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The Presence of Significant Methylotrophic Population in Biological Activated Carbon of a Full-Scale Drinking Water Plant

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Methylotrophs within biological activated carbon (BAC) systems have not received attention although they are a valuable biological resource for degradation of organic pollutants. In this study, methylotrophic populations were monitored for four consecutive seasons in BAC of an actual drinking water plant, using ribosomal tag pyrosequencing. Methylotrophs constituted up to 5.6% of the bacterial community, and the methanotrophs *Methylosoma* and *Methylobacter* were most abundant. Community comparison showed that the temperature was an important factor affecting community composition, since it had an impact on the growth of particular methylotrophic genera. These results demonstrated that BAC possesses a substantial methylotrophic activity and harbors the relevant microbes.

Keywords: Methylotroph, biological activated carbon, advanced water treatment, microbial ecology

Ozone treatment and granular activated carbon (GAC) filtration are generally used in combination for advanced water treatment in drinking water production. This combined process is applied to remove undesirable organisms and biodegradable compounds of the source water [19]. Ozone pretreatment converts natural organic substances of the source water into more biodegradable forms through a partial breakdown of their C-C chains [6, 18]. It also reduces the survival of indigenous microorganisms since ozone acts as a disinfectant [22]. GAC is colonized by indigenous microorganisms, resulting in biofilm formation on GAC. As the colonizing bacteria have a number of key functionalities, their presence results in biological activation. This system is generally termed as "biological activated carbon" (BAC). Biological degradation and physical adsorption of contaminants take place concurrently within BAC filtration systems [19, 21]. It is necessary to achieve a better understanding of the biological resources contained within BAC systems in order to enhance the performance and prevent the impairment of their function.

Supplied carbons are a key determinant of the microbial community composition of biofilm formed in a system since microbes capable of metabolizing them will proliferate [10]. BAC influent contains single carbon compounds, including formaldehyde and formate [2, 6]. Ozone treatment produces a variety of byproducts such as aldehydes and organic acids from naturally occurring carbons, resulting in an increase of their concentrations in BAC influent [22]. Thus, methylotrophic bacteria that utilize C_1 compounds as a sole carbon and energy source may be present in BAC. Some of them (*e.g.*, methanotrophs) co-metabolize a wide variety of important chemical pollutants [17]. However, methylotrophs within BAC systems have not received any attention to date, although they are surely a valuable component of BAC filtration systems if present. In the present study, it was hypothesized that BAC systems are able to degrade C_1 compounds owing to the activity of methylotrophs. The methylotrophic community and potential of a BAC filter in a full-scale drinking water treatment plant were monitored for four consecutive seasons.

BAC was obtained from the Yeongdeungpo drinking water treatment plant in Seoul, South Korea (N37.53° and E126.88°). The plant train consists of river water intake/screening, coagulation, flocculation, sedimentation, sand filtration, ozonation/GAC, and disinfection. The GAC filtration system (up to 250 km^3/day) is composed of 10

down-flow GAC contactors (empty bed contact time of 15.6 min), each with a 2.5-m-deep GAC layer (307.5 m^3 each) (F400; Calgon Carbon Co., Pittsburgh, USA). The backwash procedure (every 250–300 h) was as follows: Compressed air for 1 min, compressed air and water together for 1 min (air, 1.0 m/min; and water, 0.28 m/min), then clean water for 20 min (0.4 m/min).

BAC was collected from the fifth contactor in spring (May), summer (July), autumn (September), and winter (December) in 2011, representing seasonal samples from before and after backwashing. Four sampling spots were randomly selected on the BAC filter bed, and BAC was collected at a depth of 0–100 cm using a 1-m-long steel corer. Thus, there were four samples before and after backwashing per season. CO_2 evolution experiment and DNA preparation were performed immediately after sampling.

Although some organisms other than methylotrophs are capable of metabolizing formate [7], it was used as a C_1 substrate in this study, primarily due to its relatively high concentration in BAC influent [3, 6], as well as the toxicity of formaldehyde, the logical alternative. The methylotrophic activity potential of BAC was estimated by determining CO_2 evolution from formate. Ten grams of BAC were added into sterile bottles containing 15 ml of sterile tap water with either 0 or 100 mM sodium formate. Bottles were capped with butyl-rubbers and sealed with parafilm. They were agitated with 150 rpm at 20°C for 24 h. CO_2 concentrations at the headspace were measured using GC as described previously [8]. The background CO_2 concentration (control bottles, without formate) was subtracted from the CO_2 concentrations of sample bottles (with 100 mM formate) to calculate CO_2 production occurring from the added formate. Specific CO_2 production rates were measured ($n > 10$).

