Korean Journal of Environmental Agriculture

Research Article

CrossMark Open Access

용존유기탄소의 $\delta^{13}C$ 분석시 고형화 전처리 방법 비교: 알칼린 과황산칼륨산화-탄산침전과 동결건조

전병준[1], 박현진[1], 최우정[1*], 박용세[2], 이상모[2], 윤광식[1]

Comparison of Solidification Pre-treatment Methods for the Determination of $\delta^{13}C$ of Dissolved Organic Carbon: Alkaline Persulfate Oxidation-Carbonate Precipitation vs. Freeze Drying

Byeong-Jun Jeon[1], Hyun-Jin Park[1], Woo-Jung Choi[1*], Yong-Se Park[2], Sang-Mo Lee[2] and Kwang-Sik Yoon[1]
([1]Department of Rural & Biosystems Engineering, Chonnam National University, Gwangju 61186, Korea, [2]National Instrumentation Center for Environmental Management, Seoul National University, Seoul 08826, Korea)

ORCID

Byeong-Jun Jeon
http://orcid.org/0000-0002-0738-3150

Hyun-Jin Park
http://orcid.org/0000-0002-2642-5723

Woo-Jung Choi
http://orcid.org/0000-0002-2009-8207

Yong-Se Park
http://orcid.og/0000-0002-5059-7154

Sang-Mo Lee
http://orcid.org/0000-0002-8661-6847

Kwang-Sik Yoon
http://orcid.org/0000-0003-4996-2860

Abstract

BACKGROUND: The carbon (C) isotope ratio ($\delta^{13}C$) of dissolved organic C (DOC) is an indicator of water pollution source. In this study, the potential use of two pre-treatments for the $\delta^{13}C$ analysis, alkaline persulfate oxidation coupled with carbonate precipitation (precipitation) and freeze drying (drying), were compared to suggest a more feasible pre-treatment method.

METHODS AND RESULTS: Two reference materials with different $\delta^{13}C$ values were used for the experiments; chemical grade glucose ($-12.0\pm0.02‰$) and pig manure compost extract ($-23.3\pm0.04‰$). In the precipitation method, the measured $\delta^{13}C$ values were consistently lower than the theoretically calculated values as dissolved CO_2 could not be removed due to the alkaline property of the reagents and the dissolution of air CO_2 into the alkaline solution. The drying method also resulted in more negative $\delta^{13}C$ than the calculated $\delta^{13}C$; however, the difference was systematic ($3.9\pm0.3‰$) and there was a strong correlation ($\delta^{13}C_{calculated}=0.87\times\delta^{13}C_{measured}-0.624$, $r^2=0.98$) between the calculated and measured $\delta^{13}C$. Calibration of $\delta^{13}C$ using the relationship between the calculated and the measured $\delta^{13}C$ values produced reliable and accurate $\delta^{13}C$ values.

CONCLUSION: Our results suggest that the drying method is more accurate pre-treatment method to minimize the influence of air CO_2 compared to the precipitation method for the determination of $\delta^{13}C$ of DOC.

*Corresponding author: Woo-Jung Choi
Phone: +82-62-530-2153; Fax: +82-62-530-2159;
E-mail: wjchoi@jnu.ac.kr

Key words: Carbon isotope ratio, Dissolved organic carbon, Freeze drying, Stable isotope ratio mass spectrometer, Strontium carbonate precipitation

서 론

(DOC, Dissolved Organic Carbon)

,   (Tu et al., 2011).   DOC

(Yoo *et al.*, 2012).   DOC

,   , DOC

(Zhou *et al.*, 2006).   ,

,   ,

(Ongley *et al.*, 2010; Sun *et al.*, 2012).

(Choi *et al.*, 2017).
($^{13}C/^{12}C$, $\delta^{13}C$

‰)

,   C4

$\delta^{13}C$

(Yanagi *et al.*, 2012).

C4   (,   -11.2‰,   -13.0‰)
$\delta^{13}C$ C3   (-29.1‰,   -25.3‰)
(Yanagi *et al.*, 2012), C4
$\delta^{13}C$ (-12.6‰)   C3 $\delta^{13}C$ (-26.8‰)
(Dungait *et al.*, 2010).   ,

$\delta^{13}C$

DOC $\delta^{13}C$.
DOC $\delta^{13}C$
(TOC analyzer)   (Stable Isotope Ratio Mass Spectrometer, SIRMS)
(Kirkels *et al.*, 2014),
.   DOC $\delta^{13}C$
DOC
CO_2 Gas Chromatography (GC)-SIRMS
(van Geldern *et al.*, 2013; Yu *et al.*, 2015),
(Elemental Analyzer)   EA-SIRMS   .
,   (50~60℃)   EA-SIRMS
DOC $\delta^{13}C$ (Parker *et al.*, 2010; Tu *et al.*,

2011).   ,   DOC

,   (Amiotte-Suchet *et al.*, 2007; Lambert *et al.*, 2011).

