**RESPONSE TO REVIEWERS**

***Reviewer G:***

*The major concern of submitted manuscript is that all experiments lacks internal wtCDH control. Pichia clone carrying wtCDH is used only for determining optimal time point for cell harvesting and then it disappears from experimental setting. In further experiments of purification and activity assays authors refer only to literature data on wtCDH expression in P. pastoris whereas it would be the best practice to follow wtCDH through the same experimental setting as the mutant CDHs.*

**We thank Reviewer G for the suggestion and we have included data for wtCDH in our expression system.**

*Other points:*

*1. Please use “mutant proteins”, “mutant CDHs” or “mCDHs” rather*

*than just “mutants”. Although it is used colloquially for mutant proteins, “mutant” actually refers to mutant organism, a carrier of mutated gene.*

**We have replaced term “mutant” with “mutant proteins” as suggested.**

*2. Please discuss differences in activity between immobilized and soluble*

*variants of tm, H5, and H9 mCDHs, and possible causes.*

**We discussed after Table 1. differences in activity between immobilized and soluble variants of CDH and possible causes for it. (text marked with red color)**

*3. Please discuss the improvement in expression rate of wtCDH of 950 IU L-1*

*compared to previous 221 IU L-1 in KM71H strain in two different studies.*

**We added discussion for possible higher expression rate (multiple integration transformant) at the end of paragraph below Fig. 3. (text marked with red color)**

*4. Please discuss the difference in size between native CDH (97 kDa) and*

*recombinant CDH (100 kDa).*

**We have discussed differences in size with respect of glycosylation degree in paragraph after Fig. 4. (text marked with red color)**

*Further minor points:*

*line 39-41 English should be improved*

*line 62 Reference is missing, the same group of author published article*

*that should be cited here (Appl. Sci. 2019, 9, 1413)*

*line 71 Please specify the Taq polymerase used in amplification reaction*

*line 92 3,000 rpm instead of 3.000 rpm*

*line 94-95 English should be improved*

*line 120 The name of MW standard is missing*

*fig. 6 please change one of the triangular marks to other type of mark (dot*

*or asterix) to improve figure clarity*

*line 239 English should be improved*

*line 246 H9 instead of H7*

*line 249 …recombinant enzymes were concentrated…*

*line 292 …molekulske mase od… rather than …na molekulskoj masi od…*

**All minor points were corrected as suggested. (text marked with red color)**

*REPORT:*

 *The paper “Expression, purification and characterization of cellobiose dehydrogenase mutants from Phanerochaete chrysosporium in Pichia pastoris KM71H strain” describes for the first time expression of the tm, H5, and H9 mutant variants of cellobiose dehydrogenase (CDH) enzyme in Pichia pastoris. This group of mutant variants of CDH enzyme was recently published by the same author group, and now they made a valid effort to improve enzyme yield by transferring it to a highly efficient expression system of P. pastoris. However, this study has a flaw in experimental setting that could be easily remedied rendering the paper recommendable for publication.*

*In my opinion, this manuscript should: be published after major revision and additional review*

**We corrected our article as Reviewer G suggested and hope that we answered to all questions.**

***Reviewer H:***

*There are few concerns that need to be addressed.*

*1. Is there a particular reason why wild type enzyme wasn’t expressed in parallel to the three mutant variants, for better comparison of the activity?*

**We though that wild type CDH expressed in *Pichia* was previously described a lot of times, but as both Reviewers suggested we included it for a better comparison of the activity.**

*2. Recombinant CDHs produced by P. pastoris have been shown to be differentially glycosylated, which might affect intramolecular electron transfer reaction. Have you considered deglycosylation to estimate the degree of glycosylation?*

**Degree of glycosylation was previously determined for CDH in *Pichia* and we added this explanation in a discussion of differences in sizes between native CDH and recombinant CDH in the paragraph after Fig. 4. (text marked with red color)**

*3. Line 61- The authors should give the reference for new mutants of CDH , discovered during directed evolution. If it was the result of their previous research, it should be clearly stated.*

**We stated that it was result of our previous work and cited it as reference 21.**

*REPORT:*

 *In this paper the authors describe the procedure for production of improved CDH enzyme variants using Pichia pastoris as expression host. Due to its properties, CDH has great potential for usage in electrochemical biosensors and enzymatic biofuel cells. Hence, the results of this study could help increase their efficiency.*

*Minor comments:*

*In Abstract, a short introduction sentence describing the problem, and why the study was conducted should be added.*

**We added a short introduction sentence at the beginning of Abstract describing the problem and why the study was conducted.**

*Line 81. The procedure for transformation of E.coli should be given in bit more detail.*

**We added more details of used transformation protocol.**

*Line 70- „reverse“ should stand instead of „reverses“*

**Corrected.**

*In my opinion, this manuscript should: be published after minor revision without additional review*

**We corrected our article as Reviewer H suggested and hope that we answered to all questions.**