For DNA preparation, 50 g of BAC were added to a sterile 120 ml bottle containing 50 ml of sterile tap water. Ultrasonication (10 W for 3 min at 20 kHz) was delivered to the suspension using a Q500 ultrasonic processor (Qsonica LLC, Newton, USA), and the bottle was agitated at 250 rpm for 30 min. Suspensions (1 ml) were transferred to microtubes. They were centrifuged at 16,000 ×*g* for 5 min and the supernatants were discarded. DNA was extracted from the precipitates using the NucleoSpin Soil kit (Macherey-Nagel GmbH, Düren, Germany) as described previously [9]. Ribosomal tag pyrosequencing was performed for analyzing bacterial communities of BAC, as previously described [9]. There were 32 pyrosequencing libraries (sampling depth, 653–11,955 sequences; and average read length, more than 400 bp) after filtering [11]. The pyrosequencing libraries were deposited into the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (http://trace.ddbj.nig.ac.jp/dra) under the accession number DRA000829.

Sequence reads were taxonomically assigned using the RDP classifier of the RDP pyrosequencing pipeline with >50% bootstrap values (http://pyro.cme.msu.edu/). In this study, genera with the prefix "Methyl-" were considered to be methylotrophs. Since formate oxidizing bacteria other than methylotrophs are known to be metabolically versatile [4], it is difficult to link their phylogenetic affiliation to function. In addition, non-"Methyl-" aerobic formate oxidizing bacterial genera, such as *Pseudomonas*, *Moraxella*, *Paracoccus*, and *Mycobacterium*, with NAD^+-dependence formate dehydrogenase [4, 16] were seldom observed from the BAC. The Mantel test was performed using the zt program [1] with 10,000 permutations, to determine effects of time and water temperature on the community. The normalized weighted UniFrac distance matrix between communities was calculated using the online UniFrac tool [13]. A Mantel test was also performed to determine if there was a backwashing effect on the community in each time, for which pre- and post-backwashing samples were treated as 0 and 1, respectively, to produce the distance matrix. Bacterial communities were analyzed using principal coordinate analysis (PCoA) with the UniFrac matrix. Data were analyzed using a completely randomized block design (block, time; and treatment, backwashing) using SYSTAT software version 11 (Systat Software, Inc. Chicago, IL, USA). Linear relationships of abundances and time were determined using SigmaPlot version 10 (Systat).

This study was performed in parallel with a microbial community study, of which the results revealed that the BAC community was active and dynamic, although the system was consistently functional [11]. Water temperature, turbidity, pH, and total organic carbon (TOC) were measured seasonally. Turbidity, pH and TOC, did not significantly differ over time [11], whereas temperature varied substantially (Fig. 1A). In general, temperature is perhaps the most important determinant of microbial activity. Water temperature increased from spring to autumn, and then rapidly decreased in winter (Fig. 1A). The methylotrophic potential and abundance of methylotrophs differed seasonally ($p < 0.05$), while backwashing showed no effect on them ($p > 0.05$) (Figs. 1B and 1C). The methylotrophic potential was positively correlated with temperature before and after backwashing ($p < 0.05$; r = 0.85 and 0.69, respectively). This result is consistent with previous observations that there was a positive association between the biodegradation performance of BAC filtration and water temperature [5, 12,

