)
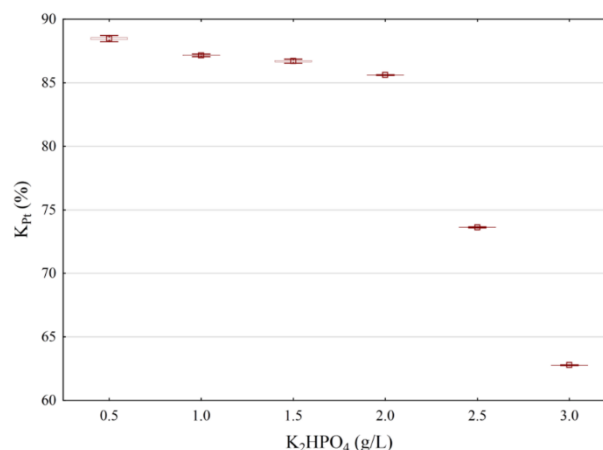

Figure 3. Effect of the different K_2HPO_4 concentrations in waste glycerol-based cultivation medium on degree of total phosphorus conversion (K_{Pt})

Homogeneous groups of experiments were established based on statistical significance of differences in the obtained xanthan concentration, its quality, the degree of conversion of glycerol to xanthan and the degree of total phosphorus conversion by using Duncan's multiple range test comparisons. The obtained results are shown in Table 3.

Table 3. Duncan's multiple range test: mean ± standard deviation for the xanthan concentration, average molecular weight of xanthan, degree of conversion of glycerol into xanthan and degree of total phosphorus conversion

K_2HPO_4 (g/L)	P (g/L)	$K_{S/P}$ (%)	M_W (10^5 g/moL)	K_{Pt} (%)
0	10.24 ± 0.36^a	63.38 ± 2.23^a	1.35 ± 0.11^a	-
0.5	10.87 ± 0.49^b	67.32 ± 3.03^b	1.82 ± 0.10^b	88.48 ± 0.12^f
1.0	11.13 ± 0.34^b	68.93 ± 2.10^b	2.26 ± 0.30^c	87.15 ± 0.05^e
1.5	11.42 ± 0.38^b	70.70 ± 2.36^b	2.84 ± 0.15^d	86.70 ± 0.08^d
2.0	12.42 ± 0.33^c	76.91 ± 2.02^c	3.02 ± 0.13^d	85.60 ± 0.02^c
2.5	13.26 ± 0.20^d	82.13 ± 1.25^d	3.38 ± 0.14^e	73.62 ± 0.02^b
3.0	13.42 ± 0.17^d	83.09 ± 1.02^d	3.43 ± 0.21^e	62.78 ± 0.01^a

Values in the same column marked with the same letter are not significantly different at α=0.05.

As it can be noticed from the Table 3, xanthan concentration in medium at the end of biosynthesis ranged from 10.24 ± 0.36 g/L to 13.42 ± 0.17 g/L. The highest xanthan concentration was obtained when K_2HPO_4 was added to waste glycerol-based cultivation medium in maximal concentration (3.00 g/L). According to the results presented in Table 3, the value of degree of conversion of glycerol into xanthan was in range from 63.38±2.23% to 83.09±1.02% and the highest value of this parameter was also achieved when K_2HPO_4 was added to medium in maximal concentration. The obtained results suggest that there is no statistically significant difference in xanthan concentration (13.26±0.20 g/L and 13.42±0.17 g/L) and degree of glycerol conversion into xanthan (82.13±1.25% and 83.09±1.02%) when K_2HPO_4 was added to cultivation medium in concentration of 2.50 g/L and 3.00 g/L, which is confirmed by the *p*-values of 0.584152 and 0.584152, respectively. Statistically significant lower xanthan concentration was obtained when cultivation of producing strain was performed on medium containing K_2HPO_4 in concentrations from 0.50 g/L to 2.00 g/L, which is confirmed by *p*-values lower than 0.05. However, the results of xanthan concentration obtained when using 1.0 g/L K_2HPO_4 (11.13±0.34 g/L) show greater success comparing to the results obtained in previous study where 7.23 g/L of xanthan was achieved by cultivation of the production strain *X. campestris mangiferaeindicae* 2103 on waste glycerol-based medium with the addition of 1.0 g/L K_2HPO_4 [4].

Based on the results presented in Table 3 it can be noticed that the lowest xanthan concentration was achieved when waste glycerol-based cultivation medium did not contain K_2HPO_4. Conversion of carbon source into product was also the lowest when cultivation of producing strain was performed on medium with the same composition. Considering the results presented in Table 3 it is evident that increasing the concentration of K_2HPO_4 in waste glycerol-based

medium for xanthan biosynthesis up to 2.50 g/L, in the applied experimental conditions, has a positive effect on the xanthan concentration and degree of glycerol conversion into xanthan. The values of both parameters obtained in this research are higher comparing to the values achieved in previous research when cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on waste glycerol-based medium containing 3.00 g/L K_2HPO_4 resulted in production of xanthan in concentration of 6.68 g/L where is 66.46% of glycerol converted to xanthan [13]. Taking into account that in industrial conditions, the degree of conversion of carbon sources into xanthan ranges from 50-85% [14], it can be concluded that within this research, a very high efficiency of bioprocess has been achieved.