$\delta^{13}C$

.

DOC

CO_2 $SrCl_2$
$SrCO_3$ (Harris *et al.*, 1997). $SrCO_3$ (DIC, Dissolved Inorganic Carbon)
(Parker *et al.*, 2010; van Geldern *et al.*, 2013), DOC
$\delta^{13}C$.   ,
DOC $\delta^{13}C$ 2
-$SrCO_3$
EA-SIRMS   DOC $\delta^{13}C$
.

재료 및 방법

표준 물질

(Glucose)
-$SrCO_3$
DOC $\delta^{13}C$.

,
1:10   500 mL
.
($^{13}C/^{12}C$)   EA-SIRMS (IsoPrime-EA, Micromass, Manchester, UK)
10            $\delta^{13}C$.

$$\delta^{13}C\ (‰) = [(R_S - R_R)/R_R] \times 1000 \tag{1}$$

R_S R_R ,
Vienna-Pee Dee Belemnite ($CaCO_3$)
0.0112372 ^{13}C atom %   (Choi and Lee, 2012). $\delta^{13}C$ -12.0±0.02‰
-23.3±0.04‰   .

과황산칼륨 산화–$SrCO_3$ 침전 후 $\delta^{13}C$ 분석 방법

-$SrCO_3$ $\delta^{13}C$
1) $\delta^{13}C$ (12.0±0.02‰)
(10 mg C/L)   (0, 5, 10, 20, 30 mL;   : G_0, G_5, G_{10}, G_{20}, G_{30})
DOC   (0, 0.05, 0.1, 0.2, 0.3 mg C)
-$SrCO_3$ $\delta^{13}C$
2) $\delta^{13}C$ 6
(10 mg C/L)   DOC

Table 1. Experimental settings for the pre-treatment of glucose samples using alkaline persulfate oxidation method and freeze-drying method

Sample code	Glucose C added (mg)	Compost extract C added (mg)	Total C added (mg)	$\delta^{13}C$ of total C calculated (‰)[a]
$G_{10}C_0$	0.10	0.00	0.10	−12.0
G_8C_2	0.08	0.02	0.10	−14.3
G_6C_4	0.06	0.04	0.10	−16.5
G_4C_6	0.04	0.06	0.10	−18.8
G_2C_8	0.02	0.08	0.10	−21.0
G_0C_{10}	0.00	0.10	0.10	−23.3

a) The $\delta13C$ was calculated using isotope mass balance equation.

$\delta^{13}C$ -SrCO$_3$
            $\delta^{13}C$ (Table 1).
                                            0.185 M K$_2$S$_2$O$_8$
0.42 M NaOH   (Cabrera
and Beare, 1993), DOC   CO$_3^{2-}$
    2 N SrCl$_2$ (Harris et al., 1997).
    50 mL   septa
            10 mL (Cabrera and Beare, 1993),   2 N
SrCl$_2$ 2 mL
        121℃   30   .
                        (SrCO$_3$)   65℃
                                    V$_2$O$_3$ 5 mg
                $\delta^{13}C$.   3

                                            $\delta^{13}C$

        (Isotope mass balance)
(Nitschelm et al., 1997).

$\delta^{13}C_{glucose+compost}=[(\delta^{13}C_{glucose}\times C_{glucose}$
$\qquad +(\delta^{13}C_{compost}\times C_{compost})]/C_{glucose+compost}$ (2)

$\delta^{13}C_{glucose+compost}$, $\delta^{13}C_{glucose}$, $\delta^{13}C_{compost}$는 각각 글루코스와 퇴비침출액 분말 혼합시료, 글루코스, 퇴비침출액 분말의 $\delta^{13}C$이고, $C_{glucose}$, $C_{compost}$, $C_{glucose+compost}$는 각각 이들의 탄소 함량이다.

동결건조 후 $\delta^{13}C$ 분석 방법
                -SrCO$_3$
        -                    (Table 1)
            .   pH   2
            DIC   ,
K$_2$SO$_4$ 100 mg   .
            $\delta^{13}C$.   3   .

