

Application for Lactic Acid Fermentation Monitoring

AS/LC-027

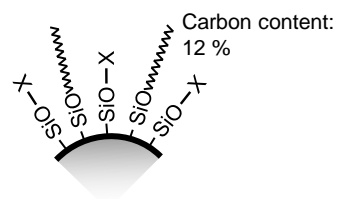
The reaction in which sugars are decomposed by lactic acid bacteria in anaerobic conditions to produce lactic acid as the decomposition product is called lactic acid fermentation. Lactic acid drinks, yogurt, and Japanese pickles are manufactured by using this lactic acid fermentation and they contain lactic acid. This time, by using the highly versatile UV detection system, the lactic acid production by lactic acid fermentation was monitored. Along with the production of lactic acid, the presence or absence of citric acid, malic acid, and succinic acid accumulation in the TCA cycle was confirmed.

As a result, it was confirmed that there is no increase in the organic acids contained in the initial culture medium during the lactic acid fermentation process. In the organic acid analysis, the column specially designed for organic acid analysis (ion exclusion mode) is generally used. However, the analysis example by using the low-cost reverse phase column from which lactic acid elutes rapidly is introduced this time. LaChrom C18-AQ column (low carbon ODS) suitable for the analysis of highly polar compounds such as organic acids was used.

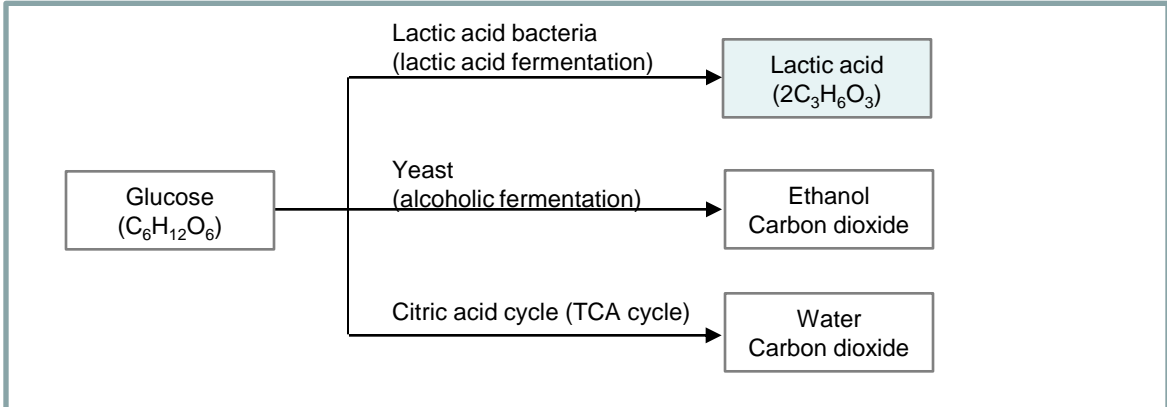
Summary for LaChrom C18-AQ Column

Low-carbon ODS column:

This is the ODS column with the suppressed hydrophobicity of silica surface to achieve the stable analysis even when used with 100% aqueous solvent. This column is useful for the separation of highly polar compounds such as sugars, nucleic acids, and organic acids. As this is a reversed phase column, it can also be washed with an organic solvent.



