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Application News

No. L588

High Performance Liquid Chromatography Nexera™ Organic Acid Analysis System

Monitoring of Organic Acids in Biomass Fermentation Process and Yeast Cultivation Process

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User Benefits

- Enables selective, high sensitivity analysis of organic acids in biomass fermentation samples, which contain many contaminants.
- Multiple organic acids such as lactic acid, formic acid, and acetic acid can be separated in a short time with high efficiency, supporting quick determination of the condition of fermentation.
- Contributes to improvement of work efficiency in research and development of biofuels, and particularly second-generation biofuels.

■ Introduction

Bioethanol is a form of carbon-neutral renewable energy and is an object of worldwide research as a measure for preventing global warming and an alternative for petroleum fuels. Due to fears for the shortage and the price increase of the food crops, development of second-generation biofuels is underway utilizing cellulose or hemicellulose from nonfood plants such as wood and rice straw. However, the production of the second-generation biofuel requires numerous processes for decomposition and fermentation of fibrous substances. A generation of organic acids such as acetic acid in those processes inhibits fermentation and thus has a large impact on the ethanol yield formed in the process. For this reason, a monitoring the behavior of organic acids during fermentation is an extremely important issue for controlling the ethanol yield.

In this article, the time-dependent variations in concentrations of organic acids in biomass fermentation samples and a yeast culture solution were monitored using the Nexera organic acid analysis system. In the post-column pH buffering-electric conductivity detection method used in this system, it is possible to detect organic acids with high sensitivity and good selectivity by mixing with a pH buffering reagent after column separation. This is the optimum system for the analysis of samples such as biomass fermentation solutions that contain many contaminants. In the experiments described here, the Nexera organic acid analysis system in combination with the Shim-pack™ Fast-OA columns for high speed analysis provided a 50% shortened analysis time and a quick checking for problems in the fermentation process.

■ Analysis of Standard Sample

The Shim-pack Fast-OA is an ion exclusion type polymer-packed column that has been optimized for use in high speed analysis of organic acids. Fig. 1 shows the chromatograms of a mixed standard sample of 9 organic acids by the conventional Shim-pack SCR-102H (2 columns in series) and the Shim-pack Fast-OA (3 columns in series). Table 1 shows the analytical conditions.

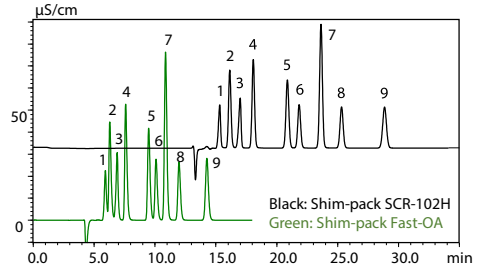

Fig. 1 Chromatograms of Mixed Standard Sample of 9 Organic Acid Components
(Top: Shim-pack SCR-102H (2 Columns in Series), Bottom: Shim-pack Fast-OA (3 Columns in Series))

Table 1 Analytical Conditions

System	: Nexera organic acid analysis system
Column (Conventional)	: Shim-pack SCR-102H (300 mm × 8.0 mm I.D., 7 μm)*1 ×2 : Guard column SCR-102H (50 mm × 6.0 mm I.D.)*2
Column (Fast)	: Shim-pack Fast-OA (100 mm × 7.8 mm I.D., 5 μm)*3 ×3 : Shim-pack Fast-OA(G) (10 mm × 4.0 mm I.D.) *4
Mobile Phase	: Reagents kit for Organic Acid Analysis System*5
Flow Rate	: 1.0 mL/min
pH Buffering Solution	: Reagents kit for Organic Acid Analysis System*5
Mixer	: MR 20
Column Temp.	: 40 ˚C
Injection Vol.	: 10 μL
Vial	: Shimadzu Vials, LC, 1.5 mL, Glass*6
Detection	: Conductivity detector

*1 P/N : S228-17893-91, *2 P/N : S228-17924-91, *3 P/N : S228-59942-41
*4 P/N : S228-59942-42, *5 P/N : S228-61465-91, *6 P/N : S228-15652-92

