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Research Article

Determination of Bicarbonate in Fermentation of Cyanobacterial by Non-Suppress Ion Chromatography

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Abstract: Separation of Bicarbonate had been investi gated using a non-suppress cation chromatography and conductivity detection. Analysis was performed on an Optimix C18/SCX Cation-exchange column coupled to a conductivity detector, with an injection volume of 25 μL and a flow rate of 0.2 mL/min. The leacheate was fermentation of cyanobacterial apart from sodium bicarbonate. Under the optimized conditions, the linear relationship of Bicarbonate was 0.99946 in the range between 01 and 500 mg/L. The relative standard derivations of Bicarbonate less than 1.0% (n = 8). The recoveries of Bicarbonate in the samples from 100.15% to 111.10% were obtained. The Limit Of Detection (LOD) was 0.5 mg/L. The result showed that the method was convenient and accurate for the determination of Bicarbonate in Cyanobacterial fermentation.

Keywords: Anion exchange chromatography, cyanobacteria, glucosylglycerol, pulsed amperometric detection, sucrose

INTRODUCTION

There are four forms of Dissolved Inorganic Carbon (DIC) in the water: CO_2, HCO_3^-, H_2CO_3, CO_3^{2-}, which can be mutually transformed and mainly affected by the pH value of water. But Colman *et al*. (2002) found that the microalgae living in the water can only absorb and utilize CO_2 and HCO_3^-. Axelsson *et al*. (1995) also said that CO_2 freely diffuses into cells and then works for the photosynthesis of microalgae; bicarbonate ion can be directly or indirectly transferred into cells and utilized by microalgae.

HCO_3^- is essential for the organisms in the water. Dou *et al*. (2008) studied that bicarbonate can relieve the stress of Vallisneria natans in high-nutrition conditions, which proves that the increase of HCO_3-DIC in the water can mitigate the impact of eutrophication on submerged plant. The research of Maberly *et al*. (2006) shows that most submerged plants can adapt to the environment of low CO_2 by utilizing HCO_3--DIC, like Vallisneria natans, Potamogeton crispus and Potamogeton malaianus, etc.

At present, Liu *et al*. (2006), Fischer (2002), Xu-Chuan (2009) and Chen *et al*. (2006) determined the carbonate utiling respectively these method of titrimetry, colorimetry, chromatography and enzymatic analysis. These methods can be applied in a certain range, but quantitative determination is more rapid and sensitive. Therefore, we propose a sort of non-suppression ion chromatography (IC) which can rapidly determine the bicarbonate contents in cyanobacteria medium.

Ion-exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. It is often used in protein purification, water analysis and quality control (Xie *et al*., 2013).

Generally, in the process of anion determination, suppressor is necessary for IC. However, because of the volatility of H_2CO_3 which is produced by H^+ and HCO_3^- eluted, bicarbonate ion cannot be quantitatively determined (Nishihara and Ackerman, 2006). Though anionic columns (AS15, AS16, AS17) were also used to detect, quantitative determination cannot be realized. So this thesis adopts non-

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suppression IC, cation exchange column of Optimix C18/SCX and matrix solution of sodium bicarbonate diluted 1000 times as leacheate.

In the study, the interference of matrix can be eliminated, which realizes the quantitative determination of carbonate contents in cyanobacteria medium. The result shows that this method has simple pretreatment, good selectivity and high sensitivity, which is appropriate for the determination of carbonate contents in cyanobacteria medium.

MATERIALS AND METHODS

Instrumentation and materials: An ICS-5000 system (Thermo Scientific Dionex, Sunnyvale, CA, USA) equipped with two pumps having capillary flow capability and pumping 18 MÙ water regenerant (flow rate, 0.03 mL/min), an AS-AP auto-sampler were used in this study. Chromeleon software (Thermo Fisher Scientific, San Jose, California) was used for system control.

All the solutions were prepared in 18.2 MΩ water (Milli-Q) with a 0.22 μm nylon membrane filter. Bicarbonate standard was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of standard solutions: 0.01 gram of Sucrose and Glucosylglycerol were prepared in 10 mL volumetric flasks with ultrapure water respectively used their stock solutions.