Analysing the values of average molecular weight of xanthan produced by cultivation of producing strain on waste glycerol-based medium with different concentrations of K_2HPO_4 (Table 3) it can be observed that the values of this indicator of biopolymer quality were in the range from $1.35\pm0.11\cdot10^5$ g/moL to $3.43\pm0.21\cdot10^5$ g/moL. The obtained results suggest that the highest average molecular weight of xanthan was obtained when the cultivation of producing strain was performed on medium containing 3.00 g/L K_2HPO_4. Xanthan of statistically insignificant lower average molecular weight ($3.38\pm0.14\cdot10^5$ g/moL) was obtained when bioprocess was performed on medium containing 2.50 g/L K_2HPO_4 (*p*=0.737000). From the results shown in Table 3 it is evident that xanthan of the lowest average molecular weight was achieved when cultivation was performed on medium with no K_2HPO_4. Considering the results discussed above, it can be concluded that increasing the concentration of K_2HPO_4 in cultivation medium up to 2.50 g/L has a positive effect on the average molecular weight of xanthan produced in applied experimental conditions.

The results of Duncan's multiple range test for the degree of total phosphorus conversion achieved after

cultivation of producing strain on waste glycerol-based medium with addition of K_2HPO_4 in concentration from 0.50 g/L to 3.00 g/L are presented in Table 3. Experiment in wich cultivation was performed on medium without any addition of K_2HPO_4 was excluded from consideration. According to the results shown in Table 3 it can be noticed that the degree of total phosphorus conversion in waste glycerol-based medium after cultivation of producing strain in applied experimental conditions ranged from $62.78 \pm 0.01\%$ to $88.48 \pm 0.12\%$. The highest degree of total phosphorus conversion was achieved when medium containing 0.50 g/L K_2HPO_4 was applied. Very high conversion of total phosphorus was also achieved in experiments where the concentration of K_2HPO_4 in the medium for xanthan production was 1.0 g/L ($87.15 \pm 0.05\%$), 1.50 g/L ($86.70 \pm 0.08\%$) and 2.00 g/L ($85.60 \pm 0.02\%$). The lowest degree of total phosphorus conversion was achieved when K_2HPO_4 was added in cultivation medium in the concentration of 3.00 g/L. The values of degree of total phosphorus conversion accomplished in this research are much higher comparing to the results obtained in previous research when degree of total phosphorus conversion during cultivation of strains of the genus *Xanthomonas* isolated from pepper leaves on waste glycerol-based media containing 3.00 g/L K_2HPO_4 was in the range from 25.70% to 31.03% [6].

The results obtained within this research show that the increase of K_2HPO_4 concentration in medium from 0 g/L to 2.50 g/L significantly contributes to the rise in xanthan concentration in media, its average molecular weight and degree of conversion of glycerol into xanthan, while further increase of phophorus source concentration has a statistically insignificant effect on the values of these parameters. Taking into account previously discussed results it can be concluded that the greatest bioprocess efficacy is achieved when the concentration of K_2HPO_4 in medium was 2.50 g/L. Waste glycerol-based medium with the initial K_2HPO_4 concentration of 2.50 g/l was suggested as the most appropriate for further research related to xanthan production.

CONCLUSION

In accordance with the defined aim, in this study the effect of various K_2HPO_4 concentrations (0-3.00 g/L) in waste glycerol-based medium on xanthan production using *Xanthomonas* strain PL 4 isolated from infected pepper leave was examined. The obtained results suggest that increasing the concentration of used phosphate salt in the cultivation medium has a positive effect on the xanthan concentration, degree of glycerol conversion into

xanthan and its average molecular weight. The highest values of aforementioned parameters were achieved when bioprocess was performed on medium containing 3.00 g/L K_2HPO_4. The obtained results also indicate that increasing the concentration of K_2HPO_4 in the cultivation medium from 2.50 g/L to 3.00 g/L does not lead to a statistically significant change in the values of analyzed parameters. On the other hand, increasing the concentration of K_2HPO_4 in the cultivation medium in the range from 0.50 g/L to 3.00 g/L has a negative effect on the degree of total phosphorus conversion and that the highest degree of phosphorus conversion was achieved when the cultivation of producing strain was performed on medium containing 0.50 g/L K_2HPO_4.

According to the results achieved in this study it can be concluded that the optimal concentration of K_2HPO_4 in waste glycerol-based medium for high efficiency xanthan production by selected producing strain is 2.50 g/L. Results obtained in this study represent valuable information for future research related to development of biotechnological production of xanthan on waste glycerol-based medium.

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