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PREPARATION OF A STAINED POLYSACCHARIDE, A POTENTIAL SUBSTRATE TO EVALUATE CERTAIN SPECIFIC ENZYMES

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Abstract
A stained biopolymer was prepared by covalently coupling of linear glucan pullulan with anthraquinone dye Remazol Brilliant Blue R (RBBR). In this way was obtained a novel stained polysaccharide material which can be used as potential substrate for evaluation of enzymes that selectively hydrolyzes glycosidic linkages that characterize this polymer.

Introduction
Pullulan is well-known neutral polysaccharide with numerous applications in the food, cosmetic and pharmaceutical industries [1]. It is widely accepted that pullulan is a linear polysaccharide with maltotriosyl repeating units joined by α-(1,6)-linkages. Alternatively, the structural formula of pullulan may be presented as a regular sequence of panoses bonded by α-(1,4)-linkages (Fig. 1). Minor structural abnormalities, like presence of maltotetraose residues or higher maltooligomers which distributed randomly along the pullulan chain not affect the overall physicochemical properties of this polymer.

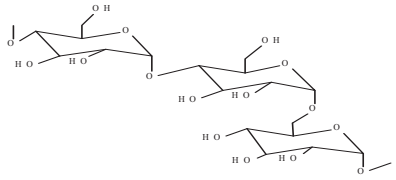

Fig 1.

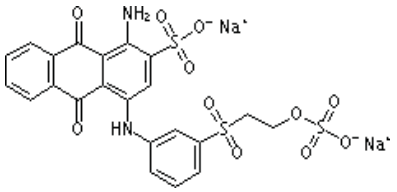

Fig.2.

Anthraquinone dye Remazol Brilliant Blue R (Fig. 2) is dye that is used for staining different substrates, some of which have commercial application [2].

In this work we reported some of our observations on the reaction covalent coupling of pullulan by reagent RBBR to give a novel stained polymer material. The aim of the present work was to prepare sample of this stained polysaccharide and to broaden our knowledge about the some characterisation of the coupling derivative pullulan-RBBR which can be used as potential substrate for evaluation of enzymes that selectively hydrolyzes characteristic linkages of this polymer.

Experimental

Pullulan used in this work is produced by the *A. pullulans*, strain CH-1 (IChTM, Collection of Microorganisms) [3,4]. Other reagents and solvents were purchased from commercial sources and used as supplied. Pullulan and RBBR were prepared separately, by dissolution in distilled water and then mixed together. The reaction mixture was stirred at 50°C for 1 h and, at different times, equal portions of Na_2SO_4 were added. After that aqueous solution of a Na_3PO_4 was added and reaction maintained at 50°C for another hour with extensive stirring. After cooling the reaction mixture was centrifuged at 4000 rpm for 15 min. The precipitate was resuspended in distilled water and excess of unreacted dye was removed by dialysis. Resulting solution was liophilized. The FTIR spectra of samples were obtained using Thermo Nicolet 6700 FT-IR Spectrophotometer. The samples were prepared in the form of KBr discs.

UV-VIS spectral characteristics of solutions of dye and stained pullulan (Fig. 3 and Fig. 4) were recorded using a GBC Cintra 40 spectrophotometer.

Results and Discussion

The coupling pullulan with RBBR was confirmed by FTIR (Fig. 3). The FTIR spectrum of pullulan-dye derivative showed a combined characteristic bands for polysaccharide and RBBR. Among polysaccharide bands at 3000-3500 cm^{-1}(ν OH), 1023 cm^{-1} ν (CO), 1155 cm^{-1} ν(C-O-C), this spectrum showed band between 670 and 870 cm^{-1}, which denote aromatic rings, band near 1650 cm^{-1} characteristic for anthraquinone rings, and bands at 1037 cm^{-1} and 1120 cm^{-1} due to of C-NH_2 vibrations.

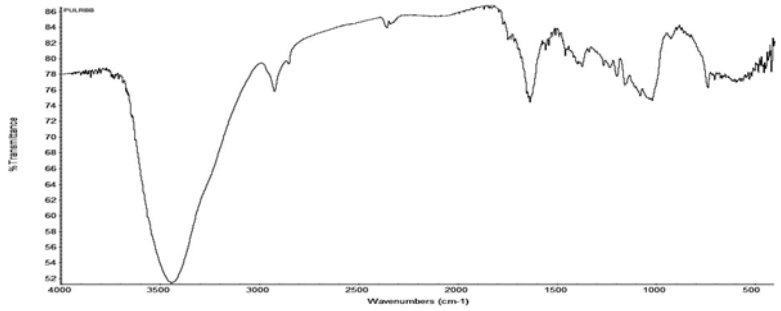

Fig.3. FTIR spectrum of pullulan-RBBR derivative.

Additional evidence that pullulan was coupled with RBBR was found in the results of elemental analysis of pullulan and pullulan-dye derivative (Table 1.). The content of dye in coupled polysaccharide is reflected by the increase of nitrogen content after reaction of this glucan with RBBR.

