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Application Note

Energy

TOC-L with TNM-L: Simultaneous Measurement of CO_2 Fixation and Nitrogen Content in Microalgal Biomass

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Example of dried microalgae

Abstract

Research and development on microalgae-derived feedstocks for sustainable aviation fuel (SAF) has been gaining momentum. Effectively advancing this research requires the rapid and quantitative assessment of microalgal CO_2 fixation capacity and nitrogen dynamics, both of which are closely linked to biomass productivity and lipid accumulation and underpin the evaluation of environmental contribution and the optimization of production processes. This Application Note introduces examples of measuring CO_2 fixation and nitrogen content in microalgal biomass using the TOC-L Total Organic Carbon Analyzer equipped with the TNM-L Total Nitrogen Unit.

1. Introduction

Global demand for sustainable aviation fuel (SAF) has been rising sharply, amid international CO_2 reduction efforts and the decarbonization goals of both Japan and the United States. Among various feedstocks, microalgae-derived bio-jet fuel has emerged as a promising candidate for SAF, with extensive research and development underway toward its industrialization. A harmonized resource assessment by U.S. national laboratories indicates that the domestic resource base could support the production of more than 75 billion liters of algal biofuel annually at full deployment of large-scale cultivation[1)]. Projections on this scale point to the magnitude of the contribution microalgae could make toward carbon neutrality, and to the industrial potential of the field.

However, realizing that potential requires the sustainability of microalgae-derived fuels to be verified by life cycle assessment (LCA). Such an assessment weighs the CO_2 fixed during cultivation against the greenhouse gas emissions generated across the entire production chain, including SAF conversion and upgrading. A key inventory item that must be established experimentally is the amount of CO_2 converted into organic carbon. Total organic carbon (TOC) analysis provides a direct measure of this quantity. Because the measurement is performed on liquid suspensions, the carbon content of a culture can be determined without a drying step[2)].

The nitrogen status of the cells is equally important. Microalgae accumulate lipids in response to intracellular nitrogen depletion, so cellular nitrogen content is closely linked to the balance between biomass productivity and lipid yield, and hence the value of the biomass as a SAF feedstock. The Shimadzu TOC-L analyzer equipped with a TNM-L total nitrogen unit measures TOC and total nitrogen (TN) simultaneously on the same sample, allowing carbon fixation and nitrogen status to be monitored together within a single workflow.

In the present study, we applied the TOC-L system to *Chlamydomonas reinhardtii* CC-1010, a model green alga whose physiological responses to nutrient limitation are well characterized, in order to evaluate its CO_2 fixation potential and to analyze how changes in cellular nitrogen content affect lipid accumulation.

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2. Method

Dry cell weight determination

Dry cell weight (DCW) was determined as the suspended solids (SS) content of the culture broth based on ASTM D5907, a method recommended by the Algae Biomass Organization (ABO) for algal biomass measurement[3]. Briefly, a known volume of *C. reinhardtii* CC-1010 culture was filtered through a Whatman GF/B glass fiber filter (Cytiva), and the collected cells were rinsed with distilled water. The filter was then dried in an oven at 100°C, and the dry weight was measured. Biomass concentration was calculated as follows:

$$Biomass\ (g \cdot L^{-1}) = \frac{Filter_{After}\ (g) - Filter_{Before}\ (g)}{Biomass\ Volume\ (L)}$$

where $filter_{Before}$ and $filter_{After}$ represent the dry weight of the filter before and after biomass collection, respectively, and biomass volume is the volume of culture broth filtered.

TOC and TN measurement

CO_2 fixation was evaluated by quantifying total organic carbon (TOC) using a TOC-L analyzer equipped with a total nitrogen measuring unit (TNM-L; Shimadzu) (Fig.1). To distinguish intracellular organic carbon incorporated into biomass from extracellular organic carbon released by the cells, such as extracellular polysaccharides secreted by some microalgal species, the culture broth was separated into a microalgal cell fraction and a supernatant fraction, and both fractions were subjected to TOC and TN analysis.

Briefly, the culture broth was centrifuged at 5,000×g for 5 min at room temperature to separate the algal cells (pellet) from the supernatant. The supernatant was retained for direct measurement of extracellular TOC and TN.