통계 분석
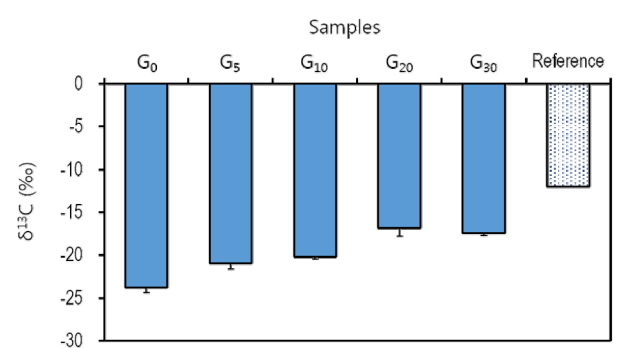   (Homogeneity of variance)

Fig. 1. The   [13]C of glucose samples processed with alkaline persulfate digestion coupled with SrCO$_3$ precipitation. 0, 5, 10, 20, and 30 mL of glucose solution (10 mg C/L) were used for sample codes, G$_0$, G$_5$, G$_{10}$, G$_{20}$, and G$_{30}$, respectively. Reference is glucose of which   [13]C was directly measured with EA-SIRMS. Vertical bars are standard errors of the means (n=3). All measured   [13]C for G0~G30 differ from the   [13]C of reference (P<0.001).

        (Normality of distribution)   Levene's
test   Shapiro-Wilk's test   ,
                                            . δ
[13]C   (   )
                            ANOVA
        .
            .   -SrCO$_3$
        $\delta^{13}C$ F-test   .
    SPSS 18.0 (SPSS Inc. Chicago, USA)
95% (α=0.05)   .

결과 및 고찰

과황산칼륨 산화−SrCO$_3$ 침전 후 $\delta^{13}C$ 분석 결과
                                    -SrCO$_3$
        $\delta^{13}C$ -23.8~-17.4‰ (-19.9‰)
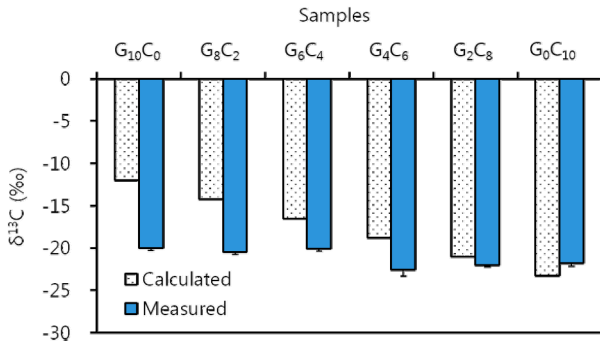(-12.0‰)   (Fig. 1).   ,

Samples

Fig. 2. The δ^{13}C of glucose+compost-extract mixed samples calculated with isotope mass balance equation (calculated) and processed with alkaline persulfate digestion coupled with $SrCO_3$ precipitation (measured). Sample codes are provided in Table 1. Vertical bars are standard errors of the means (n= 3). All measured δ^{13}C differ from the calculated δ^{13}C (P<0.001).

Samples

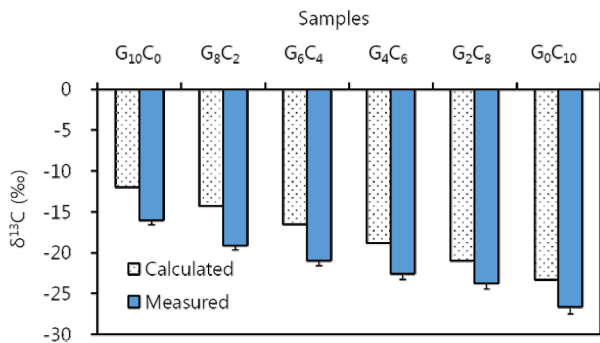Fig. 3. The δ^{13}C of glucose+compost-extract mixed samples calculated with isotope mass balance equation (calculated) and processed with freeze drying (measured). Sample codes are provided in Table 1. Vertical bars are standard errors of the means (n= 3). All measured δ^{13}C differ from the calculated δ^{13}C (P<0.001).

