Dear Prof. Dr Bojana Obradović,

We have decided to undertake the task of minor revision of original manuscript, in accordance with Your decision. All reviewers’ suggestions and corrections have been answered, and point-by-point list of responses to the reviewers’ comments are given in following text. In accordance with reviewers’ requests, four figures have been modified and have been inserted in revised manuscript. According to the reviewers’ comments, some parts of the Results and Discussion section have been altered and extended (all corrections that are made in the manuscript are highlighted in yellow). I hope You will be satisfied with quality of corrected manuscript and responses to reviewers’ comments.

Sincerely Yours,

Dr. Katarina Banjanac

Associate Researcher

Department of Biochemical Engineering and Biotechnology

Innovation centre of Faculty of Technology and Metallurgy

University of Belgrade

Karnegijeva 4

11000 Belgrade

Serbia

E-mail: [kbanjanac@tmf.bg.ac.rs](mailto:kbanjanac@tmf.bg.ac.rs)

**Answer to a Reviewers’ comments**:

**Reviewer A:**

The manuscript covers an interesting study about laccase immobilization and  
the results are important from the industrial and environmental point of  
view. Before publication I suggest minor changes:

1. **Fig 1, Fig 2, Fig 3 and Fig 4 y-axis title; authors shall change into  
   Activity of immobilized laccase and the same in the title of Figure 1**

**Answer:** We are grateful to Reviewer A for drawing our attention to this wording. In revised manuscript, all y-axis titles have been corrected accordingly, as well as the title of Fig. 1. **(page 10, Fig. 1, line192; page 12 Fig.2, line 222; page 15 Fig.3, line 285; page 16, Fig.4 line 305). All references in the text, to this term, have been corrected accordingly.**

**Reviewer B:**

Manuscript contains interesting results of study focused on development of  
novel immobilized laccases and it was proved that novel immobilized enzymes  
can be used in degradation of industrial dyes. In my opinion manuscript can  
be accepted after minor revisions described in following text.  
  
Comments:  
**1.    Within Introduction choice of industrial dyes should be more elaborately  
explained. In paragraph (lines 62-70) industrial dyes are discussed in  
general manner, authors should justify their choice of dyes.**

The authors agree with Reviewer B that the choice of dyes was not elaborated. As suggested by Reviewer B, in revised manuscript, we added information on the selected industrial dyes, with the justification on their choice (**page 5, lines 90-100)**.

**2.    Tables 2 and 3 should be merged and placed at the appropriate place in  
Introduction or Methods section.**

As suggested by Reviewer B, tables 2 and 3 are merged and placed at the appropriate place in the Methods section, and in the revised manuscript referred to as Table 2. **(page 8, line 159).** All references to this table have been corrected, as well.

**3.    At page 13, line 275 it is written “Based on the obtained data from the  
preliminary experiments performed, regarding these two types of supports,  
the immobilization of laccase on hydrophobic and epoxy activated support,  
LifetechTM ECR8285F was further optimized.” It is misleading since two  
supports, hydrophobic and epoxy activated were tested, and further reading  
is necessary to clarify that only one support – epoxy activated which is  
moderately hydrophobic - was chosen for further experiments.**

**Answer:** The authors agree with Reviewer B that the support selected for further investigations should be explained more clearly.Therefore, thepart of the manuscript containing this sentence is altered, and the explanation is provided **(page 15, line 289-292)**.

**4.    Sentences “Although, the both supports selected for further  
investigation were porous solid spheres, like amino-activated supports used  
in previous experiments, the crucial difference is that octadecyl-activated  
support gives possibility for enzyme attachment via hydrophobic interactions  
while the epoxy-activated support allows formation of covalent bonds which  
is a preferred technique over adsorption. The main characteristics of  
selected supports for this experiment are presented in Table 1.” at page  
13, line 256 should be rephrased and correctness of some phrases should be  
checked (e.g. “…in this present study…”).**

**Answer:** The authors are very grateful to Reviewer B for drawing our attention on this sentence. After reviewer’s comment we realize that such a sentence was not lucid enough, which could confuse potential future readers. Therefore, the sentence “Although, the both supports selected for further  
investigation were porous solid spheres, like amino-activated supports used  
in previous experiments, the crucial difference is that octadecyl-activated  
support gives possibility for enzyme attachment via hydrophobic interactions  
while the epoxy-activated support allows formation of covalent bonds which  
is a preferred technique over adsorption. The main characteristics of  
selected supports for this experiment are presented in Table 1.”has been rephrased in the revised manuscript **(page 14, lines 270-275).**

Also, according to the Reviewer B remark, the phrase “…in this present study…” was substituted with the phrase “in this study”, in the revised manuscript **(page 4, line 75).**

Some other phrases were corrected as well, for the purpose of clarification **(page 9, line 169 and lines 175-177, page 16, lines 297-300, page 17, line 331)**

**5.    Abstract in Serbian should be corrected - font size should be uniformed  
and spellcheck performed.**

**Answer:** As suggested by Reviewer B, in revised manuscript, Abstract in Serbian was checked and corrected, font size is uniformed, and spellcheck has been performed **(page 28 and 29, lines 538-553)**

**6.    It is necessary to uniform the used terminology on the graphs with the  
terminology in the text. For example on Fig. 2 and Fig. 4, the term  
“concentration of immobilized activity (IU/g of support)” was used and  
in text it was stated “For complete optimization of immobilization  
process, the optimal initial protein concentration (mg/g support) was  
evaluated by monitoring the expressed and specific activity (Fig. 2C).”  
(page 12, line 231) and „For complete optimization of laccase  
immobilization on this support, the optimal initial enzyme concentration was  
evaluated by monitoring protein loading and protein immobilization yield as  
well as the expressed and specific activity, like in the case of optimal  
amino-activated carrier, and results are presented in Fig 4” (page 14,  
line 287).**

**Answer:** The authors are very grateful to Reviewer B and also to Reviewer A for drawing our attention to this discrepancy in terminology. As suggested by Reviewer A, in the revised manuscript, only the term “activity of immobilized laccase” was used in the text and also in the Figures 1, 2, 3 and 4 **(page 10, Fig. 1, line192; page 12 Fig.2, line 222; page 15 Fig.3, line 285; page 16, Fig.4 line 305; page 4, line 85, page 13, lines 241 and 245, page 16, lines 301 and 307, page 17, line 312)**

**7.    Authors should rephrase and clarify the paragraph concerning the  
influence of the pH on immobilization process (page 11, line 218).**

**Answer:** The authors agree with Reviewer B that the influence of the pH on the immobilization process should be explained more clearly.Therefore, thepart of the manuscript regarding immobilization pH optimisation is altered **(page 11, lines 216-217, page 12, lines 228-237 and lines 228-237, page 13, lines 238-239)**.