The cell pellet was resuspended in an equal volume of ultrapure water relative to the original sample volume to remove residual extracellular material, and the suspension was centrifuged again at 5,000×g for 5 min at room temperature. The resulting pellet, representing the purified intracellular fraction, was collected and resuspended in ultrapure water. Each prepared sample was homogenized by sonication prior to measurement with the TOC analyzer.

Total carbon (TC), inorganic carbon (IC), and total nitrogen (TN) were measured using the TOC analyzer. Calibration curves were prepared using potassium hydrogen phthalate for TC, sodium bicarbonate for IC, and potassium nitrate for TN (all from Nacalai Tesque) (Tables 1, 2). Injection volumes were set at 50 µL for TC and TN, and 250 µL for IC. TOC was calculated by subtracting IC from TC.

FAME extraction and GC-MS/MS analysis

Fatty acid methyl ester (FAME) composition analysis of the microalgae-derived lipids was performed in accordance with Application Note No.106, with FAME content used as a measure of intracellular lipid content[4]. Lipids were extracted from lyophilized microalgae cells by disruption with 0.1-mm zirconia beads in 1 mL of $CHCl_3$/MeOH (2:1) containing tripentadecanoin as an internal standard (2,500 rpm; 1-min disruption with 2-min intervals, repeated for 8 cycles). Following cell disruption, an additional 3 mL of the extraction solvent ($CHCl_3$/MeOH, 2:1) and 2.5 mL of Tris buffer (50 mM Tris, 1 M NaCl, pH 7) were added. The mixture was vortexed for 5 min and centrifuged, and the organic (lower) phase was recovered. This extraction step was repeated with an additional 1 mL of the extraction solvent until the residue was colorless. The collected extracts were dried under a stream of nitrogen gas.

