Recent Advances in Online Microbial Cell Biomass Monitoring

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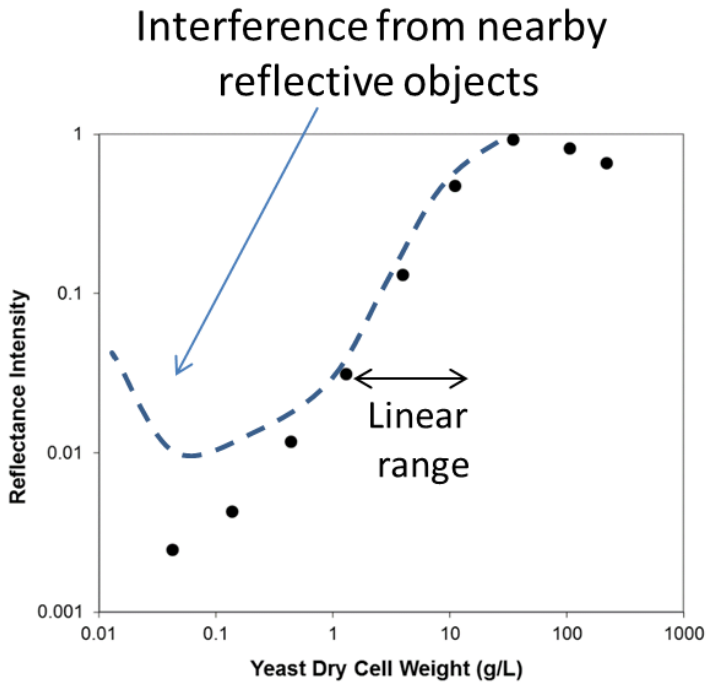
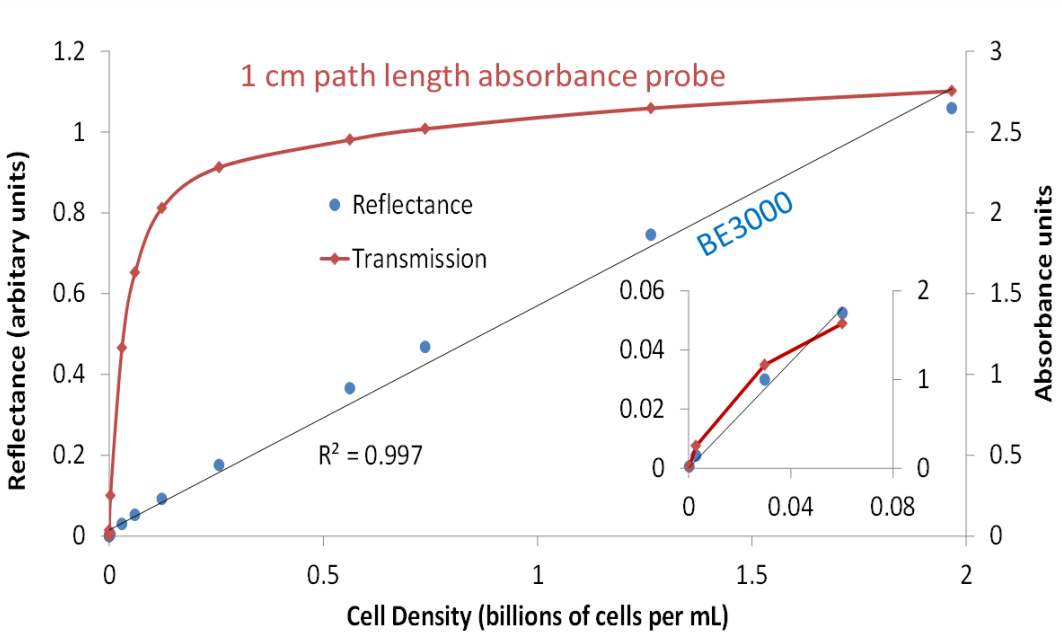
The recent trend in bioprocess development towards small fermentors and high cell densities presents challenges for accurate on-line monitoring of liquid cell cultures. Traditional on-line optical probes, such as transmission probes (also known as absorbance or optical density probes) and reflectance probes (also known as back-scatter or turbidity probes), typically require a 12 mm or larger diameter port into the bioreactor vessel and are limited in their linear response to about one or two order of magnitude of cell biomass range. In small bioreactors, such as 250 mL capacity vessels, the number of available large diameter ports is necessarily limited. Monitoring microbial cell biomass from inoculation (e.g. < 0.1 g/L dry cell weight) to harvest (e.g. > 100 g/L) frequently requires at least 3 orders of magnitude of cell biomass sensitivity.

Several factors contribute to the limited range of linear response of traditional transmission and reflectance probes. For reflectance probes, a frequent complication at low cell density is interference from reflective objects, such as impellers or other probes in the bioreactor. Such interference can lead to an S-shaped response curve, wherein interference at low biomass is gradually overtaken by reflectance from cells at higher biomass as shown on the graph below.