■ Analysis of Fermentation Samples of Real Biomass Saccharification Solution

Fig. 2 shows the chromatograms of the organic acids in fermentation samples of a real biomass saccharification solution before fermentation and after fermentation for 48 h. Fig. 3 shows the variations in the peak area of each organic acid monitored using the screening application. The improved yeast (Toyota XyloAce®) used here efficiently forms ethanol from acetic acid, which is generally considered to be a fermentation inhibitor. From Fig. 3, it can be confirmed that the acetic acid concentration was reduced by 97% or more after fermentation for 48 h. Increased concentrations of formic acid and lactic acid, which were considered as fermentation inhibitors formed during metabolic pathway, were observed. It may also be noted that a negative peak originating from the formed ethanol can be observed at around 17 min.

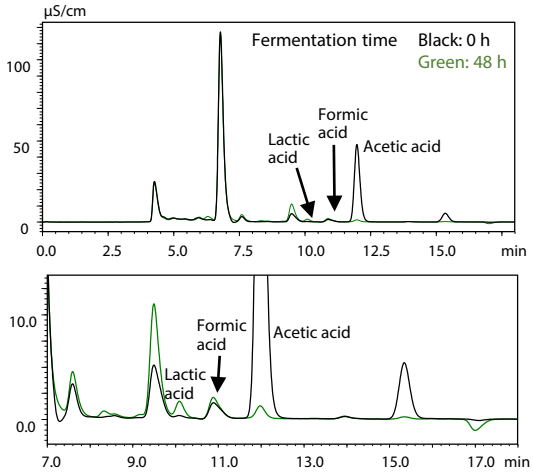

Fig. 2 Chromatograms of Fermentation Samples of Real Biomass Saccharification Solution
(Top: Full Chromatogram, Bottom: Enlargement)

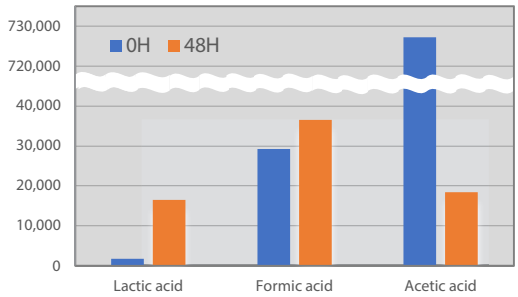

Fig. 3 Variation of Peak Areas of Organic Acids Before/After Fermentation of Real Biomass Saccharification Solution

■ Analysis of Fermentation Samples of Lignocellulosic Biomass Saccharification Solution

Fig. 4 shows the chromatograms of a saccharification solution of lignocellulosic biomass (plant matter derived from trees and grasses) after fermentation for 6 h to 40 h, and Fig. 5 shows the results of fermentation monitoring. As in the previous section, the concentration of acetic acid was decreased, and the concentrations of formic acid and lactic acid were increased slightly with the increase in fermentation time. Even very small time-dependent variations of these organic acids were able to be observed by the contribution of the high resolution of the Fast-OA columns and the high selectivity of the pH buffering-electric conductivity detection method, although lactic acid and formic acid are normally prone to overlap with contaminants.

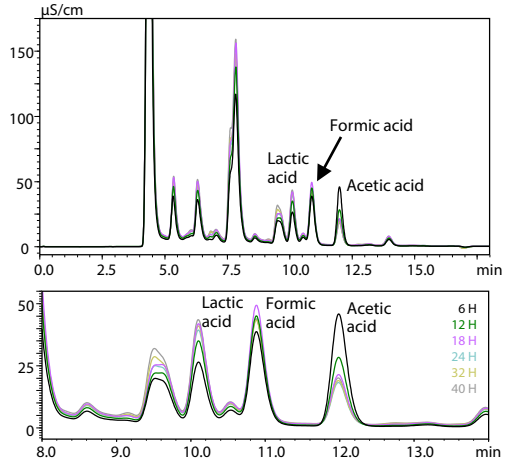

Fig. 4 Chromatograms of Fermentation Samples of Lignocellulosic Biomass Saccharification Solution
(Overlay of Chromatograms of Samples After Fermentation for 6 h to 40 h; Top: Full Chromatogram, Bottom: Enlargement)

