HPLC Analysis for the Monitoring of Fermentation Broth During Ethanol Production as a Biofuel

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Introduction

Increased ethanol production as a biofuel is leading to a paradigm shift around the world. Renewable biological resources that can be converted to biofuels are rapidly gaining interest in the energy industry as potential alternative fuel sources. This is not just a U.S. phenomenon – it is accelerating globally. In particular, resources such as corn, sugar beets, sugar cane, grains, sorghum, molasses and others (all renewable energy sources) are being converted into ethanol at an ever increasing scale.

The production of ethanol utilizes a fermentation process, in which yeast and enzymes convert the fermentable carbohydrates (dextrin, maltotriose, maltose, glucose) into ethanol. The resulting fermentation broth is a complex mixture, consisting of living yeast cells, nutrients, bacteria, cell debris and other products/byproducts of the fermentation process. This broth needs to be monitored to optimize the quantity and quality of ethanol being produced. During the fermentation, it is known that the ethanol concentration is inversely proportional to the carbohydrate concentration. Therefore, the monitoring of carbohydrate levels serves as a key indicator in determining when to stop the process. In addition, other unwanted byproducts, such as lactic acid, acetic acid, carbonic acid and glycerol are also produced. To maintain productivity, these byproducts must also be monitored. During fermentation, as the composition of the broth changes, so does the chemistry. Therefore, adjustments to the fermentation broth are often required to ensure optimal ethanol yields.

This HPLC application has been designed so that, during the fermentation process, three key parameters, including eight components, can be easily monitored and quantitatively analyzed:

1) The amount of ethanol being produced
2) The amount of fermentable sugars (dextrin, maltotriose, maltose and glucose) in the fermentation broth
3) The concentration of unwanted byproducts (lactic acid, acetic acid and glycerol) produced during the fermentation process

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precisely.

Experimental conditions

The application was performed on a PerkinElmer® Series 200 HPLC System, consisting of an Isocratic Pump, Vacuum Degasser, Autosampler, Column Oven and Refractive Index Detector. TotalChrom® Chromatography Data Systems (CDS), version 6.3.1, was used as the control/data-acquisition software. The column used was a BIO-RAD Aminex® Fermentation Monitor column (150 x 7.8 mm, 5 µm).

The analytical conditions, shown below, were optimized to produce the shortest analysis time, while maintaining sufficient resolution between components for proper identification and quantification. Using these conditions, all components can be quantitatively analyzed in less than 10 minutes.

Table 1. Conditions.

Mobile Phase:	0.001 M H_2SO_4
Flow:	0.8 mL/min
Temperature:	60 °C
Detector:	Refractive index @ 40 °C
Injection Volume:	10 µL

Results

An example of an actual 24-hour fermentation-broth sample that was taken during ethanol production is shown in Figure 1. From the chromatogram, it can be seen that the ethanol is well separated from all the other individually separated sugars and byproducts found in this particular sample.

Conclusion

In conclusion, as part of the fermentation process in the production of ethanol as a biofuel, a simple ten-minute HPLC method was developed to routinely monitor ethanol, carbohydrates and byproducts. During the process, this analysis is important to help ensure that the broth chemistry is optimized to produce the maximum yield of ethanol.

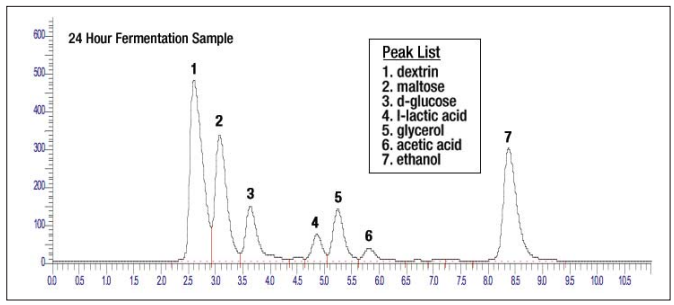

Figure 1. Actual 24-hour fermentation sample from ethanol production monitoring.

Reference

U.S. Department of Agriculture – *www.usda.gov*

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