Analysis of aliphatic carboxylic acids in anaerobic digestion process waters by ion-exclusion chromatography

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Abstract - The analysis of seven aliphatic carboxylic acids, formic, acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids in anaerobic digestion process waters for biogas production was examined by ion-exclusion chromatography with dilute acidic eluents benzoic acid, perfluorobutyric acid, PFBA and sulfuric acid and non-suppressed conductivity/ultraviolet UV detection. The columns used were a styrene/divinylbenzene-based strongly acidic cation-exchange resin column TSKgel SCX and a polymethacrylate-based weakly acidic cation-exchange resin column TSKgel Super IC-A/C. Good separation was performed on the TSKgel SCX in shorter retention times. For the TSKgel Super IC-A/C peak shape of the acids was sharp and symmetrical in spite of longer retention times. In addition, the mutual separation of the acids was good except for iso- and n-butyric acids. The better separation and good detection was achieved by using the two columns TSKgel SCX and TSKgel Super IC-A/C connected in series at lower concentrations of PFBA and sulfuric acid as eluents non-suppressed conductivity detection and UV detection at 210 nm. This analysis was applied to anaerobic digestion process waters. The chromatograms with conductivity detection were relatively simpler compared with those of UV detection. The use of two columns with different selectivities for the aliphatic carboxylic acids and the two detection modes was effective for the determination and identification of the analytes in anaerobic digestion process waters containing complex matrices.

Key words - ion-exclusion chromatography, IEC, strongly and weakly acidic cation-exchange resin columns, perfluorobutyric and sulfuric acids eluents, non-suppressed conductivity and ultraviolet UV detections, aliphatic carboxylic acids, anaerobic digestion process waters

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In anaerobic digestion processes which are recently re-evaluated as not only water treatment process but also energy production process with methane generation, the total complex organic substances proteins carbohydrates and lipids are converted to soluble biodegradable organics amino acids, sugars, fatty acids further to the aliphatic acids such as formic and acetic acids etc. and finally to methane and carbon dioxide as carbon 12. Thus analysis of the acids which are process intermediates is one of important items as a measure for controlling anaerobic digestion processes.

Various analytical methods e.g. ion-exclusion chromatography, IEC, ion-exchange chromatography, ion-pair chromatography, gas chromatography and capillary zone electrophoresis are principally applicable for this process control.

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Gas chromatography is often employed for the determination of aliphatic carboxylic acids after complex and time-consuming sample pretreatments such as extraction and derivatization. IEC is an effective technique for the separation and sensitive detection of aliphatic and aromatic carboxylic acids with direct sample injection just after filtration. The separation of the acids is performed on various types of cation exchange columns in the H⁺ form. The characteristics of the chromatography is that inorganic anions such as chloride, sulfate and the dissociated acids are excluded from the columns via the electrostatic repulsion effects therefore are not retained on the columns. On the other hand, the associated acids and partially dissociated acids with higher relative molecular masses can be retained on the cation-exchangers in the H⁺ form due to the various interactions such as ion-exclusion effects, size-exclusion effects and hydrophobic interaction. These effects lead to relatively simple and good chromatograms for the determination of carboxylic acids in various real samples. Conventionally, detection of the acids has been carried out by single detection system. However, for the samples with complex matrices the dual detection system will be useful together with the optimization of separation system.

The purpose of present study was to examine and optimize the separation and detection of seven carboxylic acids in anaerobic digestion process waters by ion-exclusion chromatography with dual conductivity and ultraviolet UV detections. Two polymer-based cation-exchange resin columns with different selectivities for the analytes and dilute acidic eluents of perfluorobutyric acid PFBA and sulfuric acid were employed for this purpose.

1 Experimental

1.1 Instrumentation

The ion chromatographic system employed was consisted of Shimadzu LC-6A or LC-10AD pumps Shimadzu Kyoto Japan a Shimadzu CTO-10AC column oven equipped with a 50-µL sample loop a Tosoh CM-8000 conductivity detector Tosoh Tokyo Japan a Shimadzu SPD-10Avp UV-VIS detector and a Shimadzu C-R8A data processing system.

The separation columns used were a Tosoh TSKgel strong cation-exchange SCX column 150 mm x 6.0 mm 5 µm polystyrene/divinylbenzene PS/DVB-based strongly acidic cation-exchange resin ion-exchange capacity 1.5 meq/mL and a Tosoh TSKgel Super IC-A column 150 mm x 6.0 mm 3 µm polymethacrylate-based weakly acidic cation-exchange resin ion-exchange capacity 0.2 meq/mL. A Tosoh TSKgel OApak P column 50 mm x 4.2 mm 5 µm PS/DVB-based strongly acidic cation-exchange resin exchange capacity 1.5 meq/mL was used as a precolumn for trapping mono- and divalent-inorganic cations in real samples. Dilute acidic eluents were used at a flow rate of 1.0 mL/min. Column temperature was maintained at 40 °C. Sample injection volume was 30 µL.

