Fabrication of bioreactor modules, inline bacterial growth sensing and the inclusion of µcosm inline sensing

Jack Bradley
Dr. Somenath Bakshi
Department of engineering.

Introduction:

- Fabrication of bioreactor modules and inline OD (Optical Density) sensor(s) to allow for real-time bacterial growth monitoring.

- The inline sensor allows for a bacterium to be in a controlled environment whilst growth is measured in parallel.

Summary:

Inline sensors allow for a more time resolute method of monitoring the growth of a bacteria in a controlled environment; minimum dt between data points is 800 ms; the typical standard is 30 minutes per data point (up to 2500 x increase in sampling).

Utilisation of microfluidics such as the mother machine for single cell analysis to help further characterisation and support OD measurements.

Modular designs:

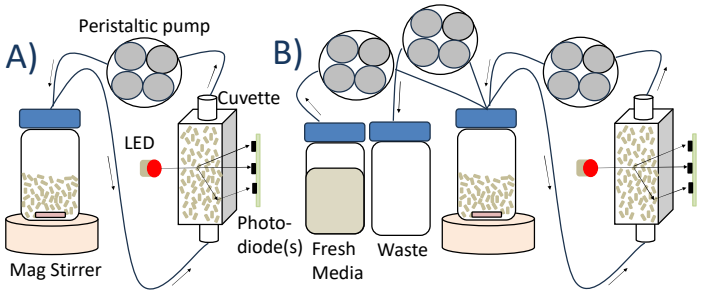

Fig 1. designs of the current two modules – spatially limited due to being housed inside an incubator. A) OD Sensor B) OD control extension.

Control methods:

- Simplistic methods such as on-off, PID and/or MPC can be used for the control of temperature.

- OD is more complex requiring differing methods of reaching the target set as instantaneous action does not affect values under the target.

One of the methods for a lower variance near the target is duration based. This requires a single calibration points of the motors. U(t) being the control output:

$$M1 = 2.5 \text{ ml/s}, M2 = 1.75 \text{ ml/s}$$

$$\alpha = 1/(M1+M2),\ M1P = (1-M1*\alpha),\ M2P = (1-M1*\alpha)$$

$$\text{Where, } U_1(t) = M1P*U(t),\ U_2(t) = M2P*U(t)$$

Other methods include level-based control and power-based control.

Bacterial growth preliminary results:

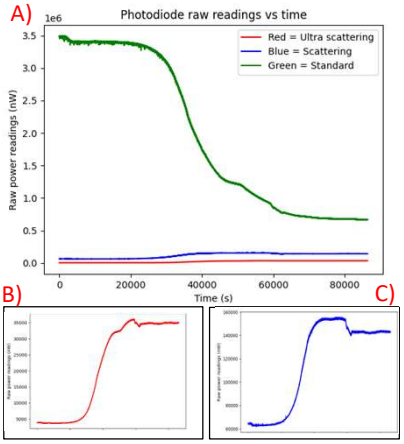

Fig 2. A) All results B) Ultra scattering C) Scattering. – 750nm LED.

- Experiment was done with E coli. over a 24-hour period.

- Noise in measurement was present in all diodes at the beginning of the run.

- Temperature was at 27°C, however a small shift occurred near the end of about 0.5°C.

- The disturbances (at 40k to 60k) are unusual; it could be attributed to a Diauxic shift within bulk populace [1].

What can single cell imaging do for us: µcosm:

- Measuring OD through transmittance is typical however, has its flaws: Aggregation of cells, differing cell sizes, etc…

- Comparing OD to single cell level data is useful to build an evidence basis.

- Cellular size allows for growth stage interpretation, mostly can be correlated to OD.

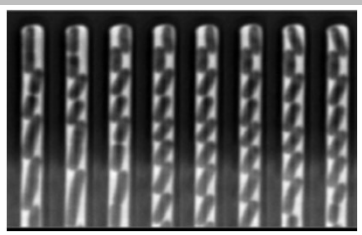

Fig 3. Imaged cellular data from mother machine. [2]

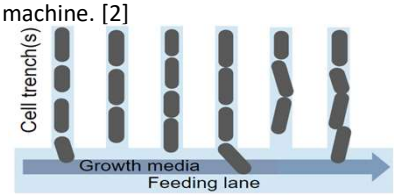

Fig 4. mother machine PDMS chip.

Conclusion:

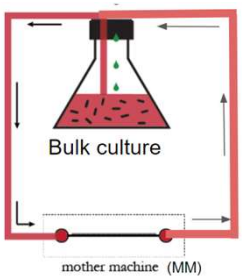

Fig 4. Module A with MM inline sensor. [3]

- Investigation into the correlation between the transmittance OD values (Fig 2.) and the cellular size data (Fig 3.) will be conducted (with and without OD control). Holding OD at a specific target over a temporal frame would allow single cell level insight to perturbation in cell characteristics such as size/stress.

- Turning multiple OD sensor + control modules into an array that can monitor multiple cellular populations in parallel.

References:

[1] Stahl et al., PubMed 2004
[2] Hardo *et al.*, bioRxiv, 2022
[3] Bakshi *et al* 2021

Acknowledgement

This work was supported by the Engineering and Physical Sciences Research Council and AgriFoRwArdS CDT [EP/S023917/1]