Addendum

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1. Name of the candidate: Dhruba Jyoti Sarma

2. Roll No: MBP13105

3. School: Sciences

4. Department: Molecular Biology and Biotechnology

5. Title of the thesis: Isolation and Purification of Cytochrome P450 from *Bacillus stratosphericus* for Development of an ENFET Device for n-Hexadecane Sensing.

Comments/Corrections/Modifications suggested by Examiner-1		
Sl. No.	Comment	Response
1	Chul-Ho Yun, et al., The bacterial P450 BM3: a prototype for a biocatalyst with human P450 activities. Trends in biotechnology (2007) 25(7),289-298. https://doi.org/10.1016/j.tibtech.2007.05.003 Comment: P450 BM3 is the most characterised molecular species of P450, which is a self-sufficient catalytic activity without additional P450 reducase, isolated from *Bacillus megaterium*. Accumulated knowledge about structure/ function relationship with this enzyme is worth to read. When you think about P450 from *Bacillus stratosphericus*. This concise review is not so new but still worth to read.	Thank you for the observations, and also it is very much appreciated for finding it worth reading.
2	Laura N. Jeffreys, et al., Novel insights into P450 BM3 interactions with FDA-approved antifungal azole drugs (2019) Scientific reports, 9, 1577. https://doi.org/10.1038/s41598-018-37330-y Comment: Drug-enzyme interaction is investigated in detail based on the 3D structure determined by X-ray crystallography in this paper. Those information and way of thinking may give	Thank you for suggesting such an interesting paper. I will surely follow up with the findings reported in this work.

	some ideas for application of your biosensor for pharmaceutical fields.	
3	Victoria V. Shumyantseva et al. From electrochemistry to enzyme kinetics of cytochrome P450 (2018) Biosensors and Bioelectronics 121, 192-204. https://doi.org/10.1016/j.bios.2018.08.040 Comment: Enzyme kinetics and possibilities of applications were reviewed in a concise article, It is readble article written by A. I. Archakov's group.	Thank you for suggesting such an interesting paper.
4	A. Molaei et al, Aphron applications- A review of recent and current research (2015). Advances in colloid and interface science. 216,36-54, https://doi.org/10.1016/j.cis.2014.12.001	Thank you for suggesting such an interesting paper.
Comments/Corrections/Modifications suggested by Examiner-2		
Sl. No.	Comment	Response
1	The figure legends and their respective reference in the text exhibit discrepancy. For e.g. third paragraph of Chapter 1, Section 1.2.2. refers to "fig. 1" for elucidation of catalytic reaction, whereas the same has been specifically elucidated in "fig.1.2" Also there is lack of clarity of symbols in a certain figure, fig. 2.7. The dot symbol for isolated bacteria TM14001 and TM14007 are indistinguishable. Fig. 2.10 seems to have incomplete fig legend, "Colony PCR amplified product of 16s rRNA for isolated bacteria TM14001" This should include TM14007 as well as the result shown for both the bacteria. The legends of fig 4.7 (a) and (b) need to be changed from "…9days.." to "…3 days…" as	In view of the examiner's suggestions, in the third paragraph Chapter 1, section 1.2.2. for elucidation of catalytic reaction was referred as fig.1 by mistake. Which has been corrected to fig. 1.2. (Pg. 9, line 2) The symbol for isolated bacteria TM14001 and TM14007 changed for better understanding as suggested. (Pg. 62) As per the suggestions given by the respected examiner the isolate has also been included in the legend. (Pg. 64)

	the data shown in the graph corresponds only to three days.	The legends of fig 4.7 (a) and (b) nine days have been changed to three days. (Pg. 106 and Pg. 107)
2	The thesis work documents the use of SiO_2 as the gate material during the fabrication of ENFET during immobilisation of the partially purified enzyme via agarose. However, a shift of the gate material to Si_3N_4 has been observed during immobilisation of the partially purified enzyme using polyaphrons. Then, performance of both the sets has been compared and an improvement of the device reported. However, there is no explanation as why to this change of gate material was made. Under such scenario, the parallel drawn between the performances of the two sets of ENFET would have been more evident.	The dimensions of surface areas of the gate region for the Silicon dioxide (SiO_2) device is 5x5 mm (agarose method), which was larger than the silicon nitride (Si_3N_4) gate (1x1 mm, polyaphron method). For stabilization of the immobilised polyaphorons, a smaller surface area was more appropriate and hence the smaller device was used. Further, SiO_2 even though biocompatible, it suffers from secondary effects such as drift. This effect is seen lesser amount in Si_3N_4. Further, it was seen that it was not possible to develop an ISFET device with SiO_2 as gate material in smaller dimension in our fabrication setup in the Electronics and Communication Engineering (ECE) department. Hence, the conscious decision to change the material was done for stabilisation of the polyahorns and for the better sensitivity of the device.
3	The thesis reports that the bio sensing layer exhibited more response when the crude protein extract was used as the sensing layer, when compared to the partially purified enzyme. What is the plausible reason? Further, any statement on the interference (as CYP450 monooxygenase has a variety of targets, aliphatic and aromatic hydrocarbons)?.	The plausible reason for better sensitivity of the crude enzyme may be attributed to the complex nature of the CYP450 enzyme. The enzyme might have lost some of its functionality during the current purification process, further comprehensive studies will be required for better elucidation. The present set of experiments, reported in the thesis, have been

		conducted in ideal environment with only substrate as "n-hexadecane". For better understanding of the interference, further studies are needed to be carried out with different targets.
4	There are typos in the text which can sometimes change the meaning of the intended sentence viz Pg. 103, last paragraph, it should be "production of n-hexadecanol" instead of "production of n-hexadecane". Pg. 130, section 5.4.6, it should be "with the increase in temperature there was decrease in output V_{GS}" instead of "with the increase in output voltage there was decrease in output V_{GS}" Pg. 140 conclusion no. 9, the construction of the sentence needs to be reviewed.	As per the suggestion given by the respected examiner in the Pg 103, "production of n-hexadecane" has been changed to "production of n-hexadecanol". In Pg. 130, section 5.4.6, "with the increase in output voltage there was decrease in output V_{GS}" has been changed to "with the increase in temperature there was decrease in output V_{GS}" In the Pg. 140 conclusion no. 9, the construct has been change from "So far their use as biological recognition elements the smaller band (band1) didn't show any significant machine output. The larger band (band 2) showed some activity, however the sensitivity was maximum for the crude." to "When used as biological recognition element, the smaller band (band1) didn't show any significant machine output. The larger band (band 2) showed some activity, however the sensitivity was maximum for the crude."
5	Apart from the above comments, a spell check needs to be run through the entire document viz. spelling of "abbreviation" in the abbreviation page, and plenty others viz	As suggested by the respected examiner the word "abbreviation" have been corrected. Further a detailed spell check has been run

	spelling of "variety", "enough" and "ENFET" throughout the document. Uniformity regarding use of symbols, viz. hyphen in "n-hexadecane" throughout the text would be appreciable. Also, substitute "ul" by "µl" wherever applicable.	through the entire document and spellings have been corrected. For n-hexadecane the hyphen is inserted throughout the document, also, all the "ul" has been substituted by "µl"
Comments/Corrections/Modifications suggested by Examiner-3		
Sl. No.	Comment	Response
1	On the 3rd page of the abstract in line number 4, the word 'improvisation' should be replaced with improvement	As suggested by the esteemed examiner the word has been replaced.
2	On the 3rd page of the abstract in line number 10, the word 'alkene' should be replaced with alkane	As suggested by the esteemed examiner the word has been replaced