1.2 Reagents and samples

All reagents of analytical grade were purchased from Kanto Chemicals Tokyo Japan except for PFBA Aldrich St. Louis MO USA. The eluents employed were prepared from 4 mmol/L stock solutions. The standard solutions of aliphatic carboxylic acids and their mixtures were prepared from each of 0.1 mol/L stock solution. All solutions were prepared using deionized water. Anaerobic digestion process waters were obtained from a mesophilic anaerobic digestor for poultry manure waste vegetables and fruits.

2 Results and discussion

2.1 Separation of aliphatic carboxylic acids on cation-exchange columns

In a preliminary study the separations of seven aliphatic carboxylic acids on a TSKgel SCX column and a TSKgel Super IC-A column were examined using 0 – 4 mmol/L benzoic acid as eluent. The resolution of the carboxylic acids with water as the eluent was very low and the peaks were fronted on both columns. The peak of each acid consisted of the associated and dissociated
forms of the acids and the ratio depended on the acid dissociation constant \( pK_a \) of each acid and the pH of the eluent \( 5\% \text{NaCl} \). The addition of dilute benzoic acid in the eluent led to sharp peak shape of each carboxylic acid and also improved separation with increased retention time due to the increase of the associated form ratio.

Fig. 1 shows the separation of the analytes with 0.5 mmol/L benzoic acid eluent. The elution orders of the analytes by the two columns were the same but different at three points. The retention time of each carboxylic acid on TSKgel SCX column was relatively short compared with that on TSKgel Super IC-A/C column. For TSKgel Super IC-A/C, the peak shapes of the acids were a little sharper and more symmetrical in spite of longer retention times. For example, the retention times of \( n \)-valeric acid were 22.0 and 11.2 min on TSKgel Super IC-A/C and TSKgel SCX, respectively. Both the half-width and tailing of \( n \)-valeric acid peak on TSKgel Super IC-A/C were ca. 20% smaller compared with those on TSKgel SCX. In addition, the mutual separations of the acids were good except for iso- and \( n \)-butyric acids. The separation of iso- and \( n \)-butyric acids with the same \( pK_a \) value of 4.63 was achieved peak resolution 0.99 on TSKgel SCX column due to the difference in hydrophobic interaction between the acids and the stationary phase styrene/divinylbenzene copolymer. Linear-chain hydrocarbons of \( n \)-butyric acid have stronger hydrophobicity to stationary phase compared with branched-chain hydrocarbons of iso-butyric acid. This pattern is strengthened for iso- and \( n \)-valeric acids with longer-chain hydrocarbons. These conclusions were also obtained from both PFBA and sulfuric acid eluents.

### 2.2 Separation and detection of aliphatic carboxylic acids by PFBA and sulfuric acid eluents and conductivity/UV detection

Based on the results of the previous section, the separation and detection using TSKgel SCX and TSKgel Super IC-A/C columns connected in series were examined. PFBA and sulfuric acid in the range of 0 – 4 mmol/L were used as eluents. Since both PFBA and sulfuric acid are UV transparent, no UV detector response conductivity and UV detection at 210 nm were used. For PFBA, there were four characteristics. For conductivity detection, the sensitivity of carboxylic acids decreased rapidly when PFBA concentration increased due to the increase in background conductivity. 43 \( \mu S \) 0.1 mmol/L PFBA \( \text{pH} 3.92 \) to 1379 \( \mu S \) 4 mmol/L \( \text{pH} 2.36 \) without change in retention times. Whereas, almost the same sensitivities were obtained by UV detection in the concentration range of PFBA examined. The system peak of PFBA approached to that of formic acid when PFBA concentration increased leading to lower resolution. The difference of retention times between iso- and \( n \)-butyric acids was 0.92 min for two columns connected in series. The resolution was 1.01. Lower PFBA concentration 0.1 mmol/L was effective as shown in Fig. 2. Also, the same pattern of separation and detection of the analytes was obtained with sulfuric acid eluent and good results were obtained with 0.1 mmol/L sulfuric acid. However, the sensitivity of conductivity detection was a little lower compared with that of PFBA due to its higher background conductivity. 91 \( \mu S \) 0.1 mmol/L \( \text{H}_2\text{SO}_4 \) \( \text{pH} 3.64 \).
Fig. 2  Ion-exclusion chromatographic separation of aliphatic carboxylic acids with 0.1 mmol/L PFBA as eluent

a. non-suppressed conductivity detection  b. UV detection.
Precolumn TSKgel OApak P column TSKgel SCX + TSKgel Super IC-A/C.
Peaks and concentrations were the same as in Fig. 1.