δ^{13}C (‰)-calculated (true value)

y = 0.87x - 6.24
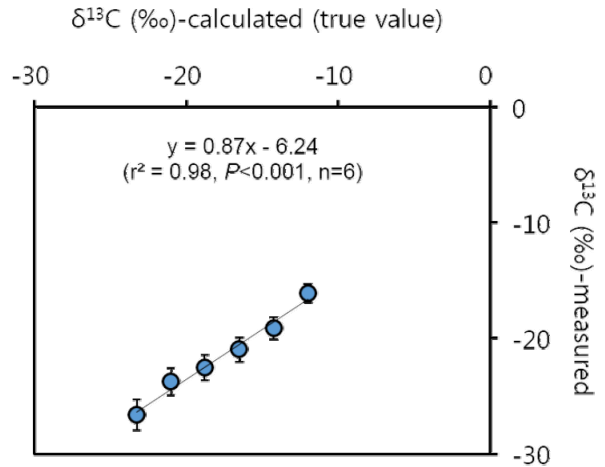(r^2 = 0.98, P<0.001, n=6)

Fig. 4. Regression analysis between the δ^{13}C calculated (true value) and measured for freeze drying pre-treatment using data shown in Fig. 3. Vertical bars are standard errors of the means (n=3).

-10‰   ,

                              CO_2 δ^{13}C -20‰
        (Choi and Lee, 2012).
                                        δ^{13}C -23.8‰
(Fig. 1),   .
    Harris *et al.* (1997) CO_3^{2-} $SrCl_2$
$SrCO_3$ EA-SIRMS δ^{13}C
        $SrCO_3$ CO_2
        δ^{13}C   ,   -13.6‰
            $SrCO_3$ CO_2 δ^{13}C
                          .   ,
                  CO_2 $SrCl_2$ $SrCO_3$
        δ^{13}C CO_2 δ^{13}C
        .   ,   -$SrCO_3$
δ^{13}C   DOC
                  .

-23.2～-12.0‰   ,   -22.6～-20.0‰
        (Fig. 2).   ,   (Fig. 2 C_0C_{10})
    ,   -$SrCO_3$ δ^{13}C
            .
                  DOC δ^{13}C
pH 2.0   DIC CO_2
        (Yu *et al.*, 2015).   ,
-$SrCO_3$ DOC CO_2

              DIC   .   ,
                              CO_2

(Harris *et al.*, 1997).
                      CO_2 δ^{13}C

동결건조 후 δ^{13}C 분석 결과
                  δ^{13}C -26.7～-16.1‰
    -$SrCO_3$ δ^{13}C (-23.8～-17.4‰)
        ,   -$SrCO_3$
(-23.3～-12.0‰)   (Fig. 3).   ,
                                  (r^2) 0.98
(Fig. 4),   3.9±0.3‰

(Systematic error)
        .   ,   (Fig. 4)
              (3.9±0.3‰)
        δ^{13}C   (Fig. 5).
                  DOC δ^{13}C
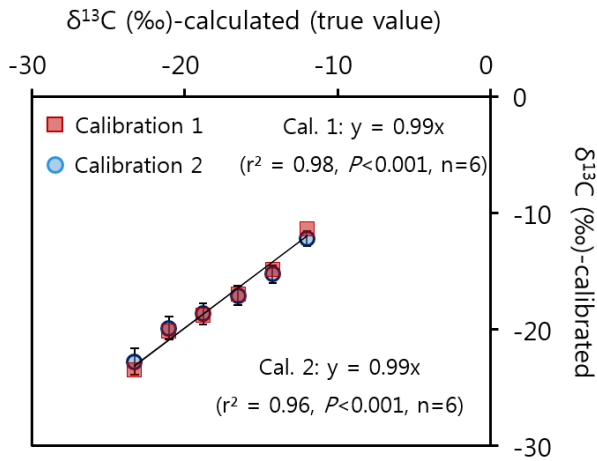(Amiotte-Suchet *et al.*, 2007; Lambert *et*

Fig. 5. Regression analysis between the ^{13}C calculated (true value) and calibrated using the regression equation in Fig. 4 (Calibration 1) and using the difference (3.9‰) between the calculated and measured ^{13}C for freeze drying pre-treatment. Vertical bars are standard errors of the means (n=3).

al., 2011), δ^{13}C
                                                        .

        DOC δ^{13}C
, δ^{13}C

                        .

요 약

    DOC δ^{13}C
                .
(EA-SIRMS)   DOC δ^{13}C
                    (-SrCO$_3$
)   .
(δ^{13}C; -12.0±0.02‰)   (δ^{13}C;
-23.3±0.04‰)   .   -SrCO$_3$
                        DIC
CO$_2$
            .   ,   DIC
        ,
                .   , EA-SIRMS
    DOC δ^{13}C

        .

Acknowledgement

from the Yeongsan and Sumjin River Watershed Management Committee, Ministry of Environment, Republic of Korea.

References

Amiotte-suchet, P., Linglois, N., Leveque, J., & Andreux, F. (2007). ^{13}C composition of dissolved ogranic carbon in upland forested catchments of the morvan mountains (France): Influence of coniferous and deciduous vegetation. Journal of Hydrology, 335(3-4), 354-363.