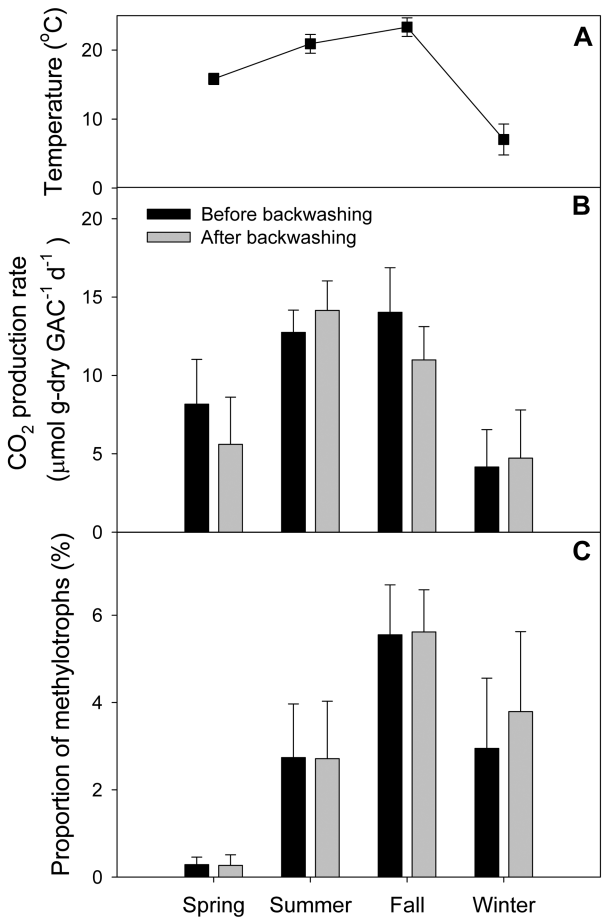

Fig. 1. Methylotrophic potential and relative abundance of methylotrophs.
(**A**) Water temperature; (**B**) CO_2 production rate from formate; and (**C**) proportion of methylotrophic bacteria in the total bacteria.

20]. The methylotrophic potential before backwashing increased from 8.2 μmol·g^{-1}·d^{-1} in spring to 14.0 μmol·g^{-1}·d^{-1} in autumn, and then rapidly reduced to 4.16 μmol·g^{-1}·d^{-1} in winter (Fig. 1B). The formate removal capacity of a single contactor BAC (307.5 m^3) was extrapolated to be up to 92.7 kg formate per day in autumn. Consistently, the methylotrophic proportion increased to 5.6% by autumn, followed by a reduction (Fig. 1C). Strong correlation was shown between the methylotrophic potential and proportion ($p < 0.05$; r = 0.80), and quantitative PCR of total bacteria indicated that the bacterial density increased from spring to summer, and did not change thereafter (data not shown). These results indicated that the methylotrophic density reflected the methylotrophic potential. Methylotrophic proportion before backwashing had a positive correlation with water temperature ($p < 0.05$; r = 0.51). These combined results demonstrated that BAC contains methylotrophs as an important inhabitant, and that these systems have of significant methylotrophic activity.

A total of 12 methylotrophic genera were observed in the BAC process (Table 1), and seven of them were methanotrophic, accounting for up to 48–100% of the methylotrophic community. Methanotrophic bacteria utilize methane as a sole carbon and energy source. Phylogenetic and physiological properties classify methanotrophs into types I and II groups, belonging to Gamma- and Alphaproteobacteria, respectively [17]. Type II members (*Methylocystis* and *Methylosinus*) were rarely observed within the BAC. The methanotrophic proportion increased rapidly from spring to summer and retained thereafter. *Methylosoma* was the most abundant, accounting for up to 64.4% of the community, followed by *Methylobacter* (21.5%). Interestingly, these most abundant genera are known to be obligate methanotrophs. Therefore, it was speculated that C_1 compounds other than formate, such as formaldehyde, might be used for a carbon source of the relevant bacteria in the BAC environment where methane production was limited. Community organization (diversity and evenness) varied seasonally, while backwashing did not affect the organization. Both diversity and evenness reduced from spring to autumn, with no other significant seasonal changes observed, and this trend of diversity and evenness was inverse to that of methylotrophic abundance. Therefore, water temperatures were associated with the growth of particular methylotrophic genera, resulting in a seasonal variation in the community organization. The temperature decline did not induce an immediate change of community organization, since community tolerance to temperature is greater when decreasing the temperature [15]. The relative abundances of *Methylibium*, *Methylophilus*, and *Methylosoma* varied over time, but the relative abundances of *Methylobacter* and *Methyloversatilis* were stable. The strong correlation between the methylotrophic density and activity indicated that the addressed methylotrophs were likely to be responsible for the methylotrophic activity, although their individual activities were not determined.

Water temperature is a significant factor to influence microbial community composition in drinking water biofilters [14]. Mantel test results indicated that backwashing had no effect on community composition, and hence, the backwashing effect was neglected for further Mantel tests. The degree of the dissimilarity between communities (*i.e.*, the change in the bacterial community) increased with time difference ($p < 0.05$; R = 0.43), but not with water temperature distance. The dissimilarity degree increased linearly by 7.5°C difference (from spring to autumn) (r = 0.57), then reduced with time,

Table 1. Relative abundances of known methylotrophic bacterial genera observed in the BAC (unit = %).

