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Application Note

Identification of Biofuel Producing Bacteria Through Temperature Resistance

Demonstration of the High Temperature Capabilities of BioTek's Microplate Readers

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The use of microplates is driven by a demand to increase throughput in many different biological systems. While microplate reader design has provided for elevated temperature control for some time that was sufficient for typical cell-based studies, an extended range was needed for some biological applications. For example, bacterial strains useful for the generation of biofuels require bacteria that are temperature resistant. The search for these strains requires that growth curves of multiple bacterial clones be monitored at high temperatures over long periods. Towards that end, BioTek has improved their microplate readers' incubator design to accommodate temperature control up to < 68ºC. Here we demonstrate the utility of the PowerWave™ XS Microplate Reader to perform absorbance measurements at high temperatures.

Introduction

There is an increasing requirement for microplate readers that can provide temperature control at high temperatures. One example is the screening of bacterial clones for their ability to convert cellulose into biofuels. Bacteria are typically cultured at 37ºC, but the formation of biofuels from cellulosic biomass requires growth at much higher temperatures, often exceeding 50°C. Normally, the screening for high temperature variants requires separate cultures that are sampled and measured individually in a spectrophotometer. The ability to perform bacterial growth studies directly in a microplate reader provides a vast increase in throughput, as well as eliminating any manual intervention during the experiment. Here we demonstrate some of the temperature capabilities of the PowerWave™ XS at 65ºC and provide a demonstration of its utility in identifying bacterial growth at high temperature.

The PowerWave XS Microplate Spectrophotometer is a single channel absorbance microplate reader that has the capability of measuring absorbance in 96- and 384-well microplates (Figure 1). This monochromator-based instrument, which has a wavelength range of 200-999 nm, requires no filters and can perform spectral scans of substances in increments as small as 1 nm. A Xenon-Flash lamp is used to illuminate a high precision diffraction grating monochromator. The monochromatic light is then split into experimental and reference channels. The experimental channels are then focused onto the microplate, while the reference channel is directed to the dedicated reference silicon diode detector. After passing through the experimental sample, unabsorbed light is focused onto silicon diode detectors. Elevated temperatures are regulated by a four-zone control system that assures superior temperature uniformity up to 68°C.

Figure 1. PowerWave™ XS Microplate Spectrophotometer

Materials and Methods

Initial temperature monitoring experiments were performed using a temperature test plate data recorder (Innovative Instruments Inc., Wake Forest, NC) to monitor temperature within the reading chamber of the reader. This test plate allows a non-intrusive temperature determination at five locations (four corners and the center) on a microplate. In order to examine the increase in temperature from ambient to 65°C, the test plate was incubated in the reader's read-chamber immediately after the incubators were turned on. After a 5-second delay to allow transport of the plate into the reader, the temperature was recorded every minute for 100 minutes. Temperature stability data was obtained at 65°C with a reader that had already achieved equilibrium. In these experiments, data was recorded every 30 minutes for a total of 50 hours. After recording, data was downloaded to a PC using the test plate manufacturer's software, exported to GraphPad Prism (San Diego, CA) and the data plotted.

Bacterial growth was monitored at 600 nm using the PowerWave XS reader's temperature set at 68°C. An increase in absorbance is indicative of bacterial growth. Unknown bacterial clones were used in each well with the expectation that some were capable of growth at 68°C, while others were not. Reader parameters and data acquisition was carried out with Gen5™ Data Analysis Software (BioTek Instruments, Winooski, VT). Plate Data was expressed using Gen5 Data Analysis Software.

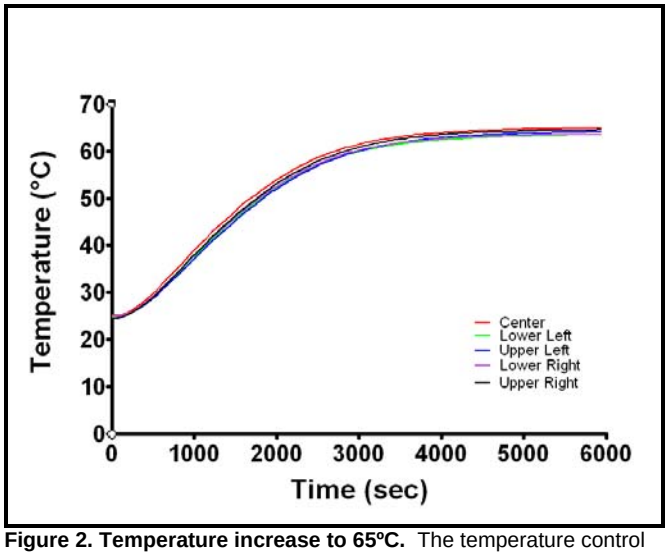

Figure 2. Temperature increase to 65ºC. The temperature control of a PowerWave XS was changed from ambient to 65ºC and the temperature was monitored for 100 minutes at five plate locations using a test plate.

Results

The BioTek readers utilize a four-zone control system that provides uniform temperatures across the entire microplate. As shown in figure 2, temperatures on each corner and the center of the plate follow a uniform increase when the reader's temperature control system is turned on. Maximal temperature of 65°C is achieved within 50 minutes and maintained a constant temperature thereafter. While the center region of the microplate had slightly higher temperatures than the peripheral corners, the difference was less than 2°C during ramp up and less than 1.5°C once equilibration had occurred.