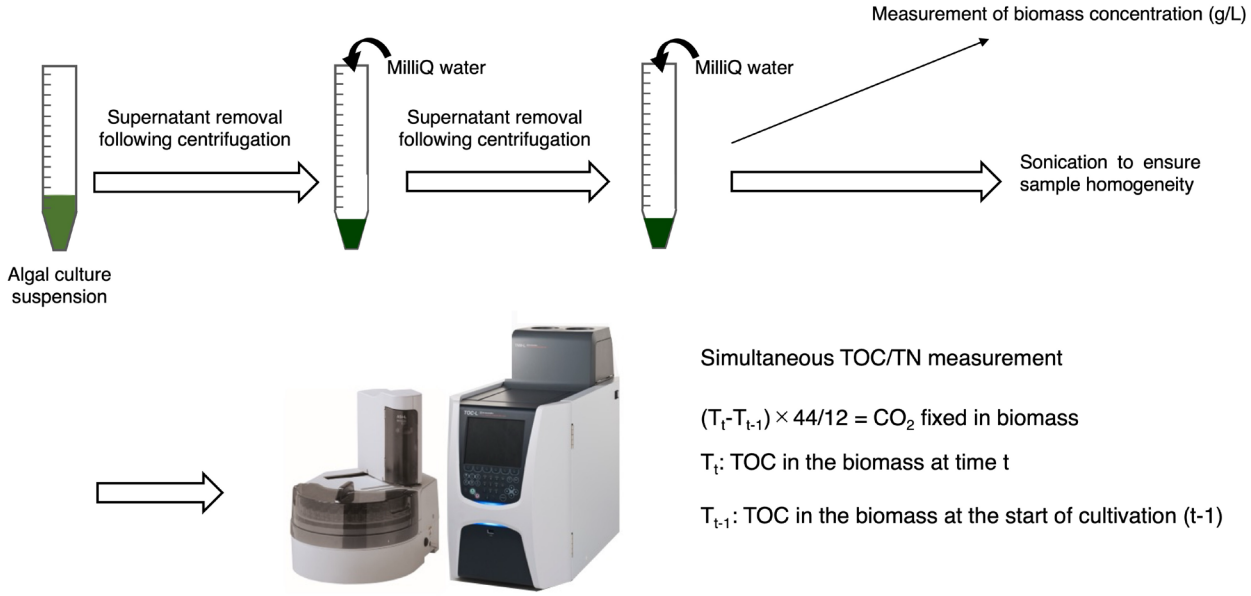


Fig. 1 Measurement procedure for organic carbon in microalgal biomass

Table1 Measurement conditions for TC and TN

Analyzer	:	TOC-L_{CPH} total organic carbon analyzer + TNM-L total nitrogen unit
Oxidation Method	:	TC: Combustion catalytic oxidation/NDIR TN: Thermal decomposition/chemiluminescence
Catalyst	:	TOC/TN catalyst
Measurement Items	:	TC and TN
Injection Volume	:	50 µL
Calibration Curve	:	TC: 4-point calibration curve with 0–500 mgC/L of potassium hydrogen phthalate aqueous solution TN: 4-point calibration curve with 0–100 mgN/L of potassium nitrate aqueous solution

Table2 Measurement conditions for IC

Analyzer	:	TOC-L_{CPH} total organic carbon analyzer
Oxidation Method	:	Acidification and sparging/NDIR
Measurement Items	:	IC
Injection Volume	:	250 µL
Calibration Curve	:	IC: 4-point calibration curve with 0–100 mgC/L of sodium bicarbonate aqueous solution

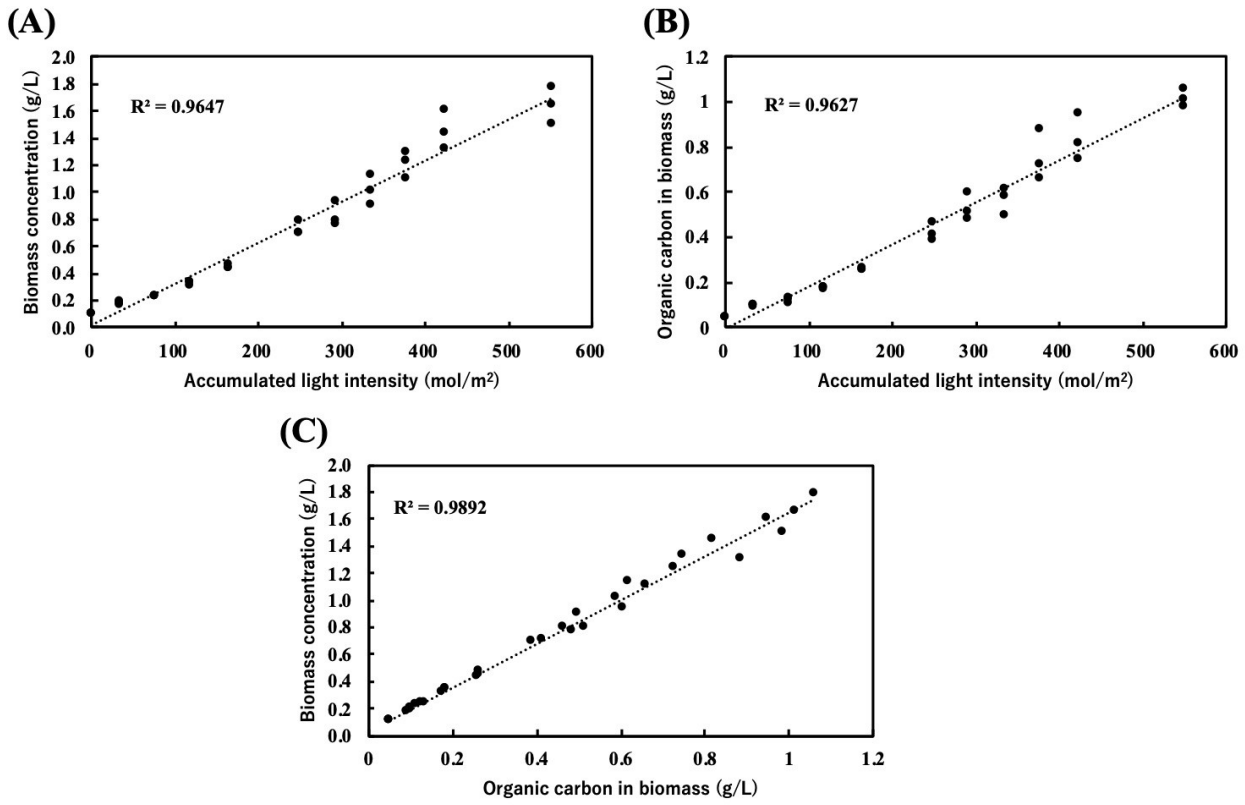


Fig. 2 Relationships between accumulated light intensity and biomass concentration (A) or organic carbon in biomass (B), and between organic carbon in biomass and biomass concentration (C)

