Meeting slides: Milica Radisic Interview

Advice:

- Make this as small as possible to reduce amount of bacteria required to grow
- Will want a microfluidic bioreactor that can work with small amount of cultures
    - Should be looking for volumes of few hundred microlitres -> scale down
    - 96 is not a lot of bioreactors, since screening a lot of enzymes
    - Need to minimize transfer steps
- Ideally doing spotting instead of bioreactor for high throughput assay
- Also avoiding the transfer and drawing up of E. coli; realistic cycle time is 2 weeks
- Volume depends on application
- Can get well plate based bioreactors
- Look for 96 deep well plates -> 5 day cycles
- Use film membrane instead of plastic lid to allow cells to grow better
    - Also buy bottomless deep well plates then add a permeable membrane to make the bottom also better air -> PDMS bottom
- Channels etched into bottom that connects to 3 wells??? (someone in her lab is working on this. Also her previous PhD → Postdoc left and is now doing a company to design and manufacture these wells to be sold)
- Be careful w/ sterility; everything going in needs to be sterilized

Overview:
The meeting with Milica lasted an hour ish and was insightful. She knew of iGEM and was like “everyone knows about iGEM 🤩” (LOL). But then we started talking about the project and overall we spent some time to explain the reasoning behind the bioreactor.

The 2 core reasons: Making this low cost and high throughput means that we can achieve scale that other industrial or even current solutions cannot reach. By stripping out the excessive components not needed for this project we can reach a cost of <$150 or even <$100 which would be impressive and unlock scale. Our goal for the iGEM cycle is to reach 8. Now this inherently unlocks lab bench side testing. We can create these parallelized systems to work on a lab bench and just test out enzymes rapidly.

The other core reasoning and arguably more important reasoning behind bioreactors is that you can do long horizon scale experiments with very fine grained and programmatic control over conditions. You can also do ‘directed evolution’ in these containers by allowing for mutagenesis with a faulty polymerase or some other means of mutagenesis. Something that is not possible in an incubator or a well or any other medium. You can do programmatic growth curves or gradients or a variety of other measures that can give you very rich data on kinetics and truly enable you to draw out the Michaelis-Menten rate for example or calculate the binding affinity or the Vmax for example.

Milica understood this and saw the potential in this scale of thinking but did not agree with the physical scale we are working with. And I see her point now. We initially have a pre-screen to go from large scale data mining down to ~1000 candidate enzymes that can break down plastic (this is wet lab) and then for hardware we can think about this in 2 ways. We need an intermediate screen that can then help you choose the ~50-100 or so enzymes that are really really good and efficient and then you use the bioreactors to grow them out for weeks on end and find their local optima. So you can 'hill climb' each specific enzyme you find.

Her recommendation was that this needs to be done at a smaller scale. Right now our reactors are about 40 mL but we need to get down to 1mL and work at the microliter scale. This will reduce the space requirement and the enzyme volume bottleneck and allow us to do that intermediate screen. Yes, the 40 mL is good for directed evolution so we then decided to continue down this path for iGEM and beyond. But she is also interested in the microliter scale and is going to find an undergrad student in her lab who is in their 4th year thesis and can help us out and be a part of igem for the rest of this sem and even for the next cycle maybe.

TL;DR I think the space of bioreactors is really really cool and has immense promise. We have found this niche where our reactors can sit on a lab bench and unlock very fast and easy testing for any enzyme. There is a niche for smaller and more parallel testing with say the deep well plates and then having channels in it to then enable 384 well plates as mini reactors. But overall, this is very promising and we can have **monthly** calls with Milica to show her progress.

Base questions in order of priority:
Potential bioreactor improvements?

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What features would be most useful in a device for you as a researcher? In industry?

- Safety is very important
- Easy to sterilise
- Reusability
- Not too many connectors (Not too hard to set up)
- Good way to remove gases or bubbles to accumulate in cultures
- As few parts as possible (but also easy to customise; modular)

UI feedback:

- Overview of condition of modules to improve modularity
- Constant OD? How can we keep OD constant? -> may need some feature development for this

Modules:

- Additional colour sensor for plastic degradation
    - You actually can't use the same OD channel for colour dye
    - If dye is contaminating your OD readings you can't estimate the bacteria density
    - Plastic degrading activity per unit cell

    - Select different diodes to measure different wavelengths of light

Per cell vs per enzyme:

- We can tag enzyme using GFP, etc. to ensure a per enzyme count

Data collected through all colour channels:

- Down the line; different plastic substrates: one reaction and measure multiple plastic substrates at once
- Put different microplastics together and measure activity for all
- Do reading on flow cytometry
- Multiplex dyes together!!!!
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How can high throughput or high dimensional experiments help in your field?

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Suggestions and challenges you foresee around designing temp, pH, etc. control systems?

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What can bioreactors be used for in your field?

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Next steps:

- Artem: Bacteria, can run it for 8 hours, pause it, resume the next day–doesnt have to be continuous lol
- GFP signals caused by enzyme expression and not background noise of the system
- Stress test all electronic components
- More experiments to see how OD calibration reliable is