Season	Spring		Summer		Autumn		Winter	
Backwashing	Before	After	Before	After	Before	After	Before	After
Methylibium	6.3 ± 12.5	8.6 ± 11.4	n.d.	n.d.	n.d.	0.2 ± 0.4	0.1 ± 0.2	n.d.
Methylobacter[a]	2.8 ± 5.6	17.0 ± 6.8	23.7 ± 12.8	23.3 ± 19.0	13.6 ± 6.9	21.9 ± 6.4	40.8 ± 35.1	28.8 ± 23.5
Methylobacterium	n.d.	1.0 ± 1.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methylocaldum[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.4	2.0 ± 3.9
Methylocystis[a]	n.d.	3.3 ± 3.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methylomonas[a]	n.d.	n.d.	n.d.	n.d.	0.8 ± 1.5	n.d.	n.d.	0.3 ± 0.5
Methylophilus	32.3 ± 20.3	22.1 ± 7.6	3.0 ± 4.1	11.7 ± 9.0	0.0 ± 0.0	n.d.	1.2 ± 0.9	1.0 ± 1.5
Methylopila	6.3 ± 12.5	1.0 ± 1.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methylosinus[a]	n.d.	n.d.	n.d.	n.d.	0.8 ± 1.5	n.d.	n.d.	n.d.
Methylosoma[a]	45.4 ± 26.1	47.1 ± 15.3	72.7 ± 10.7	64.4 ± 20.7	83.9 ± 5.9	77.0 ± 5.4	57.3 ± 36.5	67.4 ± 28.6
Methyloversatilis	7.0 ± 9.3	n.d.	0.7 ± 1.3	n.d.	0.4 ± 0.8	0.2 ± 0.4	0.1 ± 0.2	n.d.
Methylovulum[a]	n.d.	n.d.	n.d.	0.6 ± 1.2	0.6 ± 1.2	0.7 ± 1.1	0.3 ± 0.6	0.5 ± 1.0
Sum	100	100	100	100	100	100	100	100
Methanotrophs	48.2	67.4	96.4	88.3	99.7	99.6	98.6	99.0
Diversity	0.99 ± 0.34	1.25 ± 0.29	0.65 ± 0.08	0.79 ± 0.26	0.50 ± 0.11	0.57 ± 0.04	0.55 ± 0.24	0.62 ± 0.35
Evenness	0.83 ± 0.22	0.85 ± 0.12	0.68 ± 0.21	0.67 ± 0.21	0.48 ± 0.14	0.57 ± 0.18	0.45 ± 0.08	0.55 ± 0.16

n.d., not detected.

[a]Methanotrophic genera.

because the temperature drop did not alter the community composition (data not shown), which was corroborated by the PCoA result of phylogenetic community assemblages (Fig. 2). Therefore, the dissimilarity could not be explained by a linear relationship with water temperature difference. Fig. 2 shows a temporal change in the methylotrophic community. The first and second axes of PCoA explained 62.3% and 28.9% of the total community variation, respectively. Bacterial communities were not distinctly grouped as seasonal groups based on water temperatures. The PCoA 1 provides a general indication that the temperature increase altered the community composition, but the temperature drop did not (Fig. 2A). PCoA 1 values varied over time ($p < 0.05$), while backwashing did not affect these values (Fig. 2B). The PCoA 1 value increased as the temperature increased, whereas the temperature decline did not affect the value as much as the temperature increase did. Thus, water temperature may be an important

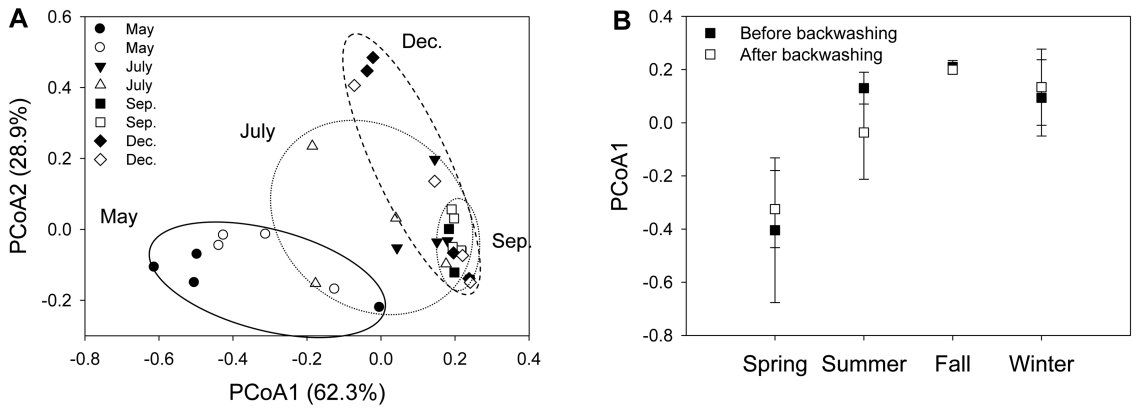

Fig. 2. Principal coordinate analysis of methylotrophic communities (**A**) and PCoA 1 values on sampling times (**B**).
Closed symbols, before backwashing; and open symbols, after backwashing.

factor influencing the methylotrophic activity and community of BAC.