The dried samples were then subjected to methyl esterification by adding 5% H_2SO_4/MeOH (3 mL) and incubating at 70 ℃ for 3 h. After the reaction, ultrapure water (3 mL) and hexane (3 mL) were added, and the mixture was centrifuged to facilitate phase separation; the hexane layer (upper phase), containing the target FAME products, was then collected. Anhydrous sodium sulfate was added to the recovered hexane phase for dehydration. The resulting FAME samples were analyzed using a GCMS-TQ™8040 NX. Further details are provided in Application Note No.106.

3. Results and Discussion

Accurately quantifying the CO_2 fixation capacity of microalgae, that is, their ability to convert CO_2 into organic carbon, is essential for evaluating both the environmental contribution and practical viability of microalgae-based SAF production. In addition, combining this evaluation with assessments of biomass productivity and lipid accumulation capacity enables a more comprehensive assessment of practical applicability. In this study, we therefore used a TOC-L/TNM-L analyzer (Shimadzu) to measure the total organic carbon content of the microalga *Chlamydomonas reinhardtii* CC-1010, while also investigating its characteristics, including its biomass production potential in relation to varying cumulative light intensity and intracellular nitrogen content.

Because cumulative light intensity is a primary factor of photosynthetic carbon fixation, we first examined its effect on the biomass concentration and total organic carbon (TOC) content of *C. reinhardtii* CC-1010.

The results showed that both biomass concentration and TOC content increased with increasing cumulative light intensity during CC-1010 growth (Fig. 2A, B). Furthermore, TOC content was found to be positively correlated with biomass concentration (Fig. 2C). These findings are consistent with previous reports indicating that optimizing biomass productivity in microalgae contributes to improved CO_2 utilization efficiency[5,6].

While photosynthetic efficiency and biomass productivity are critical factors directly linked to CO_2 fixation, the amount of lipid accumulated in algal cells is important for fuel applications. Microalgae are known to increase lipid content in response to intracellular nitrogen depletion, and this variability in lipid content is an essential consideration when evaluating microalgal biomass as a fuel feedstock. We therefore examined the effects of intracellular nitrogen content, in addition to cumulative light intensity, on lipid content and CO_2 fixation. Lipid content increased in response to decreasing nitrogen content but was not appreciably affected by cumulative light intensity (Fig. 3A). In contrast, TOC content decreased slightly with decreasing nitrogen content but increased with cumulative light intensity (Fig. 3B). A similar trend was observed for TOC in the culture supernatant (Fig. 3C). These results suggest that the CO_2 fixation and lipid production capacities of microalgae are intricately regulated by nutrient and light conditions. CO_2 fixation efficiency in microalgae is known to increase in proportion to cell concentration[5,6], and lipid accumulation induced by restricted cell proliferation under nitrogen-deficient conditions has also been widely reported[7–11].

