

**DOTTORATO DI RICERCA IN**

**BIOINGEGNERIA E BIOINFORMATICA**

**UNIVERSITA’ DI PAVIA**

IL COORDINATORE

**PhD THESIS EVALUATION FORM**

*Confidential to the PhD Final Evaluation Committee*

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| **Year\*\*** | **2024** |
| **PhD Student** | **Francesca Usai** |
| **Reviewer** | **Dr. Yonatan Chemla** |
| **Reviewer Affiliation** | **Massachusetts Institute of Technology** |
| **Date of the review** | **December 3, 2024** |

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| **Title of the PhD thesis\*** | **Engineered living materials for sustainable****and green applications** |

**Overall Assessment. Please suggest a possible outcome of the evaluation among the choices:**

🞎 PhD thesis not ready to be defended;

**🞎 PhD awardable;**

🞎 PhD awardable *cum laude* (top 10%)

*Evaluation Table 1 of 2 (Please tick as appropriate: 4 – Excellent, 3 – Very Good, 2 – Good, 1 – Fair, 0 – Poor,Not App: Not Applicable). Please add a short comment if the evaluation is Fair or Poor*

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| **Scientific soundness and significance** | **4** | **3** | **2** | **1** | **0** | **Not App** | **Comment** |
| Wide relevance/interest of the research theme  | **x** |  |  |  |  |  |  |
| Objectives well defined and scientifically supported |  | **x** |  |  |  |  |  |
| Adequacy of the methodological approach |  | **x** |  |  |  |  |  |
| Quality of the experimental setup |  | **x** |  |  |  |  |  |
| Novelty of the approach |  | **x** |  |  |  |  |  |
| Contribution to knowledge in the field |  | **x** |  |  |  |  |  |
| Quality of the results  |  |  | **x** |  |  |  |  |
| Discussion and conclusions valid and properly supported |  |  | **x** |  |  |  |  |

*Evaluation Table 2 of 2 (Please tick as appropriate: 4 – Excellent, 3 – Very Good, 2 – Good, 1 – Fair, 0 – Poor, Not App: Not Applicable). Please add a short comment if the evaluation is Fair or Poor*

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| **Written Document** | **4** | **3** | **2** | **1** | **0** | **Not App** | **Comment** |
| Quality of the Abstract (is it exhaustive?) |  | **x** |  |  |  |  |  |
| Document organization. Suitable balance of the component parts of the thesis |  | **x** |  |  |  |  |  |
| Adequacy of the references |  |  |  | **x** |  |  | **Requires many more references** |
| Clarity  |  | **x** |  |  |  |  |  |
| Communication effectiveness |  |  | **x** |  |  |  |  |
| Properly supported discussion and conclusions |  | **x** |  |  |  |  |  |

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| **Comments/Notes** |
| *Please add here any further comments/notes that might be useful to the PhD Candidate for improving the final version of the thesis.**PLEASE NOTE THAT THE FOLLOWING SECTION WILL BE FORWARDED TO THE PhD CANDIDATE**Use additional pages if needed*Thesis review:Francesca Usai’s thesis and research are impressive and well deserving of a PhD. The scope of her research is wide and encompassing. The ideas are creative and impactful and the results can be infuential for the field of ELM and carbon capture. I commend her for the good work and it was a pleasure reading the thesis.I recommend without hesitation to award a PhD. Below are my comments and recomendations to improve the thesis.Major comments:1. Missing a lot of references across all sections, many claims or methods are unsupported.
2. Section 4 is missing an introduction and a discussion about what is the research question or motivation?

Minor comments:1. Figures can be made more consistent and informative, mainly add genetic circuit diagrams, and mention what is the chassis for each.
2. I think call-outs to your published papers or papers that are under preparation would be useful for future reference.
3. Minor typos are present throughout, would benefit from proofing.

Section 1:General:1. Introduction requires more citations in many instances. For example in page 4 there are many claims but only one citation refereeing to only the last paragraph.
2. Author should attribute statements like “fluorescent proteins commonly used in whole-cell biosensors…” to the original papers showing them. Also there is no citation to the development of fluorescent proteins, etc.
3. Page 3 second P, ‘operator’ or operon would be more appropriate than ‘cassette’
4. Page 3 Bottom - Not necessarily proteins, can be any genetic part, or knocking out parts
5. Missing bioluminescence in Fig. 1.2 and in the text.
6. Author does not define how LOD is calculated in page 5)
7. Missing a better connection between Biosensors and ELMs, early in the background section.
8. Missing the limitations of outside the lab and in the field.
9. Page 9 first paragraph – this was not the main reason the bacteria were encapsulated in alginate.
10. Cant find reference for Ma et al (Page 9)
11. Page 10 – need a better discussion on what is the actual risks of release of GMOs, including references.
12. Page 14 - The need for cyanobacteria surface display should be better explained. From what is written its not clear why one would need the system specifically in cyano and not in other bacteria, which after selection of my protein I will move it cyano for production. I understand the need for secretion but not for surface display.
13. Page 18 - Very well defined and important thesis questions. Which are surprisingly under researched in the field.