Nine standards of Sucrose and Glucosylglycerol were prepared respectively by pipetting 1, 5, 10, 20, 50, 100, 200, 500, 1000 μL, respectively of their stock solutions into 10 mL volumetric flasks and diluting to volume with ultrapure water. The concentrations of standard series were both 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 ppm.

Sample preparation: Cultures were sampled by taking 1.5 mL aliquots that were centrifuged at 6000 r/min for 10 min to obtain cell pellets and supernatants. The supernatants were diluted 5 times and filtered using 0.22 μm water nylon membrane using 3 mL solution.

Chromatographic condition: IC separation was performed using a capillary column (Optimix C18/SCX, 2.1 mm×30 mm×3 um, Agela Technologies) and conductivity detection; the flow rate of MSA eluent was 0.2 mL/min at 30°C; the injection volume was 50 μL.

Chromatographic condition: Matrix solution of Cyanophyta with sodium bicarbonate diluted 1000 times is prepared into 500 mg/L standard stock solution. The different concentrations of standard solutions can be seen from Table 1. Samples are injected according to the chromatographic condition of below section. And standard curve is drawn with peak area y (nC×min) as ordinate and mass concentration x (mg/L) as abscissa.

Experiment of repeatability and recovery: 20, 100, 500 mg/L sodium-bicarbonate standard solutions are prepared. Samples are separately put into 4 mL centrifuge tube and 1.5 mL samples are taken out for chromatographic analysis, injected 8 times repeatedly.

The residual standard solutions are centrifuged at the speed of 6000 r/min for 5 min. Supernate is taken and diluted 5 times. Then 3 mL solutions are filtrated by 0.22 μm aqueous-phase membranes for chromatographic analysis.

RESULTS AND DISCUSSION

The choice of chromatographic column: The conductivities of bicarbonate and bicarbonate ion are the lowest in inorganic anions. So the leacheate with high background conductance and cation exchange column of Optimix C18/SCX were used to produce negative chromatographic peak and baseline-resolved columns are chosen for further detection and determination.

Cation exchange column of Optimix C18/SCX is used for separation, 5 mm methanesulfonic acid as leacheate, conductivity for detection and sodium bicarbonate for baseline separation. As shown in Fig. 1.

Selection of moving phase concentration: The impact of 300, 500, 800, 1000 mmol/L NaOH leacheate to separation effect of Sucrose and GG is respectively investigated and the result shows that, at concentration of 300 mmol/L and 500 mmol/L, Sucrose and GG can be separated, but separation time is fairly long and exceeds 1h; for leacheate of 800 mmol/L, Sucrose and GG can be separated within 30 min, for leacheate of 1000 mmol/L, separation effect of Sucrose and GG is not ideal. Therefore NaOH of 800 mmol/L is selected as moving phase.

Table 1: Mass concentration of standard solution (unit: mg/L)

Number	1	2	3	4	5	6	7	8	9	10
Sodium bicarbonate	1	5	10	20	50	100	200	300	400	500

Table 2: The evaluation data of this method

Analyte	RSD (%) (n = 8)	Coefficient of association	Equation of linear regression	Linearity range	Limit of detection (mg/L)	Recovery (%)
Bicarbonate	0.32-0.85	0.99946	$Y = 0.4440X+0.1355$	1-500	0.5	100.15-111.1

Table 3: Content of different culture solutions of Bicarbonate

Analyte	Sample 1	Sample 2	Sample 3	Sample 4
Bicarbonate	13.0667	12.0515	11.3120	10.0735