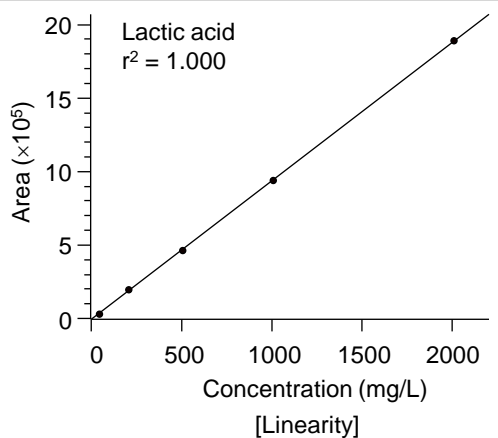
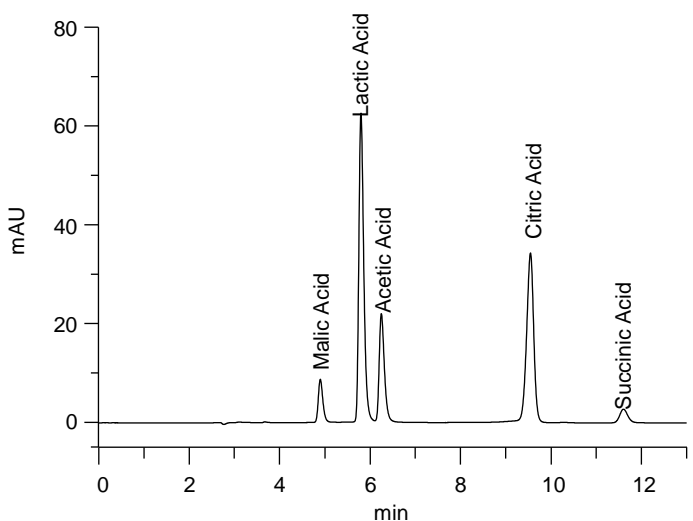
Summary for Lactic Acid Fermentation



Analysis Example of Organic Acid Standard Samples (Reversed-phase Mode)

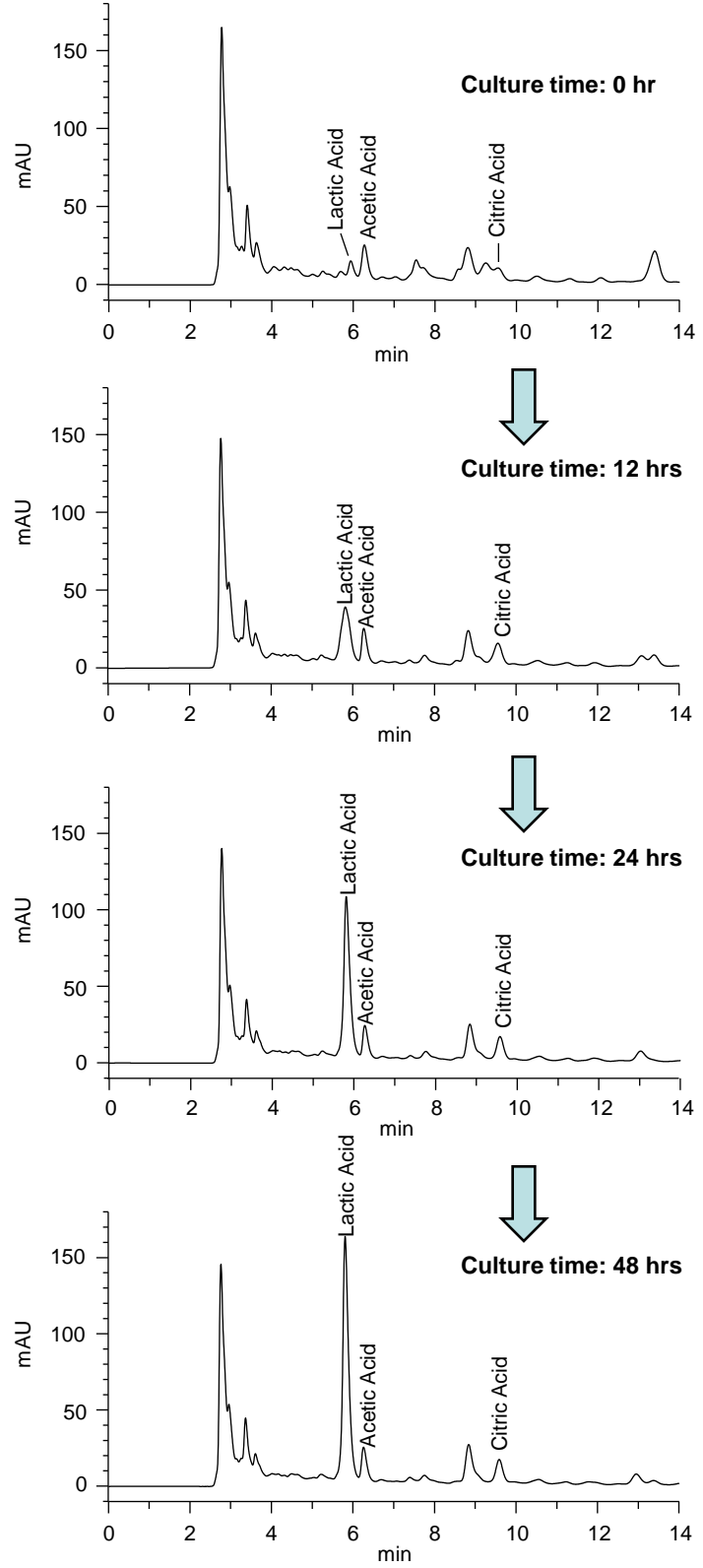
Component name	Malic acid	Lactic acid	Acetic acid	Citric acid	Succinic acid
Concentration (mg/L)	50	500	250	250	50

