INSIDE: MAXIMIZE ETHANOL YIELD WITH HPLC TESTING

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Minimize enzyme and antibiotic use, maximize ethanol yield with accurate HPLC testing

By Sam Ellis and Emma Murphy, Thomson Instrument Company

Ethanol-based biofuels, like those produced by fermenting corn mash, have the potential to revolutionize our fuel industry. Significant improvements in production can be made to show that this will be a more feasible and profitable fuel source. One of the current challenges is how to accurately assess the conversion of biomass to ethanol in a timely manner. This calculation is important because it determines the additional levels of enzymes (gluco-amylase and alpha amylase) and antibiotics, which can make or break yield and budget.

A well-controlled rate of digestion of the starches from corn to sugar by the amylase enzymes is key to good ethanol fermentation. Too much free sugar encourages bacterial growth in the fermentation tank and will increase the amount of antibiotics needed. Much of the guesswork can be taken out of enzyme and antibiotic additions by the eXtreme Filter Vials (patented) from Thomson Instruments. Specially designed for the biofuels industry, the eXtreme Filter Vials are a labor- and cost-saving tool that provides a more accurate high-performance liquid chromatography (HPLC) ethanol measurement than the current methods.

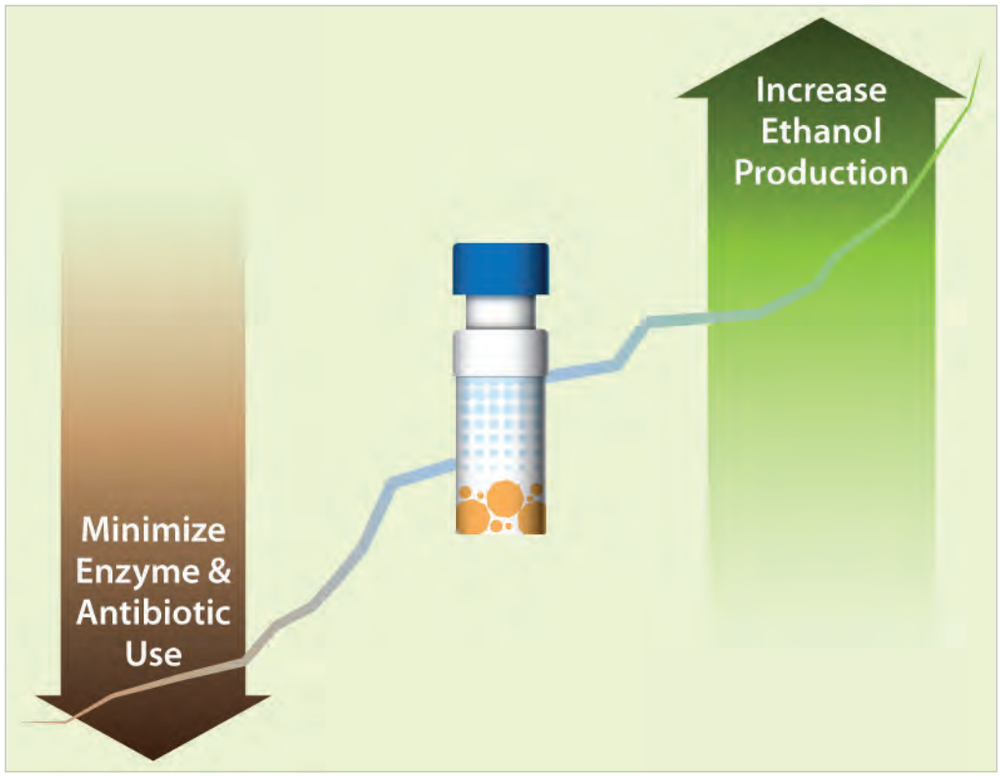

Figure 1.

Currently, the most common method to prepare a fermentation sample for ethanol analysis starts with gravity filtration through a Kim wipe (or similarly porous material) into a 50 mL tube. This can take from 10-20 minutes. Since ethanol is a volatile substance and evaporates quickly, this process is highly inaccurate for assessing ethanol production and subsequent enzyme and antibiotic additions because the ethanol evaporation will result in skewed sugar to ethanol ratios. To finish preparing the sample for HPLC, 10 mL of the gravity-filtered material is filtered through syringe filters. This can take up to 4 syringe filters to complete and the repetitive motion can cause hand fatigue, especially when monitoring multiple fermentations. The final sample is then deposited in an HPLC vial and capped for analysis. This method is time consuming and uses an excessive number of lab disposables.

Accurate sugar analysis is needed to minimize the amount of free sugar in the tank. Free sugar leads to a bacterial bloom, dramatically increasing the amount of antibiotics needed. Ethanol evaporation factors cannot be determined; however, when only minimal evaporation time has been allowed, HPLC can be used to obtain an accurate ethanol to sugar ratio.

Thomson eXtreme Filter Vials can tackle the filtration in a single step, reducing tech time and hand fatigue, as well as materials costs. The eXtreme Filter Vials are a completely contained system and sample preparation takes a fraction of the time, mitigating ethanol evaporation. After pulling a corn mash sample from the fermentation tank, it should be spun down in a centrifuge. A sample from the top layer is then loaded into the bottom chamber of the vial and completely filtered by simply pressing down the inserted plunger. The eXtreme Filter Vials are designed to clearly let the operator know when the enzyme ratio should be increased, since the filter membrane will clog with solids. It has been observed that if the vial fails to filter the centrifuged sample, then more enzymes need to be added in early fermentation.