Cabrera, M. L., & Beare, M. H. (1993). Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal, 57(4), 1007-1012.

Choi, W. J., Kwak, J. H., Lim, S. S., Park, H. J., Chang, S. X., Lee, S. M., Arshad, M. A., Yun, S. I., & Kim, H. Y. (2017). Synthetic fertilizer and livestock manure differently affect δ^{15}N in the agricultural landscape: A review. Agriculture Ecosystems & Environment, 237(1), 1-15.

Choi, W. J., & Lee, K. H. (2012). A short overview on linking annual tree ring carbon isotopes to historical changes in atmospheric environment. Forest Science & Technology, 8(2), 61-66.

Dungait, J. A. J., Bol, R., Lopez-Capel, E., Bull, I. D., Chadwick, D., Amelung, W., Granger, S. J., Manning, D. A. C., & Evershed, R. P. (2010). Applications of stable isotope ratio masss spectrometry in cattle dung carbon cycling studies. Rapid Communications in Mass Spectrometry, 24(5), 495-500.

Harris, D., Porter, L. K., & Paul, E. A. (1997). Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. Communications in Soil Science and Plant Analysis, 28(9-10), 747-757.

Kirkels, F. M. S. A., Cerli, C., Federherr, E., Gao, J., Kalbitz, K. (2014). A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. Ⅱ: optimization and assessment of analytical performance. Rapid Communications in Mass Spectrometry, 28(23), 2574-2586.

Lambert, T., Pierson-Wickmann A. C., Gruau, G., Thibault, J. N., & Jaffrezic, A. (2011). Carbon isotopes as tracers of dissolved organic carbon sources and water pathways in headwater catchments. Journal of Hydrology, 402 (3-4), 228-238.

Nitschelm, J. J., Lüscher, A., Hartwig, U. A., & van Kessel, C. (1997). Using stable isotopes to determine

soil carbon input differences under ambient and elevated atmospheric CO_2 conditions. Global Change Biology, 3(5), 411-416.

Ongley, E. D., Xiaolan, Z., & Tao, Y. (2010). Current status of agricultural and rural non-point source pollution assessment in China. Environmental Pollution, 158(5), 1159-1168.

Parker, S. R., Poulson, S. R., Smith, M. G., Weyer, C. L., & Bates, K. M. (2010). Temporal variability in the concentration and stable carbon isotope composition of dissolved inorganic and organic carbon in two Montana, USA rivers. Aquatic Geochemistry, 16(1), 61-84.

Sun, B., Zhang, L., Yang, L., Zhang, F., Norse, D., & Zhu, Z. (2012). Agricultural non-point source pollution in China: causes and mitigation measures. AMBIO, 41(4) 370-379.

Tu, C. L., Liu, C. Q., Lu, X. H., Yuan, J., & Lang, Y. C. (2011). Sources of dissolved organic carbon in forest soils: evidences from the differences of organic carbon concentration and isotope composition studies. Environmental Earth Sciences, 63(4), 723-730.

Van Geldern, R., Verma, M. P., Carvalho, M. C., Grassa, F., Delgado-Huertas, A., Monvoisin, G., & Barth, J. A.

C. (2013). Stable carbon isotope analysis of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in natural waters-Results from a worldwide proficiency test. Rapid Communications in Mass Spectrometry, 27(18), 2099-2107.

Yanagi, Y., Hirooka, H., Oishi, K., Choumei, Y., Hata, H., Arai, M., Kitagawa, M., Gotoh, T., Inada, S., & Kumagai, H. (2012). Stable carbon and nitrogen isotope analysis as a toll for inferring beef cattle feeding systems in Japan. Food Chemistry, 134(1), 502-506.

Yoo, S. H., Ro, H. M., & Choi, W. J. (2012). Soil and water qualities affected by six decades of agricultural paradigm shifts in Korea. Proceedings of Korean Academy of Science, 51(1), 127-157.

Yu, K., Gan, Y., Zhou, A., Han, L., & Liu, Y. (2015). A persulfate oxidation method for stable isotope analysis of dissolved organic carbon and the influence of inorganic ions on the results. International Journal of Mass Spectrometry, 392(1), 63-68.

Zhou, J., Wu, Y., Zhang, J., Kang, Q., & Liu, Z. (2006). Carbon and nitrogen composition and stable isotope as potential indicators of source and fate of organic matter in the salt marsh of the Changjiang Estuary, China. Chemosphere, 65(2), 310-317.