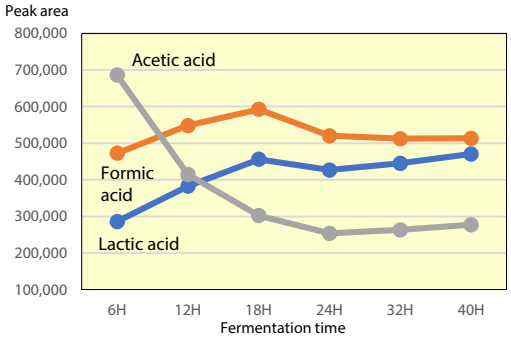

Fig. 5 Results of Fermentation Monitoring of Lignocellulosic Biomass Saccharification Solution

■ Analysis of Molasses Culture Solution

Fig. 6 shows the chromatograms of samples cultivated in molasses, and Fig. 7 shows the cultivation monitoring results. Here, the large decreases in lactic acid, formic acid, and acetic acid with the increase in cultivation time was observed. These showed that saccharide was depleted due to multiplication of the yeast by cultivation, and the organic acids formed as byproducts of yeast cultivation were metabolized.

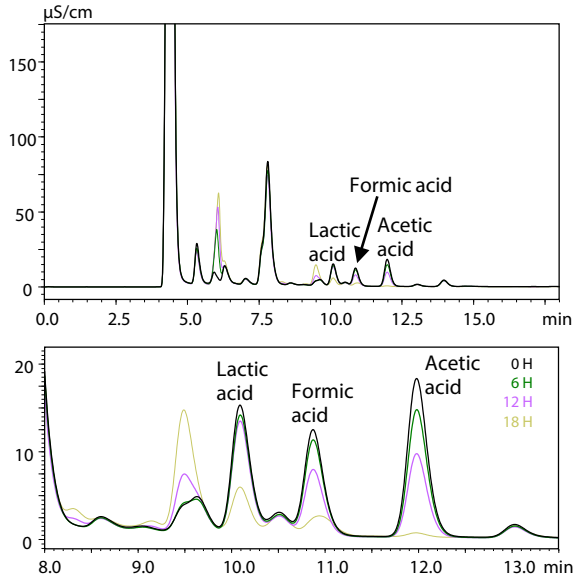

Fig. 6 Chromatograms of Molasses Culture Solution
(Overlay of Chromatograms of Samples Cultivated for 0 h to 18 h; Top: Full Chromatogram, Bottom: Enlargement)

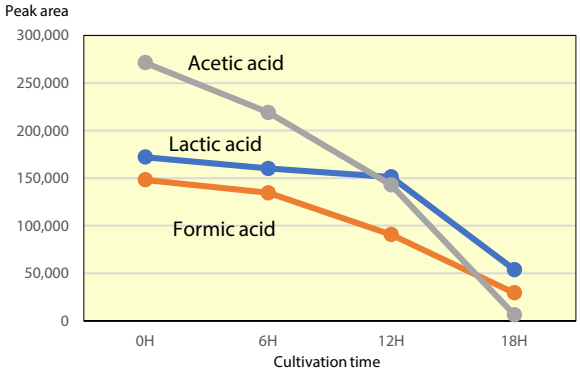

Fig. 7 Results of Cultivation Monitoring of Molasses Culture Solution

■ Conclusion

This article introduced the efficient and highly accurate analyses of organic acids in biomass fermentation samples that contain many contaminants using a combination of the Nexera organic acid analysis system and the Shim-pack Fast-OA columns. This system makes an important contribution to improvement of work efficiency in research and development of new biomass fuels, which are being promoted widely all over the world.

<Acknowledgement>
We wish to thank Ms. Noriko Yasutani of the Agriculture & Biotechnology Business Div. TOYOTA MOTOR CORPORATION, for providing the samples used in the preparation of this article.

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First Edition: Feb. 2021