For absorbance probes, the well-known break-down of the Beer-Lambert law is manifested as a flattening of the absorbance curve at high concentrations as depicted in the graph below. A past approach to extending the useful range of optical probes has been to fit a curve to the response beyond the linear range. But this technique suffers from increasing inaccuracy as the response increasingly deviates from linearity, especially if the causes of non-linearity are not consistent across bioreactor runs.



Extending the Linear Response Range

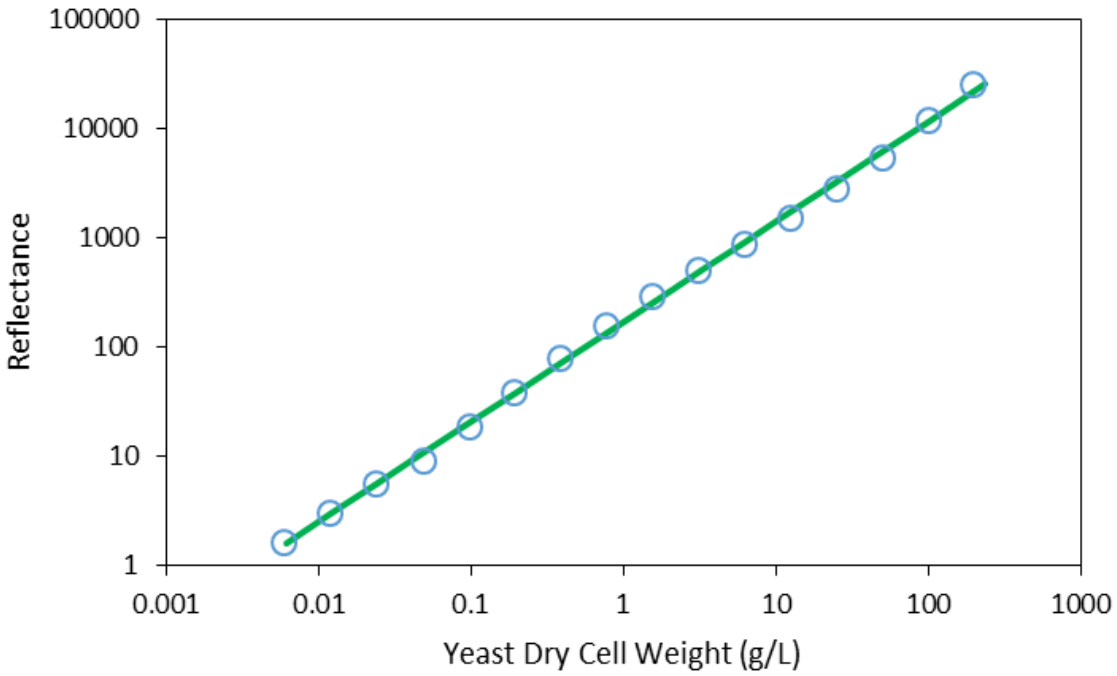
The center of the linear biomass range of an optical probe is determined by the path length that light must travel through the medium to get from the source to the detector. Decreasing this source-detector separation has the effect of shifting the linear response range to higher biomass. One approach to extending the range of biomass is to use several probes with different path lengths to monitor the same process. This technique is typically precluded in small scale bioreactors due to space limitations.

An alternative approach is to measure cell biomass reflectance through the bioreactor wall, such as the BE2100 sensor manufactured by BugLab LLC. This non-invasive method eliminates the risk of contamination from the sensor. Three source-detector pairs are incorporated into a single sensor and are automatically arbitrated between in order to maintain linear response to biomass over three orders of magnitude cell biomass.[1] This approach has been successfully employed in vessels having volumes of 500 mL and larger.

In order to accommodate the trend towards smaller vessels, a more recent approach (BE3000 probe and miniBE sensor manufactured by BugLab LLC), uses a small diameter (e.g. 3 mm) fiber optic probe or non-invasive sensor measuring back-reflectance at 1310 nm and 1330 nm, respectively. At this wavelength, water absorbance limits optical penetration into the medium to 3 cm or less.[2,3] At shorter wavelengths (i.e. towards the visible region of the spectrum) the penetration depth increases due to weaker water absorbance. For example, at 850 nm the penetration depth into the medium can be more than 10 cm. Particularly in small vessels, this can make it difficult to avoid interference from other probes and objects such as impellers. At longer wavelengths, the penetration depth into water rapidly diminishes

due to increasing absorbance of light by water. For example, at 1450 nm, the penetration depth is less than 1 mm. This has the effect of reducing the effective measurement volume to such an extent that the sensitivity to cell biomass is substantially diminished. At 1310-1330 nm the measurement volume is small enough so that measurements can be made in small vessels (e.g. as small as 50 mL in a 250 mL vessel), while at the same time maintaining a very wide linear range of sensitivity to biomass.[3] Using an infrared laser source also avoids light absorbance by colored media components, colored vessels, and visible-light absorbing chromophores such as photosynthetic algae.

