Online Fermentation Analyzer Optimizes Production

By George E. Barringer and Brian O'Flaherty

Ethanol, most of which is derived from corn feedstocks, is now marketed as a mainstream component of transportation fuel. Demand for the fuel continues to rise unabated. With increasing interest in ethanol, there is an ever-growing need and demand—technically, economically and politically—to improve the overall efficiency and yield of the entire process from growing corn to transportation and delivery of the final product.

A vital key to transforming ethanol-based fuel to a sustainable and viable product will come from improvements in the fermentation process. To reach this goal, research is focusing on a number of fronts: improved corn feedstocks to achieve higher starch content, enzymatic processing to achieve faster conversion of starch to sugar and mechanical processing to achieve more easily accessible starch and thus faster fermentation cycles. We will discuss here the additional opportunity to achieve higher yields and faster processing times through the application of online automated monitoring and assay instrumentation, which permits careful, accurate and timely control of the process.

Process Automation History

At line and online physical sensors (e.g., temperature, pressure and flow), chemical sensors (pH and O_2), separation systems (online gas chromatography) and end-point detection instruments (automatic titrators) have historically been key to the development of lean, efficient manufacturing within many industries. These monitoring tools have been broadly implemented to improve quality and yield in industries such as petroleum and petrochemical processing, food and beverage processing, detergent manufacturing, semiconductor processing, and pharmaceutical manufacturing. Food and beverage processing make liberal use of these monitors to control temperature, flow and pressure through closed feedback loops. Similarly, semiconductor facilities now operate at six sigma quality levels—3.4 defects per million parts—due in large part to extensive, fully automated chemical and physical monitors employed in the production process.

This level of production quality signals the value of real time in process control and tight statistical control of the processes—namely reduced unit production cost, increased utilization of capital equipment and lower overhead. Major gains in efficiency and yield in ethanol fermentation processes can be similarly enjoyed through use of automated process control.

In-line ultraviolet, visible or near infrared spectroscopy (NIR) optical monitors have been useful tools for measuring a host of products' physical and chemical properties including those directly related to ethanol production. For instance, NIR with multivariant statistical data reduction techniques has been useful in monitoring properties such as moisture, protein, fat and fiber in raw material, and could be applied in fuel ethanol production to ensure the quality of corn and other feedstocks.

The Groton automatic reactor sampling system provides four or eight sterile connections between ethanol fermentors and the Agilent 1200 Series LC System for online high-performance liquid chromatography assays.

Fermentation Monitoring

Fermentation is the core technology applied for use in biopharmaceutical and ethanol production. Therefore, advances currently employed in biopharmaceutical production may be quickly and effectively transferred to ethanol production with similar economic and technical benefits. Biopharmaceutical production processes have historically used a number of *in situ* probes to measure and control dissolved oxygen, carbon dioxide, temperature, pressure, pH and agitation rates. Recent developments now permit direct, automated molecular measurements on bioreactors. In the fermentation stage of biopharmaceutical processing, it is now common to measure not only biomass production but to directly detect and quantify the desired active pharmaceutical ingredient and feedstock ingredients by automated online separation instruments such as high-performance liquid chromatography (HPLC).

Ethanol facilities now use offline HPLC as a technique to monitor the progress of ethanol production during the fermentation process. HPLC permits monitoring of the complete cycle; conversion of starch to glucose, glucose to ethanol and the byproduct/degradation process of ethanol to acetic acid. An automated analytical instrument can be used in ethanol fermentation to directly monitor ethanol production and feedstock consumption. Ultimately, like the semiconductor industry, the application of online automated reaction monitoring instrumentation will lead to closed-loop feedback control of the entire ethanol fermentation process with corresponding improvements in the quality of product and reduction in both cycle time and labor allocations in the fermentation process.

More details on the analysis of liquid biofuel fractions can be found in the compendium of standard biomass analytical methods published by the National Renewable Energy Laboratory.

The Automated Online ARS-HPLC System

It is now possible to fully automate an HPLC system to perform online analysis of an ethanol fermentation process with an automatic reactor sampling (ARS) system. The ARS is a fully programmable, software-controlled autosampler designed to connect a fermentor tank to external assay instruments. It controls the collection and extraction of a liquid sample from the fermentation tank and delivers the sample aliquot to the connected analytical instrumentation while maintaining the sterile condition and environment of the process.

Any sampling system, whether manual or automatic, must maintain the sterility of the fermentation system to which it is attached. Many approaches used to ensure manual sampling sterility in fields other than biofuels, such as biopharmaceutical production, wouldn't be appropriate in ethanol manufacturing. For instance, flaming the sample port to pyrolyze contaminants before sample extraction is a standard and typical method in the biopharmaceutical industry would be ill-advised in biofuel applications.

Automatic sample systems have been developed by others that utilize *in situ* filtration elements to exclude the entry of external contaminants to the fermentor. These filters are sized to exclude, by mechanical filtration, particles of a certain diameter. Current practice in *in situ* sterile filtration employs filter elements with effective pore sizes or 0.22 micrometers. While effective in dilute solutions, these filters are known to fail through the clogging of filter pores. In a high-solids, high-mass application typical of yeast fermentation, this filter is particularly ineffective as it clogs quickly and permanently, and wouldn't be recommended for use in fuel ethanol fermentation.

The ARS is an effective interface between a fermentor and an analytical instrument (e.g., an HPLC used for ethanol concentration determinations) because it incorporates an effective clean-in-place (CIP) subsystem that cleans and sanitizes the ARS before, during and after each sample acquisition cycle. This process ensures that the fermentor sample valve and all components of the sampling system aren't exposed to airborne or fluid-borne contaminants that may infect the fermentor.

A second and vital component of the ARS system, which further protects the fermentor and assures sterility, is the ARS reactor interface module. The module is located on, at or near every fermentor sample port and provides the mechanism to clean the sample transfer lines and the valves in the module that first isolates, but also controls, the flow of sample from the fermentor to the ARS. This interface provides complete isolation of the fermentor from the ARS, and permits cleaning and flushing of all components in contact with the sample up to and including the fermentor port isolation valves, thus protecting the fermentor (and samples) from contamination. The ARS has been successfully combined with HPLC systems to provide researchers and producers with an automated online system for monitoring feedstock components and final product in operating ethanol fermentors.

Online monitoring of ethanol production by the ARS-HPLC online instrument is proven to be an effective production tool that lowers cost, increases yield and improves quality of the final product—ethanol. The producer can more effectively utilize capital equipment, feedstocks and resources to yield higher-quality product at optimum throughput while minimizing production costs. Application of the ARS and other online analytical tools, such as NIR, to improve and optimize the production process will ensure that fuel ethanol becomes and remains a sustainable component of the vital biofuel initiative.

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