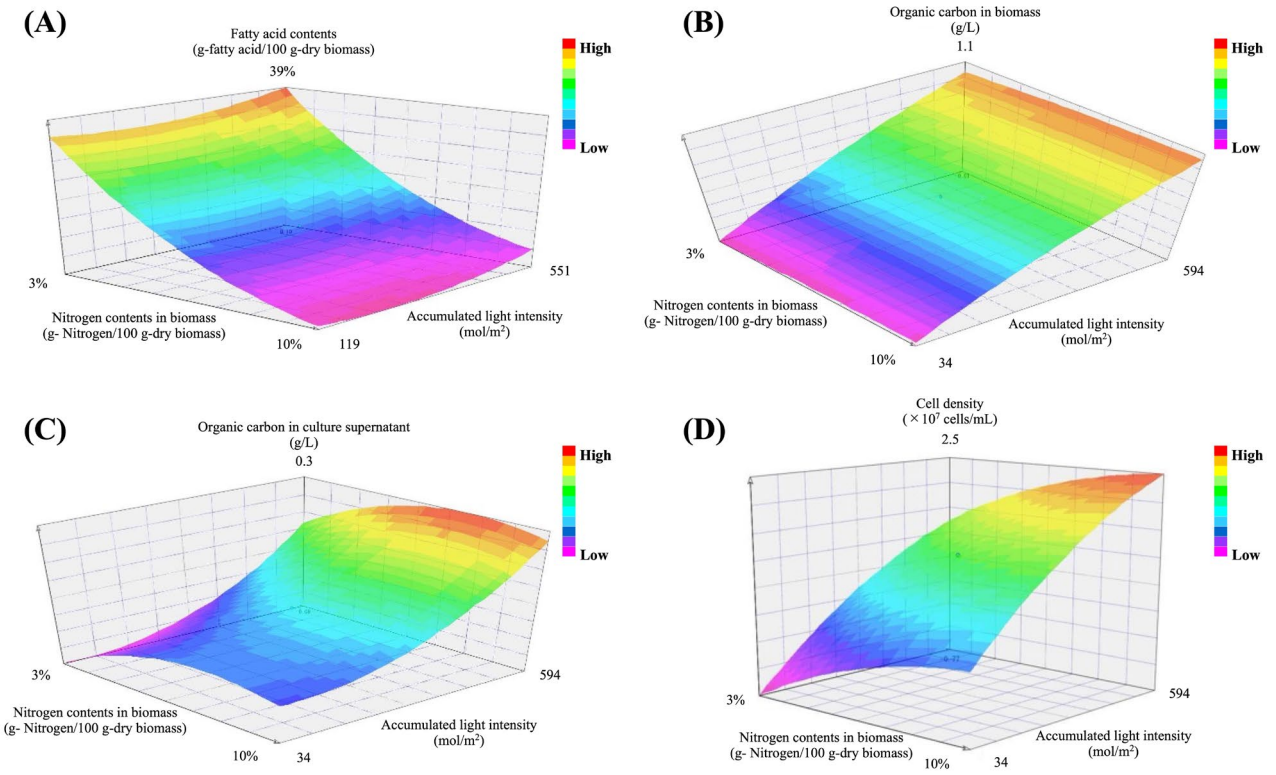


Fig. 3 Response surfaces of fatty acid content (A), organic carbon in biomass (B), organic carbon in culture supernatant (C), and cell density (D) of *Chlamydomonas reinhardtii* CC-1010 as functions of nitrogen content in biomass and accumulated light intensity

Consistent with this, we found that cell concentration decreased with decreasing nitrogen content (Fig. 3D). Taken together, these findings suggest that the decrease in TOC content under nitrogen deficiency reflects a trade-off between lipid accumulation and carbon fixation. A decline in photosynthetic activity may reduce carbon fixation per cell and, through restricted cell proliferation, further lower the fixation capacity of the culture as a whole. Nitrogen depletion is also known to trigger remodeling of membrane lipids and their conversion into triacylglycerol[12, 13], which would increase lipid content through redistribution of existing carbon rather than through additional fixation.

These results indicate that appropriately monitoring nitrogen availability during the exponential growth phase and preventing its depletion are critical for improving both biomass productivity and CO_2 fixation efficiency. They also suggest that adjusting the initial nitrogen concentration so that nitrogen becomes depleted once cell concentration plateaus can effectively promote lipid accumulation. Understanding the physiological characteristics of the target microalgal species and applying appropriate nutrient control tailored to each cultivation phase, guided by continuous monitoring, may thus enable simultaneous improvement of lipid productivity and CO_2 fixation efficiency.

4. Conclusion

Meeting the growing global demand for SAF requires two key elements: maximizing the lipid productivity of microalgae, and quantifying CO_2 fixation for use in LCA.

This application note used the Shimadzu TOC-L, equipped with a TNM-L total nitrogen unit, to evaluate CO_2 fixation in the microalga *Chlamydomonas reinhardtii* CC-1010, while also monitoring intracellular nitrogen content as an indirect indicator of lipid accumulation. The results suggest that appropriate management of nitrogen availability during the exponential growth phase is linked to sustained biomass productivity and CO_2 fixation efficiency, and that nitrogen depletion following the rise in cell density may serve as an effective switch for inducing lipid accumulation. The TOC-L, capable of measuring TOC and TN simultaneously, enables integrated monitoring of these complex phase transitions between growth and lipid accumulation, thereby supporting precise nutrient management.