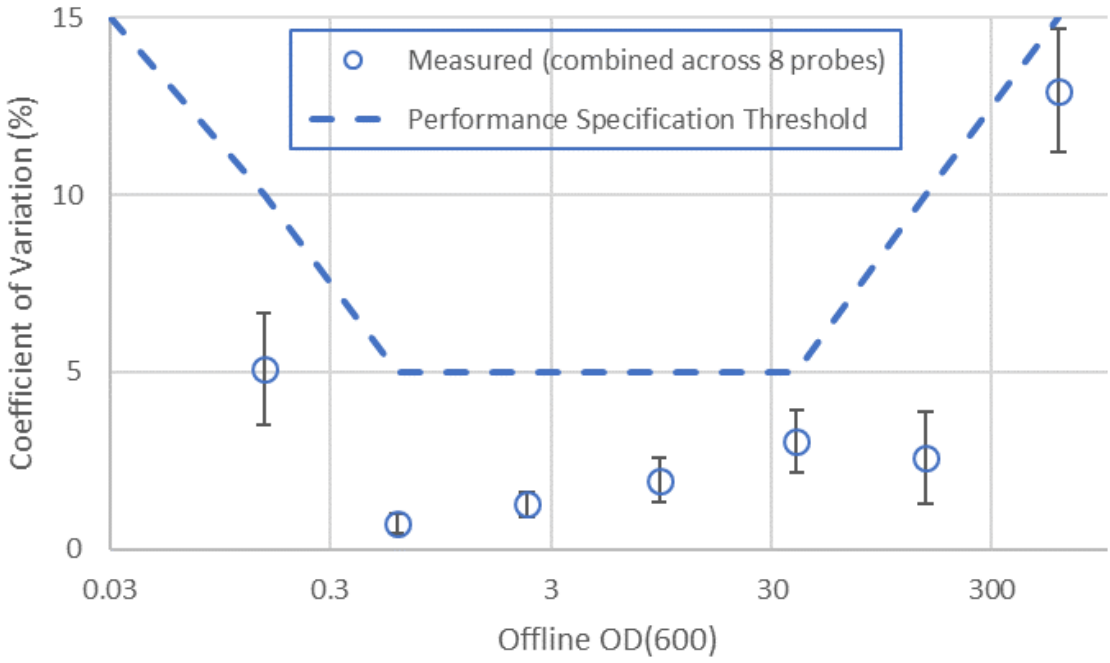
This graph below shows the optical reflectance of a liquid culture of Saccharomyces cerevisiae as a function of yeast dry cell weight. The culture was maintained in a 250 mL bioreactor, and optical reflectance was measured at 1310 nm. Notice that both optical reflectance and yeast dry cell weight vary over four orders of magnitude.



Mitigating the Effects of Bubbles

One of the most well-known sources of interference with online optical measurements of microbial cell biomass is bubbles. For microbial cells grown in bioreactors, very high gassing and stirring rates are often employed. By using a small fiber optic probe and a source wavelength having limited penetration depth, the proBE 3000 and miniBE measurement volumes are limited to approximately 200 μL or less. The number of microbial cells in this volume will be in the thousand to millions at the lowest concentrations of interest, and will range up into the millions to billions at high concentrations. As a result, the individual microbial cells that are inside the optically sampled volume will change over time as the cells move through the medium, but the mean number of cells will be nearly constant. Bubbles are generally larger and less numerous than the cells, so the number of bubbles within the optically

sampled volume will vary widely as a function of time. By creating a 2 dimensional map of biomass as a function of the reflectance distribution and central value, the effects of changing bubbles and biomass can be effectively separated.[3] By applying this map to new measurements, accurate biomass prediction is achieved over four orders of biomass magnitude, despite widely varying agitation and sparging conditions.[3]



The graph above shows the process-induced variation in yeast dry cell weight prediction across process conditions spanning the full range of a typical aerobic fermentation (200 mL vessel, agitation: 750-2000 rpm, sparge: 0-2 vvm).

The bulk scattering effects measured by transmission and reflectance probes is affected by the size of the scattering particles. For this reason, calibration is required in order to report biomass in absolute units such as dry cell weight or cell counts. The proBE 3000 and miniBE instruments come pre-calibrated for dry cell weight of various organisms such as *Saccharomyces cerevisiae*, *E. coli*, and the micro-algae *Chlorella vulgaris* as well as NTU (Nephelometric Turbidity Units). Calibration to other unicellular organisms or off-line methods is a straight-forward process.

The BE3000 probe is available in a range of diameters: 3-12 mm, and insertion lengths: 120-325 mm. The miniBE sensor is easily attached to the side of the vessels with a small footprint (24 x 21 mm) disposable universal adapter. Since optical penetration into the medium is limited, the BE3000 probe and miniBE sensor require less than 3 and 2.5 cm of fluid depth, respectively, and are ideal for mini benchtop bioreactors. The BE3000 probe is autoclavable and CIP/SIP compliant. Multiplexing options allow for parallel biomass monitoring in multiple vessels. The instrument base units have both digital USB and analog output as well as process connectivity options for various bioprocess controllers, automation software and process information management systems.

Additional information is available at www.buglab.com Please direct any inquiries to info@buglab.com

References

[1] US Patent 8,603,772

[2] US Patent 8,405,033

[3] US Patent 9,752,974, 10,054,532, 10,408,730