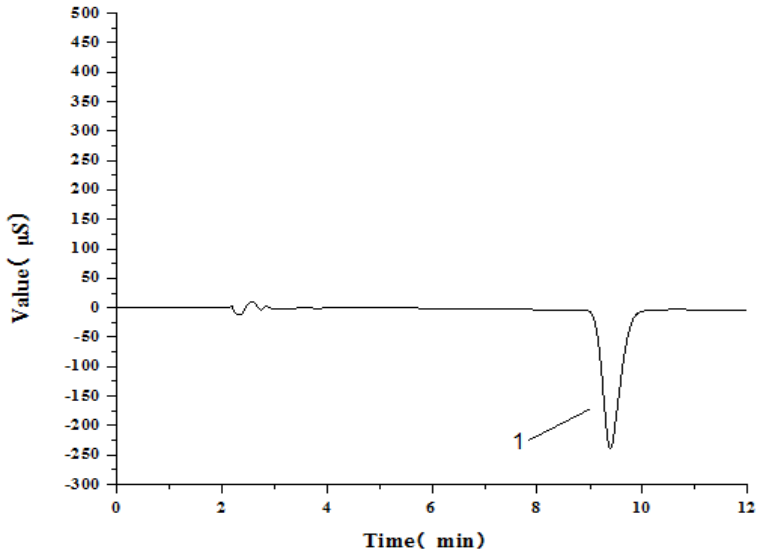

Fig. 1: Chromatogram of sodium bicarbonate (1-sodium bicarbonate)

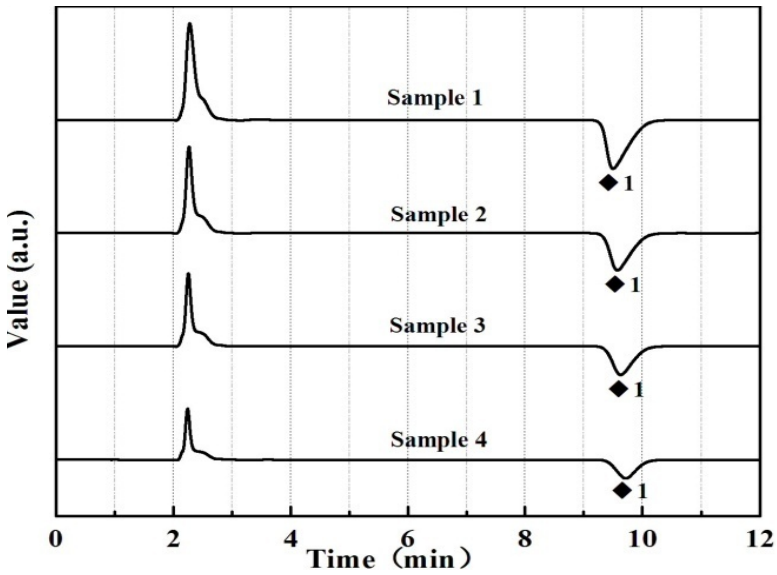

Fig. 2: Chromatogram of different culture solutions of Bicarbonate; 1-Bicarbonate; Sample 1-cultivated 24 h; Sample 2-cultivated 48 h; Sample 3-cultivated 72 h; Sample 4-cultivated 96 h; Sample 5-cultivated 120 h

Evaluation of method: Mixed standard solutions listed in Table 1 are successively injected. According to linear regression with peak area Y as ordinate and mass concentration of standard solution X (mg/L) as abscissa, the linearly dependent coefficient is measured to be 0.99946. NO. 4, 6, 10 standard solutions in Table 1 are successively injected 8 times and the relative Standard deviation is measured to be less than 1.00%. Samples are added into the NO. 4, 6, 10 standard solutions and are processed by sample handling method of section 2.2. The average recovery rates are ranged from 101.15-111.10%. Equation of linear regression, coefficient of association, relative standard deviation, recovery and limit of detection are all listed in Table 2.

Analysis of practical samples: Four cyanobacteria medium samples with different reaction times are pretreated by the method mentioned in above section. The sodium bicarbonate in the samples are well separated with sharp peak. The chromatogram of four practical samples can be seen in Fig. 2 and Table 3.

CONCLUSION

In this study, we realizes the quantitative determination of carbonate contents in cyanobacteria medium. The result shows that this method has simple pretreatment, good selectivity and high sensitivity, which is appropriate for the determination of carbonate contents in different system.

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