Ethanol
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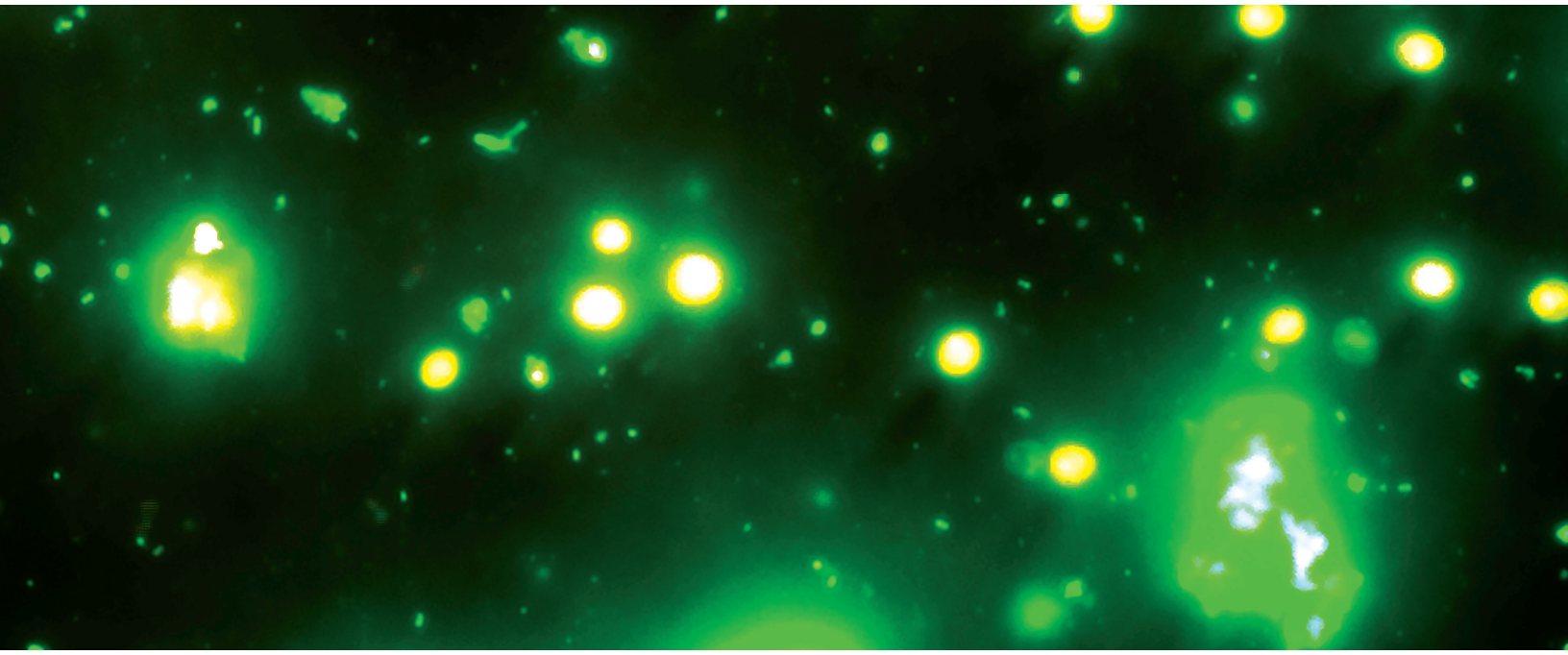

SEEING SPOTS: Some ethanol plants use fluorescence microscopes to detect bacterial infections and maintain yeast health. The microscopes allow technicians to see a mosaic of greens and reds that indicate the type and amount of cells in a sample.

PHOTO: LALLEMAND BIOFUELS AND DISTILLED SPIRITS

Going Small for Big Gains

Fluorescent microscopy provides colorful insights on bacteria and yeast.

By Chris Hanson

Microscopes have long been the ethanol industry's gateway to miniature worlds of bacterial infection and yeast concentration. Standard light microscopes are currently used in labs to count yeast cells and identify bacteria strains, but the processes can take as long as four days to complete. Fluorescent microscopes and automated counters are working to reduce that time, however, and provide a more accurate picture of the tiny organisms that impact ethanol production.

Fluorescent microscopes work by, first, staining cells with primarily green or red nucleic acids. The chemicals in the green acid penetrate living and damaged cell membranes, while the red acid stains dead or dying cells.

The sample is placed on the stage, a small platform near the front of the microscope. When the microscope is turned on, ultraviolet light hits a dichroic mirror to reflect specific light wavelengths down to the specimen, which in turn causes the cells to glow. The image is then picked up by the objective lens, goes through the dichroic mirror and light filters to produce what is seen in the eyepiece. What the researcher observes is a mosaic of greens and reds that can help indicate what type and how many cells are in the sample.

> 'One of the advantages of using the fluorescence microscope is it gives you an immediate indication of live or dead bacteria in your system, whereas an ordinary light microscope will only indicate if bacteria are present.'

Researchers at Lallemand Biofuels and Distilled Spirits use fluorescence microscopes to troubleshoot primarily in the fuel and beverage industries. "One of the advantages of using the fluorescence microscope is it gives you an immediate indication of live or dead bacteria in your system, whereas an ordinary light

microscope will only indicate if bacteria are present," says Francois van Zyl, technical service team leader at Lallemand. "With this we can conduct complete hygiene checks when a problem arises with short response times, usually between one to three hours. It is also of great use to have monthly hygiene audits incorporating bacteria counts using a separated calibration."

In addition to examining possible bacterial infections, fluorescence microscopes are able to inspect yeast cultures. "Having this equipment is also helpful when looking at yeast health or identifying wild yeast in your system," says van Zyl. While examining yeast health, researchers are able to study yeast budding sequences, maturity stages and budding scars, he adds. "This requires different sampling methods with an applicable stain. It is also of great use when we audit CO_2 scrubber systems for potential wild yeast, but not limited to this vessel," says van Zyl.

Going beyond the fluorescence microscope, some developers use the technology to create automated cell counters. Nexcelcom Bioscience LLC develops its Cellometer which can examine yeast samples. "Traditionally, people use some sort of manual enumeration technique and a microscope to look at cells and try to determine concentration and viability. It's very tedious and subjective process and it takes a lot of time." explains Peter Li, CEO of Nexcelcom.

Over the years, Nexcelcom received input from interested parties that led to the development of an automated system that can be user friendly and produce reliable data quickly and accurately. Although the Cellometer is primarily used for life sciences, such as drug research, it does have implications for use in the biofuel industry.

The implementation of fluorescent microscopy technology in the ethanol industry is relatively limited. One of the obstacles of getting the technology to the ethanol lab is the price tag, Li says. He says some customers felt other models were too costly, which led Nexcelcom to develop more budget-friendly models, such as the Cellometer X2. "We feel it's more suitable for people's budget and the reason we did this was we are able to do some cost reduction on our side and to make a product that will perform well with less cost," he says.

"The fluorescence microscope is a very versatile tool that can be used in real time to identify and correct potential issues within the production facility, thus saving time, money and avoiding costly infections," says van Zyl.

Li hopes that by offering a more cost-effective product, it can benefit the ethanol industry. "The way we use the Cellometer to measure yeast cells viability, especially in the corn mash samples, has been very effective," he says. "So if we can be helpful to the industry that would be great."

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