Understanding the physiological characteristics of the target microalgal species and monitoring nutrient status across these distinct cultivation phases are essential to achieving this balance between lipid accumulation and carbon fixation performance. By providing rapid analytical data, the TOC-L is well positioned to support process optimization and carbon-neutrality assessment in the production of SAF feedstocks.

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References

1) "Algae Harmonization Study: Evaluating the Potential for Future Algal Biofuel Costs, Sustainability, and Resource Assessment from Harmonized Modeling" 2017 Technical Report.
2) Lim, Y. A., Chong, M. N., Foo, S. C., Ilankoon, I. M. S. K., Analysis of direct and indirect quantification methods of CO_2 fixation via microalgae cultivation in photobioreactors: A critical review. *Renew. Sustain. Energy Rev.*, 137, 110579. 2021.
3) American Society for Testing and Materials. D5907: Standard Test Methods for Filterable Matter (Total Dissolved Solids) and Nonfilterable Matter (Total Suspended Solids) in Water. *ASTM International*, 2019.
4) Marutani, T., Yoshino, H., Aoyanagi, T., Nishio, Y., Nagao, Y., GC-MS-Based Evaluation of Fatty Acid Composition in Microalgae. *Shimadzu Corporation Application Note* No. 106, 1–7. 2024.
5) Anjos, M., Fernandes, B. D., Vicente, A. A., Teixeira, J. A., Dragone, G., Optimization of CO_2 bio-mitigation by Chlorella vulgaris. *Bioresource Technol.*, 139, 149–154. 2013.
6) Sun, Z., Bo, C., Cao, S., & Sun, L., Enhancing CO_2 Fixation in Microalgal Systems: Mechanistic Insights and Bioreactor Strategies. *Mar Drugs*., 23(3), 113. 2025.
7) James, G. O., Hocart, C. H., Hillier, W., Chen, H., Kordbacheh, F., Price, G. D., Djordjevic, M. A., Fatty acid profiling of Chlamydomonas reinhardtii under nitrogen deprivation. *Bioresour Technol.*, 102(3), 3343–3351. 2011.
8) Boyle, N. R., Page, M. D., Liu, B., Blaby, I. K., Casero, D., Kropat, J., Cokus, S. J., Hong-Hermesdorf, A., Shaw, J., Karpowicz, S. J., Gallaher, S. D., Johnson, S., Benning, C., Pellegrini, M., Grossman, A., Merchant, S. S., Three acyltransferases and nitrogen-responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in Chlamydomonas. *J Biol Chem*., 287(19), 15811–15825. 2012.
9) Yang, D., Song, D., Kind, T., Ma, Y., Hoefkens, J., & Fiehn, O., Lipidomic Analysis of Chlamydomonas reinhardtii under Nitrogen and Sulfur Deprivation. *PloS one*, 10(9), e0137948. 2015.
10) Fakhry, E. M., & El Maghraby, D. M., Lipid accumulation in response to nitrogen limitation and variation of temperature in Nannochloropsis salina. *Bot Stud.*, 56(1), 6. 2015.
11) Rios, L. F., Klein, B. C., Luz, L. F., Jr, Maciel Filho, R., & Wolf Maciel, M. R., Nitrogen starvation for lipid accumulation in the microalga species Desmodesmus sp. *Appl Biochem Biotechnol.*, 175(1), 469–476. 2015.
12) Li, X., Moellering, E. R., Liu, B., Johnny, C., Fedewa, M., Sears, B. B., Kuo, M. H., & Benning, C. A galactoglycerolipid lipase is required for triacylglycerol accumulation and survival following nitrogen deprivation in Chlamydomonas reinhardtii. The Plant cell, 24(11), 4670–4686. 2012.
13) Bhatnagar, V. S., Saldat, M., Zienkiewicz, A., Du, Z. Y., & Zienkiewicz, K. Triacylglycerol synthesis in microalgae under abiotic stress: an updated integrative review. Plant & cell physiology, 67(8), 1263–1286. 2026.

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