Microbial

Capacitance has been used to measure cell density in a wide array of microbial applications. One study used capacitance to monitor viable Xanthomonas bacterial cells within a 2000L seed fermentor. Kelco UK was a company using 2000L fermenters in order to create Xanthomonas bacterial seeds to be transferred to a production vessel. Aber Futura systems were initially used in order to first better understand the growth of the seeds, then it was later used to help better understand when to extract the seeds. The graphs below show data collected from 8 different runs. The first 5 runs were done with their original operating conditions. The capacitance trend showed two distinct growth phases. After medium substate changes, the final 3 runs showed more consistent and speedier growth for seed transfer.

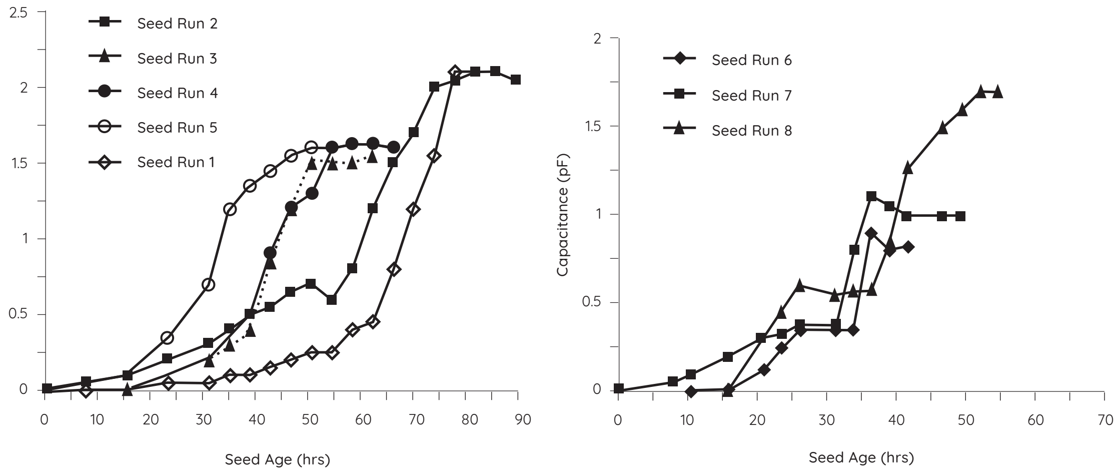

Figure 1: Capacitance trends following original operating conditions followed by trends after medium substrate changes (Data courtesy of Kelco UK).

The use of the real time biomonitoring capacitance allowed for efficient changes to the process that allowed for:

- Seed transfer to the production vessel 30% earlier than the procedure originally instructed.
- Hold onto the seed for 3 times longer without loss of viable cell concentration. The latter point allowed them to maintain ideal cell seed transfer density even when the production vessel was not readily available.

Further adjustments using data collected from the Aber Futura allowed for a 5% increase in production without the need to buy equipment that would increase production capacity. The ability to measure viable bacteria on-line has clearly benefited the Kelco UK site in terms of:

- Better understanding of their seed fermentation.
- Improved seed growth rates by up to 30%.
- Increase holding time by up to 200%.

- Subsequent increase in productivity by 5%.
- Hold off further capital investment that would have been required to achieve this increase in productivity.

This information has been supplied by courtesy of Nutrasweet Kelco Company, Penrhyn Road, Knowsley Industrial Estate, Prescot, Merseyside L34 9HY, UK.

In situ scanning capacitance sensor with spectral analysis reveals morphological states in cultures for production of biopharmaceuticals

Capacitance (via dielectric spectroscopy) was used to monitor cell growth and morphological changes of microbial cells used to produce human insulin and Green Fluorescence Protein (GFP) within a 6L fed-batch bioreactor. Morphological changes were determined using the full spectra of radio frequencies used to measure capacitance (0.1 to 15MHz). It was expected that within the data there would be a period of flat growth within the cells creating a plateau of cell density. During this period, the cells are expected to grow in size as they create the necessary protein. This growth in cell size should be seen on the capacitance curve despite the plateau in cell concentration.

To determine the change in growth, induced and uninduced cells were compared. During the measurement of the induced insulin producing cells it was found that the Dielectric spectroscopy trend had a two to six-times higher slope with a 40-49% increased signal when compared to uninduced cells. This change is met with results from scanning electron microscopy that shows uninduced cells maintain an average cell size of approximately 1.5µm while induced cells average cell size grew from 1.5 to 3.0µm. It was determined that there was no significant size increase when comparing induced and uninduced green fluorescence protein producing cells.

Addressing the challenge of optimum polyhydroxyalkanoate harvesting Monitoring real time process kinetics and biopolymer accumulation using dielectric spectroscopy

Polyhydroxyalkanoates (PHAs) are produced intracellularly by bacteria as a natural way to store carbon and energy. PHA is of interest in an industrial sense as a material to help replace polypropylene. Complications arise during the manufacturing of the material due to cells' natural degradation of the product, which varies between feedstock and bacteria. Aber Futura measuring capacitance via dielectric spectroscopy was introduced as a way to identify the most appropriate time to stop fermentation in real time. This was tested by monitoring real time capacitance of a line of Cupriavidus necator bacterial cells within a fermenter. It was determined that dielectric spectroscopy followed along the same curve as total PHA. This allowed peak capacitance to correlate with peak extraction point. The understanding of peak extraction point allowed for an increase in yield and better understanding of the process for even further optimization.

References

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