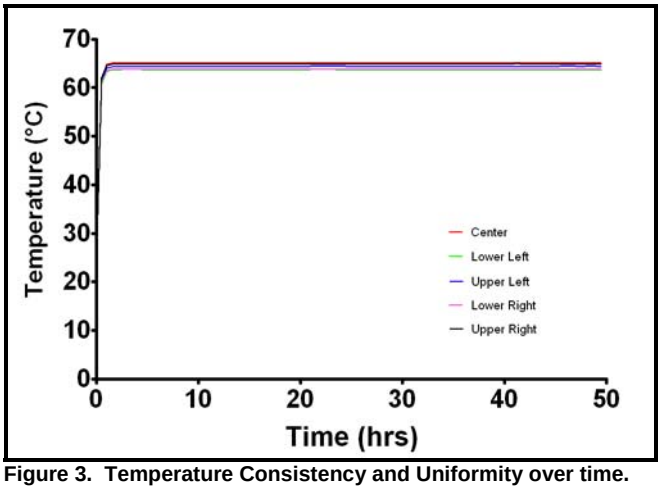

Figure 3. Temperature Consistency and Uniformity over time.

Temperature consistency over a long period of time was also examined. With the PowerWave XS reader equilibrated at 65°C, the temperature test plate was used to monitor temperature over a period of two days. Once the test plate equilibrated (approximately 30 minutes), temperatures remained constant over the 50 hour period examined (Figure 3). Typical temperature variation between the minimum and maximum measurements at any time point was 1.5°C or less. The rapid increase in measured temperature during the initial portion of the experiment is the result of the temperature test plate warming from ambient to the set point of the reader of 65°C.

Using the PowerWave XS reader at 68°C, unique bacterial clones were tested for temperature resistance by measuring growth using light scatter at 600 nm. Bacteria growth in liquid-broth behaves as a colloidal suspension and causes the light beam to be diffracted or scattered. The result is that despite not truly being absorbed, less light is measured at the detector and recorded as absorbance. Bacterial growth is indicated by an increase in the absorbance output at 600 nm. As demonstrated in Figure 4, bacteria with different growth characteristics can be identified with this technique. Using the kinetic curve display of Gen5 Data Analysis Software, bacteria with significant growth changes over time can easily be distinguished from those with little or no growth.

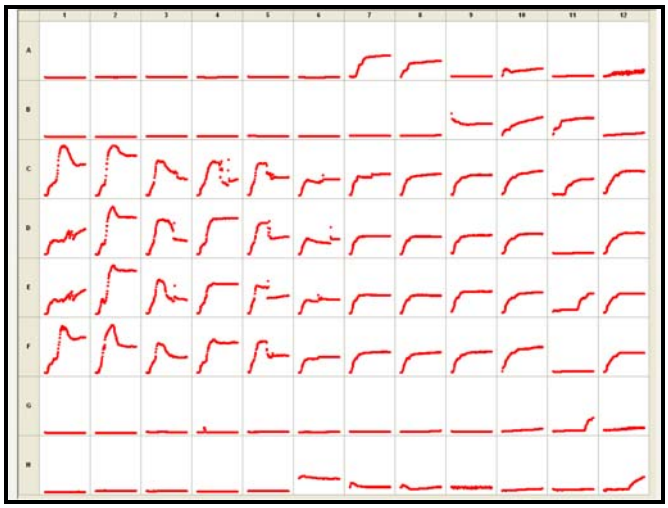

Figure 4. Growth Curves from different bacterial clones.
Different bacterial clones were incubated at 68ºC for 100 hours and the absorbance at 600 nm monitored every 10 minutes.

These growth curves can also be examined in closer detail using the "well zoom" feature of Gen5 (Figure 5). Examination of several of these bacterial clones with Gen5 Data Analysis Software reveals that different growth patterns over time. Some clones are obviously unable to grow at higher temperatures and their wells do not increase in absorbance over time. Other wells reach a steady state within 24 hours, but do not increase thereafter, while other samples demonstrate continued growth over 96 hours. Interestingly, a number of clones increase in a stepped growth pattern over time, possibly indicating the selection of specific variants within the well. Once identified, the attributes of each temperature resistant clone can then be further investigated.

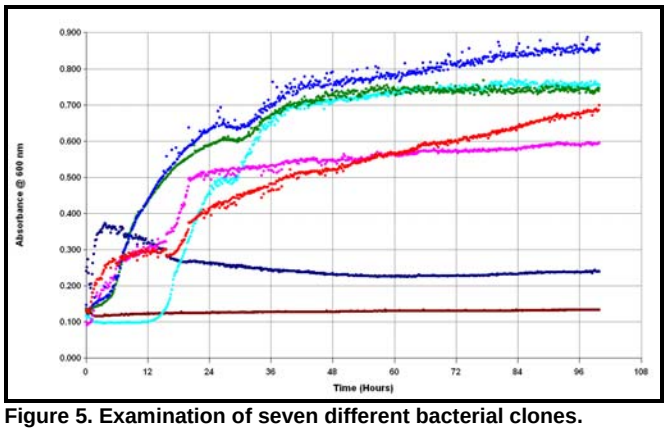

Figure 5. Examination of seven different bacterial clones.
Representative wells presented in Figure 3 were examined in closer detail using he zoom feature of Gen5 Data Analysis Software.

Discussion

BioTek readers such as the PowerWave XS Microplate spectrophotometer have always provided temperature control up to 50°C. BioTek has recently redesigned and improved on the reader incubation control allowing for an increase in the temperature range to 68±1.5°C. While the use of higher temperature incubations was demonstrated with bacterial growth selection, there are a number of other uses for these temperature capabilities. Numerous enzymatic reactions from thermophiles, such as *Thermus aquaticus and Thermococcus litoralis,* which thrive at temperatures between 50°C and 80°C, can be screened at these temperatures in microplates. Likewise, chemical reactions, which require or benefit from elevated temperatures, can be monitored using the PowerWave XS reader.

Acknowledgments

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