BAC processes can possess a substantial methylotrophic activity, since they harbor dense and diverse populations of the relevant microbes. Backwashing is a common process to avoid excess biomass accumulation in BAC processes since biomass accumulation causes clogging as well as the proliferation of undesirable organisms [19]. Backwashing had no significant effect on methylotrophic potential, abundance, community organization, and composition.

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References

1. Bonnet E, de Peer YV. 2002. zt: a software tool for simple and partial Mantel tests. *J. Stat. Softw.* **7:** 1-12.
2. Can ZS, Gurol M. 2003. Formaldehyde formation during ozonation of drinking water. *Ozone Sci. Eng.* **25:** 41-51.
3. Carlson K, Amy G. 1997. The formation of filter-removable biodegradable organic matter during ozonation. *Ozone Sci. Eng.* **19:** 179-199.
4. Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME. 2009. The expanding world of methylotrophic metabolism. *Annu. Rev. Microbiol.* **63:** 477-499.
5. Fonseca AC, Scott Summers R, Hernandez MT. 2001. Comparative measurements of microbial activity in drinking water biofilters. *Water Res.* **35:** 3817-3824.
6. Hammes F, Salhi E, Köster O, Kaiser H-P, Egli T, von Gunten U. 2006. Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water. *Water Res.* **40:** 2275-2286.
7. Jormakka M, Byrne B, Iwata S. 2003. Formate dehydrogenase – a versatile enzyme in changing environments. *Curr. Opin. Struct. Biol.* **13:** 418-423.
8. Kim TG, Lee E-H, Cho K-S. 2013. Effects of nonmethane volatile organic compounds on microbial community of methanotrophic biofilter. *Appl. Microbiol. Biotechnol.* **97:** 6549-6559.
9. Kim TG, Moon K-E, Yun J, Cho K-S. 2013. Comparison of RNA- and DNA-based bacterial communities in a lab-scale methane-degrading biocover. *Appl. Microbiol. Biotechnol.* **97:** 3171-3181.
10. Kim TG, Yi T, Lee E-H, Ryu HW, Cho K-S. 2012. Characterization of a methane-oxidizing biofilm using microarray, and confocal microscopy with image and geostatic analyses. *Appl. Microbiol. Biotechnol.* **95:** 1051-1059.
11. Kim TG, Yun J, Hong S-H, Cho K-S. 2013. Effects of water temperature and backwashing on bacterial population and community in a biological activated carbon process at a water treatment plant. *Appl. Microbiol. Biotechnol.* [Online published]
12. Laurent P, Kihn A, Andersson A, Servais P. 2003. Impact of backwashing on nitrification in the biological activated carbon filters used in drinking water treatment. *Environ. Technol.* **24:** 277-287.
13. Lozupone C, Hamady M, Knight R. 2006. UniFrac – an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* **7:** 371.
14. Moll DM, Summers RS, Fonseca AC, Matheis W. 1999. Impact of temperature on drinking water biofilter performance and microbial community structure. *Environ. Sci. Technol.* **33:** 2377-2382.
15. Pettersson M, Bååth E. 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. *FEMS Microbiol. Ecol.* **45:** 13-21.
16. Popov VO, Lamzin VS. 1994. NAD^+-dependent formate dehydrogenase. *Biochem. J.* **301:** 625-643.
17. Semrau JD, DiSpirito AA, Yoon S. 2010. Methanotrophs and copper. *FEMS Microbiol. Rev.* **34:** 1-36.
18. Siddiqui MS, Amy GL, Murphy BD. 1997. Ozone enhanced removal of natural organic matter from drinking water sources. *Water Res.* **31:** 3098-3106.
19. Simpson DR. 2008. Biofilm processes in biologically active carbon water purification. *Water Res.* **42:** 2839-2848.
20. van der Aa LTJ, Rietveld LC, van Dijk JC. 2011. Effects of ozonation and temperature on biodegradation of natural organic matter in biological granular activated carbon filters. *Drink. Water Eng. Sci. Discuss.* **3:** 107-132.
21. Velten S, Boller M, Koster O, Helbing J, Weilenmann H-U, Hammes F. 2011. Development of biomass in a drinking water granular active carbon (GAC) filter. *Water Res.* **45:** 6347-6354.
22. von Gunten U. 2003. Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. *Water Res.* **37:** 1469-1487.