New miniature on-line optical cell biomass probe with wide linear range and bubble discrimination

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Immersible on-line cell biomass probes have historically suffered from a limited range of linear response to biomass and sensitivity to process variables, such as agitation and gas sparge rate. A new optical probe has been developed that provides linear response over 4 orders of magnitude of biomass (e.g. 0.01-200 g/L yeast dry cell weight). The small probe diameter (e.g. 3 mm) and minimal optical penetration depth (e.g. <3 cm) make it suitable for a wide range of vessel types, including miniature bioreactors (e.g. 250 mL). Interference from bubbles is largely eliminated through a novel measurement technique, making the results nearly insensitive to agitation and aeration rate changes.

Key Areas of Focus

1. Optical Reflectance Constraints

![Geometric Constraints](image)

Depicted on the left is a reflectance probe with a light source having a center wavelength less than 1000 nm, immersed in a bioreactor along with the usual paraphernalia, such as an agitator and other probes. Without taking any steps to limit the measurement volume, the light can bounce over a very wide area, reflecting particularly well off of stainless steel objects, and the wall of the vessel. The effect of such un-wanted reflections is an /Oblishaped reflectance response as a function of biomass, as depicted in the graph on the right.

2. Limiting the measurement volume

![Liquid Water Absorbance vs Path Length](image)

In order to limit measurement volume, we found it useful to shift the light source wavelength further into the near infrared, where liquid water absorbance is strong. Shown here is the mean path length of light traveling through pure liquid water, as a function of wavelength. Have a look at 850 nm (the path length is greater than 10 cm). Now have a look at 1310 nm: here the mean path length is down to about 6 mm. Going even further towards the mid-infrared, the path length becomes exceedingly short, leading to diminished sensitivity at low biomass. The region from about 1100 to 1400 nm provides a range of path lengths that turns out to be optimal for maximizing sensitivity to cell biomass while minimizing sensitivity to reflections from interfering objects.1,2

3. Working with small vessels (500 mL or less)

![Effect of Bubbles on Reflectance](image)

Very high sparging rates in fermentors are a potential source of interference for all on-line biomass monitoring methods. The histograms above show the signals measured by a reflectance probe immersed in a bioreactor containing 25 g/L yeast. The data in the top graph were collected under conditions of moderate agitation and no sparge. The data in the bottom graph were collected at high agitation and sparge. Note that the histogram for the low-bubble case is relatively symmetrical, whereas for the high-bubbling case, the histogram is skewed towards high reflectance amplitudes. As a result, if simple averaging is applied to this data, the mean reflectance is skewed to higher values.

5. Bubble Correction Map

![Bubble Correction Map](image)

As shown above, by creating a 2D map of biomass as a function of the reflectance distribution and central value, we have found that the effects of changing bubbles and biomass can be effectively separated. By applying this map to new measurements, accurate biomass prediction is achieved over four orders of biomass magnitude, despite widely varying aeration and sparging conditions, as depicted in Section 6.

6. Result: 4 Orders of Magnitude of Accurate Biomass Prediction

![Result: 4 Orders of Magnitude of Accurate Biomass Prediction](image)

7. References