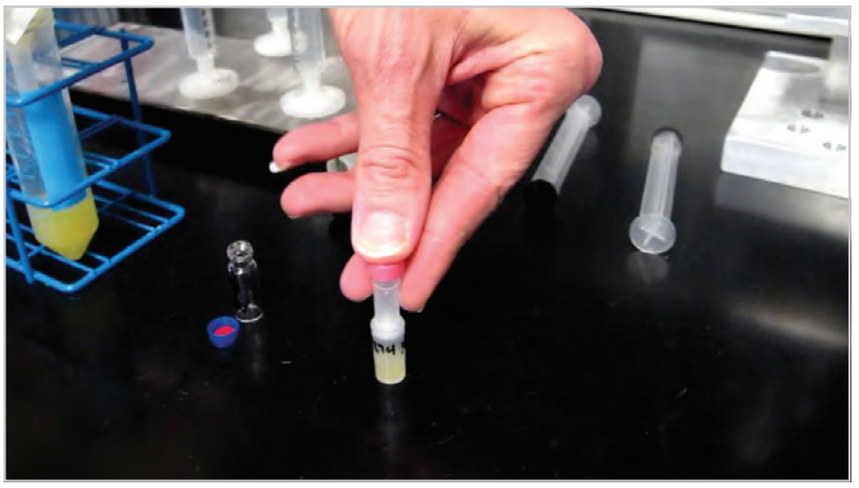

Figure 2. Insert plunger ~ 1/3 down

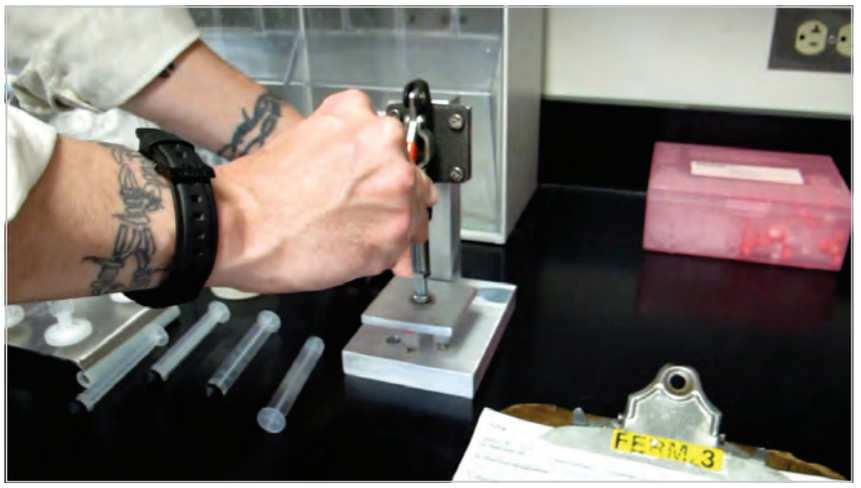

Figure 3. Toggle press slowly, over 5 seconds.

Figure 4. Place into HPLC.

The eXtreme Filter Vial containing the final sample can be loaded directly onto any HPLC machine for ethanol analysis without risk of damaging the HPLC column. (See Figures 2-4.)

The eXtreme Filter Vial method of sample preparation provides quick and accurate ethanol measurement, critical for determining the amount of amylase enzyme and antibiotics to be added to the fermentation. With monitoring samples pulled frequently, rapid assessment of what is happening in the fermentation tank is needed to make informed decisions to maximize ethanol production. An accurate picture of the biomass conversion to ethanol is needed to calculate how much of each amylase enzyme to add.

Alpha amylase facilitates the breakdown of the complex starches in corn to fermentable sugars while gluco amylase facilitates the breakdown of the complex sugars. Too little enzyme added will hurt the ethanol yield, since not all the starch will be converted to sugar. This will also make the corn harder for the machinery to process, since the amylase digestion softens the corn kernel. The corn is pulverized by a hammer mechanism, and the less digested the corn kernel, the harder they will be to break apart, which adds unnecessary wear and tear to the machinery.

Adding too much of the amylase enzymes is not an economically viable approach to solving this problem. With the average price of amylase enzymes at $3/kg, excess additions are not a feasible option since they will significantly reduce profit margins. The eXtreme Filter Vials are a cost effective tool to address these common issues in ethanol fuel production. They streamline sample preparation, yielding more accurate readings of ethanol levels in a timely manner, which gives researchers and producers more knowledge of, and therefore more control over, the fermentation process.

Ethanol analysis with Thomson eXtreme Filter Vials provides a quick and reliable way to assess biomass to ethanol conversion, allowing biofuel producers to make informed process decisions. The eXtreme Filter Vials are a single-step, closed system that allows minimal ethanol loss due to evaporation, thus providing more accurate numbers than the current methods of sample preparation. Using Thomson eXtreme Filter Vials allows for more precise additions to be made throughout the fermentation process, maximizing ethanol production.

Sam Ellis is a biochemist and molecular biologist who has worked for more than 13 years on different process development for both analytical and fermentation processes. Ellis holds more that 15+ patents worldwide, and has contributed to development of many techniques within the realm of analytical sciences and biochemistry. He serves as the vice president of Thomson Instrument Company, www.htslabs.com. He was recently co-chair of a session at Recent Advancement of Fermentation technology or Raft for the Society of Industrial Microbiology. You may contact him by e-mailing bpteditorial@woodwardbizmedia.com.

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