Table 1. Elemental analysis of starting substances and reaction product

	% N	% C	% H
Pullulan	/	44.4	6.2
RBBR	4.4	42.1	2.5
Pullulan-RBBR	0.6	47.3	6.4

UV-VIS spectral properties of RBBR coupled with pullulan did not differ from those of the free dye. Free RBBR in distilled water (2,13mg/ml) show absorption maximum at 595 nm (Fig. 4). The spectrum of pullulan-coupled RBBR (2,08mg/ml) demonstrated a identical shape of the curve with the broad maximum at 595 nm (Fig. 5).

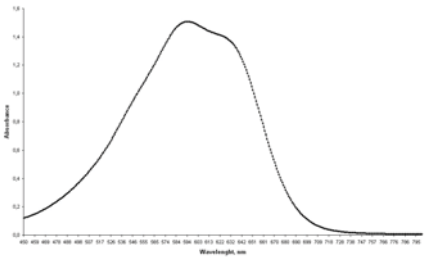

Fig.4. UV-VIS spectrum of RBBR.

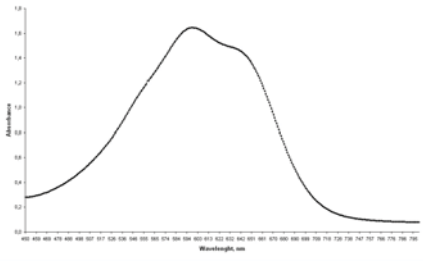

Fig.5. UV-VIS spectrum of derivative pullulan-RBBR

Conclusion

The results presented in this study show a route to produce stained polymers for potentially useful applications. The pullulan-RBBR derivative was obtained through covalent coupling of pullulan and anthraquinone dye Remazol Brilliant Blue R. This derivative can be used as potential substrate for evaluation of enzymes that selectively hydrolyze characteristic glycosidic linkages of this polymer.

Acknowledgement

This work was supported by the Serbian Ministry of Science, Projects ON 142018B.

References

[1] K. I. Shingel , Carbohyd. Res., 2004, **339,** 447–460.

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[3] D. M. Jakovljević, M. M. Vrvić, M. Đ. Radulović, M. S. Hranisavljević - Jakovljević, J. Serb. Chem. Soc., 2001, **66,** 377-383.

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INTRODUCTION

Pullulan is well-known neutral polysaccharide with numerous applications in the food, cosmetic and pharmaceutical industries [1]. Anthraquinone dye Remazol Brilliant Blue R (RBBR) (Figure 1) is dye that is used for staining different substrates, some of which have commercial application [2].

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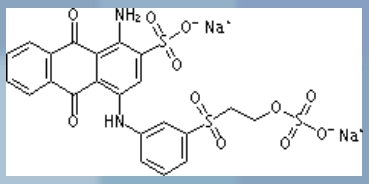

Figure 1: Structural formula of Remazol Brilliant Blue R

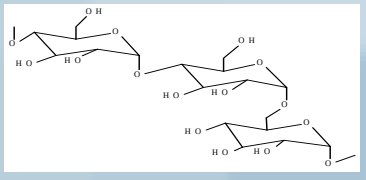

Figure 2: Structural formula of pullulan

RESULTS AND DISCUSSION

Pullulan used in this work is produced by the *Aureobasidium pullulans*, strain CH-1 (IChTM, Collection of Microorganisms) [3,4]. Other reagents and solvents were purchased from commercial sources and used as supplied.

The coupling reaction between pullulan and RBBR was confirmed by FTIR (Figure 3). The FTIR spectrum of pullulan-dye derivative showed a combined characteristic bands for polysaccharide and RBBR. Among polysaccharide bands at 3000-3500 cm^{-1} ν (OH), 1023 cm^{-1} ν (CO), 1155 cm^{-1} ν (C-O-C), this spectrum showed band between 670 cm^{-1} and 870 cm^{-1}, which denote aromatic rings, band near 1650 cm^{-1} characteristic for anthraquinone rings, and bands at 1037 cm^{-1} and 1120 cm^{-1} due to C-NH_2 vibrations.

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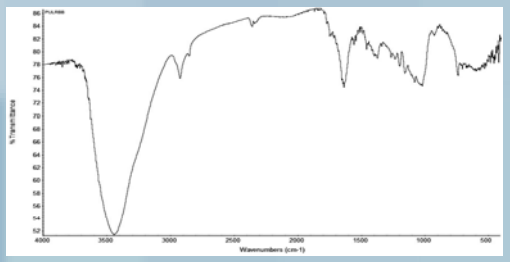

Figure 3: FTIR spectrum of pullulan-dye derivative

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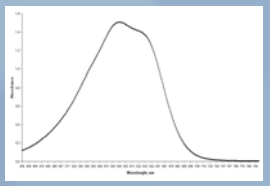

Figure 4: UV-VIS spectrum of RBBR

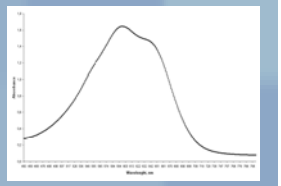

Figure 5: UV-VIS spectrum of pullulan-RBBR derivative

CONCLUSION

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