### Analytical performance parameters

Table 1 shows the detection limits D. L. signal/noise = 3 of analytes using PFBA and sulfuric acid eluents with conductivity and UV detections respectively. Higher sensitivity of formic acid with conductivity detection was obtained due to its higher dissociation by low pK values.

For 0.1 mmol/L PFBA eluent sensitivity of conductivity detection was a little higher compared with that of 0.1 mmol/L sulfuric acid eluent due to a little lower background conductivity. But the sensitivities of UV detection were relatively similar.

Calibration by peak area was examined in the range of 0.1 - 20 mmol/L. For conductivity detection, the linearity correlation coefficient $r^2$ was a little lower for formic acid and propionic acids with relatively higher sensitivity PFBA $r^2 = 0.983 - 0.994$ H$_2$SO$_4$ $r^2 = 0.987 - 0.995$ but linearity was very good for iso-butyric acids and iso-n-valeric acids PFBA $r^2 = 0.994 - 0.999$ H$_2$SO$_4$ $r^2 \geq 0.998$.

Whereas good linearity of UV detection $r^2 \geq 0.998$ for all the carboxylic acids was obtained with both PFBA and sulfuric acid as eluents. The stability of the carboxylic acids chromatograms was good. For example the relative standard deviations RSDs of peak areas of the 7 carboxylic acids with 0.1 mmol/L PFBA eluent was in the range of 0.5% - 2.0% and 0.6% - 1.9% for conductivity and UV detections respectively.

#### 2.4 Application to real samples

The samples were obtained from a pilot-scale mesophilic anaerobic digestor for poultry manure waste vegetables and fruits. The digestor consisted of two tanks an acid-forming tank volume 0.7 m$^3$ and a methanogenic tank 6.3 m$^3$ with hydraulic retention times of 5 and 45 days respectively. Fig. 3 shows chromatograms of anaerobic digestion process waters using non-suppressed conductivity and UV detections with 0.1 mmol/L PFBA as eluent. The chromatograms with conductivity detection Fig. 3a were simpler compared with those of UV detection Fig. 3b. These results suggest the presence of the organic compounds with lower sensitivity for conductivity detection. Similar chromatograms were obtained with 0.1 mmol/L sulfuric acid eluent and conductivity/UV detection for the sample waters.

The analytical results by non-suppressed conductivity detection and UV detection are shown in Table 2. Almost the same results were obtained by both the conductivity and UV detections. In an acid-forming tank Fig. 3a higher concentration of acetic acid acetate was detected and other carboxylic acids carboxylates were also detected. The concentration of iso-butyric acid isobutyrate was lower than those of propionic acid propionate and n-butyric acid n-butyrate.

<table>
<thead>
<tr>
<th>Carboxylic acid</th>
<th>0.1 mmol/L PFBA</th>
<th>0.1 mmol/L Sulfuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.003</td>
<td>0.005</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>iso-Butyric acid</td>
<td>0.008</td>
<td>0.005</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>iso-Valeric acid</td>
<td>0.010</td>
<td>0.010</td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>0.020</td>
<td>0.020</td>
</tr>
</tbody>
</table>

A non-suppressed conductivity detection B UV detection.
The concentration levels of formic acid, formate were very low. In a methanogenic tank, acetic acid acetate was almost not detected and might be converted to methane. Other carboxylic acids carboxylates were also not detected. Quantitative and stable biogas production was obtained under the optimum process conditions. These results indicated that the anaerobic digestion processes were in a good and steady condition. On the other hand, under incomplete treatment conditions, the concentration of carboxylic acids carboxylates in a methanogenic tank was relatively higher and comparable to that in an acid-forming tank, thus biogas production rate was low. The inverse correlation was obtained between gas production rate and the concentration of aliphatic carboxylic acids aliphatic carboxylates in a methanogenic tank. Thus, it is necessary to measure aliphatic carboxylic acids aliphatic carboxylates for proper control of anaerobic digestion processes. In conclusion, the IEC system was demonstrated to be effective for monitoring and optimizing the biogas production process.

### 3 Conclusions

Determination of the aliphatic carboxylic acids in mesophilic anaerobic digestion process waters was achieved by using both strongly and weakly acidic cation-exchange resin columns connected in series, dilute acidic eluents, non-suppressed conductivity and UV detections. The columns with different selectivities for carboxylic acids were useful for application to real samples. Additionally, parallel detection by conductivity and UV detection methods was valid for sample identification.

### References