<Analytical Conditions>
 Column : LaChrom C18-AQ (5 μm) 4.6 mm I.D. × 250 mm
 Eluents : 1 mmol/L H₂SO₄, 8 mmol/L Na₂SO₄
 Flow rate : 1.0 mL/min
 Column temperature : 25 °C
 Detection wavelength : UV 210 nm
 Injection vol. : 20 μL



A good linearity with the coefficient of determination of 1.000 was obtained over the range of 40 – 2000 mg/L for lactic acid.

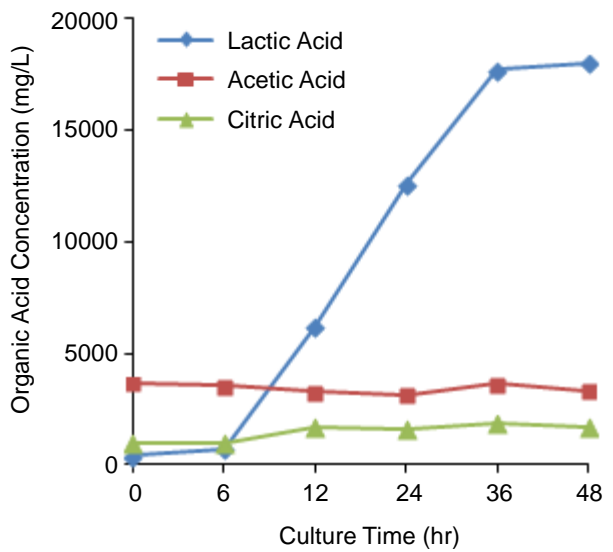
Analysis Example of Culture Sample (Monitoring of Culture Time and Lactic Acid)



[Culture Time and Change Over Time of Organic Acids]

<Preparation of Culture Sample>

- 50 g/L glucose / 10mL MRS culture medium
- ↓
- Sterilization (autoclave)
- ← Inoculation of lactic acid bacteria (anaerobic condition)
- Culture (30°C)
- ↓
- Lactic acid fermentation**
Glucose (C₆H₁₂O₆) → Lactic acid (2C₃H₆O₃)
- ↓
- Centrifuge 12000 rpm, 10 min, 4 °C
- ↓
- Supernatant
- ← Dilute to 10 times with ultrapure water
- Filtration Pore Size 0.45 μm
- ↓
- Analytical sample (20 μL)



[Culture Time and Change in Organic Acid Concentration]

Lactobacillus casei, lactic acid bacterium, was cultured under anaerobic conditions by using 50 g/L glucose as the carbon source. Lactic acid was gradually produced from the initial culture stage. It was clarified that about 18g/L of lactic acid was produced at 36 hours of the culture and the fermentation does not progress after that. The formation of other organic acids was not found through this lactic acid fermentation.

*The analysis samples were provided by Dr. Kazuhiro Hoshino, Associate professor, Biological Reaction Engineering Laboratory, Department of Life Science and Bioengineering, Faculty of Engineering, Toyama University.

Main system configuration: Chromaster 5110 pump, 5210 autosampler (with thermostat), 5310 column oven, 5410 UV detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.