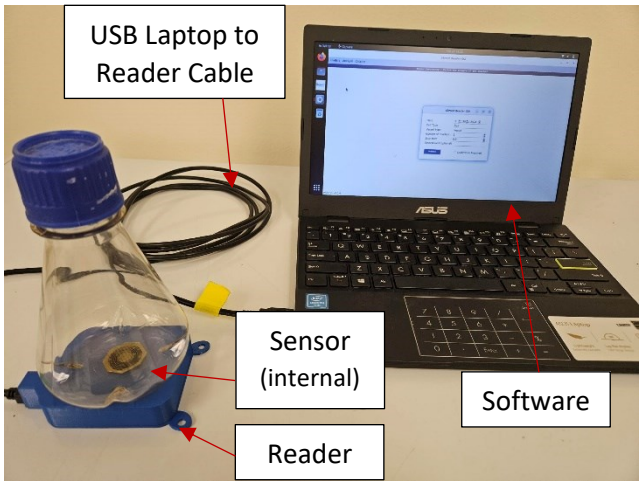
Skroot SMART Platform: Monitoring Yeast Growth Profiles in Shake Flasks

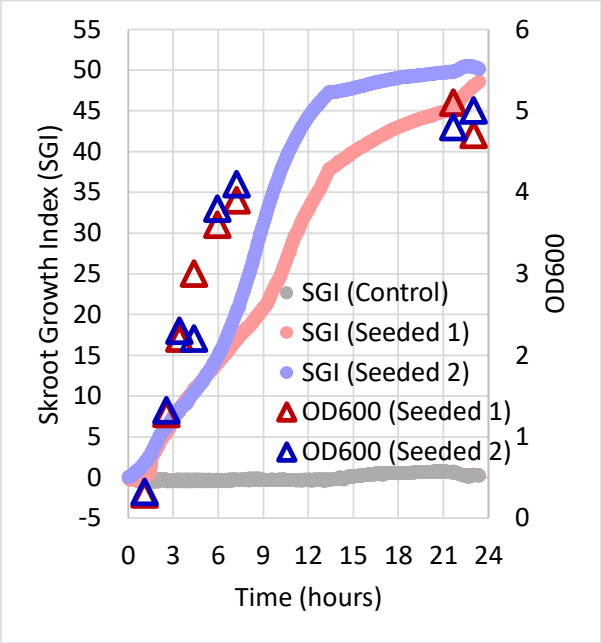
Motivation: Yeast cells, with their rapid doubling time, are an excellent platform for biomanufacturing. The growth patterns of these microorganisms depend significantly on the strain type and growth conditions. Traditionally, to monitor the growth phases (lag, log, and stationary), manual sampling of the media is conducted throughout the entire growth period. A common method for manual sampling involves using a spectrophotometer to measure light absorbance, typically at 600 nm (OD600). However, this method's accuracy is limited to around 1 OD, necessitating careful dilutions at higher cell densities. Furthermore, obtaining a comprehensive growth profile requires the presence of an operator for a challenging 16+ hours.

Solution: The Skroot Single-use, Metabolite Absorbing, Resonant Transducer (SMART) sensor provides continuous monitoring capabilities for cell culture growth. Placing the sterile Skroot SMART sensor inside a shake flask allows for minute-to-minute tracking of yeast cell culture growth without the need for manual sampling. When used in conjunction with the Skroot reader and software, the Skroot SMART sensor enables real-time reporting of yeast cell growth responses. The sensor operates by absorbing naturally secreted secondary metabolites through a cell transduction layer, thereby altering the sensor's radio frequency (RF) resonant characteristics. This process occurs wirelessly and passively, eliminating the need for onboard power in the Skroot SMART sensor. Moreover, the non-optical sensing mechanism ensures the effectiveness of Skroot SMART sensors in various media, including turbid media with high cell densities.



Demonstration: To showcase the application of Skroot SMART sensors in monitoring yeast cell growth, sensors were placed in 250 mL shake flasks with 80 mL of Terrific broth. Two flasks were inoculated with 1 vol-% of SafAle™ S-04 yeast culture, while the third flask served as a control vessel and was supplemented with Pen-Strep to prevent microbial growth. The flasks were shaken at 200 RPM and incubated at 37°C. In addition to the Skroot SMART sensor output, the Skroot Growth Index (SGI), 1mL media samples were analyzed with a spectrophotometer (OD600).

The following plot illustrates the tracking of cell growth by both the continuously-monitoring Skroot SMART sensor system as well as the manual spectrophotometer samples. While clear differentiation between the control and seeded sensor data is observed, a comparison of SGI with OD600 data shows direct correlation across all growth phases. It is also worth noting the significant uncertainty present in the manual OD600 samples.



Intellectual Property:
1.Wireless Sensor (US Patent #11105761)
2.Transduction Cell Membrane (Application at USPTO)
3.Resonant Sensor Reader (WO Patent #236534)

Preprint for more information:
https://doi.org/10.1101/2024.01.27.577601

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