Section 2:1. Page 21/22 , is the bioprinting protocol based on published studies? If so, cite them.
2. Page 22, its unclear when the bacteria were added to the alginate and how, and how they were incubated, and at what days are some of the images in Figure 2.1 are taken.
3. Pages 23, 24, and Figure 2.3 (and elsewhere), I recommend to refer to the alginate as simply ELM in the text, it is not an accurate description and may cause me confusion and promote misinterpretation.
4. Page 26 – provide the VAI standard chemical name.
5. Page 26 – provide reference for the VAI biosensor, who discovered, who first used as a biosensors, etc.
6. Page 26 - How image analysis and fluorescent measurements were done? I would recommend a sentence here even if its detailed in the methods section.
7. Page 26 – define LOD.
8. Page 26 – Give a reference for the fast degrading tag.
9. Page 27 – PAI appears in figure 2.6, and it has not been defined.
10. Page 29 – why these inducer concentrations were chosen, and did the author tried with lower concentrations?
11. Page 31 – again, why these specific concentrations of VAI were chosen?
12. Generally – across many figures, it would be beneficial to add the genetic circuit diagram of the bacteria used.
13. Page 32 - the gram positive and gram negative pathogens references needs some clarification, its unclear what it has to do with the sensors, and why it is tested.
14. Page 33 – Figure 2.9 – the sample numbers in panel A are not discussed. What is each sample?
15. Page 33 – Need references for the range of PAI levels
16. Page 36 – Figure 2.11, the diagrams showing VAI-RFP or IPTG-RFP are confusing, I recommend showing a genetic circuit.
17. Page 36 – Figure 2.11, can you quantify the effect of the sender and receiver cells?
18. I think the conclusions about sensing clinical samples should be made with more caution as only one out of the 3 samples worked. Was this reproducible? i.e., was the experiment repeated? If so, mention it, and it strengthens the conclusion.

Section 3:1. Pages 42/43: The thesis would benefit from a discussion on the specific or non-specific nature of some of the sensors that are mentioned. Are some of the sensors general stress response sensors? If so, how can they be accurately used for VOC sensing?
2. Page 43: References for ethanol below toxicity concentrations.
3. Page 45: Figure 3.4 is not mentioned in text, and its not clear what the figures shows and how its relevant to the section.
4. Generally – it is can be misleading to present RFP/OD data for sensors, as toxicity from the inducer that lowers the OD can be interpreted as higher signal. I suggest adding a senstence about it or data showing that is not the case whenever RFP/OD is presented.
5. Generally – would be helpful to add genetic circuit diagrams to figures and mention what is the chassis (E. coli?) in the figure caption to avoid uncertainty.
6. The in depth analysis of abiotic factor’s influence on sensors is very informative, and important data.
7. Page 68 – its unclear what the author means by digestate, please define.
8. Page 70 – Need a discussion around the claim: “clearly demonstrated
9. the feasibility of using glnaP2-based ELM biosensors to detect acetate” when its clear that the biosensor does not operate in the relevant sensitivities for the presented application in figure 3.17.
10. Page 76 (and other pages), whenever introducing a new material, like PDMS or PES, a sentence describing it and explaining what the abbreviation stands for.
11. Page 80/81 – I understand how a on/off kind of analysis is possible with the biosensor readout and the laser, but how is it possible to have a quantitative readout given the variability coming from the laser, camera, and the settings? I.e., wouldn’t it be hard to have a calibration curve for real world settings? I recommend to discuss it in the text.
12. Page 84 – I recommend addressing and revisiting the questions presented in the research aim section in the begging in page 18 and 19, to make the conclusions clearer.

**Chapter 4**1. Page 85. **Major comment:** Missing background and motivation to carry the research described in the chapter.
2. Page 85/86,missing references. For example, for the SLPs, and the HiBiT.
3. Page 86, Figure 4.1, is the Q31NQ2 bar have 2 or three replicates.
4. Page 86,Figure 4.1, are the bar graphs in C normalized to cell density? If so, how? Please add.
5. Page 91, Figure 4.4b, again I don’t understand the normalization if any.
6. Page 92 , line 2 , typo (lsecond).
7. Page 93 - written well and very clear.
8. Page 94 , again , missing references.
9. Page 99, figure 4.8, its unclear what are the cells in the images. And its unclear if you discuss the difference if any, between panels A and C.
10. Page 100, I’m missing a final conclusion if there are any direct evidence of the mineralization of carbon in these bricks.
11. Page 101, please provide a reference and an explanation about MMseq2.
12. Page 105, I recommend you discuss the use of secreted CAs as part of the bricks to improve carbon sequestration. Put it in the larger context.
13. Page 106, typo – *survuve*
14. Page 107 , typo sywestems
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