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Biodegradation Capacity Utilization as a New Index for Evaluating Biodegradation Rate of Methane

Kim, Tae Gwan[1], Taewoo Yi[1], Jeonghee Yun[1], Hee Wook Ryu[2], and Kyung-Suk Cho[1]*

[1]*Department of Environmental Science and Engineering, Ewha Womans University, Seoul 120-750, Korea*
[2]*Department of Chemical Engineering, Soongsil University, Seoul 156-743, Korea*

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Density of catalytic organisms can determine the biodegradation capacity and specific biodegradation rate (SBR). A new index, biodegradation capacity utilization (BCU, %), was developed for estimating the extent of actual biodegradation of a gas compound over the full capacity. Three methanotrophic cultures were serially diluted (1–1/25), and methane SBR and BCU were measured. Consistently, biomass reduction increased the SBR and decreased the BCU. Linearity ($p < 0.05$, $r > 0.97$) between the BCU and cell density indicated the reflection of biodegradation capacity by BCU. Therefore, BCU is indicative of whether the density of catalytic organisms is pertinent for SBR evaluation of low-soluble gaseous compounds.

Key words: Biodegradation, low soluble gases, methane oxidation

Methane-oxidizing bacteria, methanotrophs, have been extensively studied in many different fields as they are integral to global carbon cycling and remove chemical pollutants [2, 5]. The specific biodegradation rate (SBR, biodegradation·time^{-1}·biomass^{-1}) is commonly used for evaluating methanotrophic abilities of cultures and environmental samples [1, 4, 7, 10–12]. The density of catalytic organisms can determine the biodegradation capacity and affect the SBR, owing to being the divisor. Conventional SBR measurement of low-soluble gaseous compounds can lead to an underestimation if the biodegradation capacity is greater than the gas–liquid mass transfer, since the mass transfer is the rate-limiting step [8]. Thus, it is necessary to adjust the inoculum density to an appropriate level, where its capacity does not exceed the extent of the mass transfer. SBR measurement is time-consuming and may increase the chance of experimental error occurrence if the biodegradation capacity is too low. Therefore, estimation of the biodegradation capacity may allow for determination of the SBR without significant biases.

The main objectives of this study were to develop and evaluate an index to estimate biodegradation capacity. The term capacity utilization, used in economics, was adopted to calculate the biodegradation capacity of a given biomass in this study. Capacity utilization refers to the extent to which a unit actually uses its installed productive capacity during a certain time period [6]. In this study, biodegradation capacity utilization (BCU) refers to the extent to which catalytic microbes actually consume a gas compound as their substrate in a system for a time period. Effects of biomass on methane SBR and BCU were evaluated and their relationship was determined.

Methanotrophic cultures including *Methylocystis* sp. M6 and two methane-oxidizing consortia were used. M6 was originally isolated from a landfill site [11]. It was maintained in nitrate mineral salts (NMS) medium, supplemented with methane as the sole carbon and energy source [13]. Serum bottles (600 ml) containing 50 ml of M6 culture were capped with butyl rubber septa, and sealed with parafilm. Methane (99.9%) was injected to a final concentration of 5% (v/v) using a 50 ml syringe. Gases were periodically sampled from the bottles using a 1 ml gas-tight syringe. Methane concentrations were monitored using gas chromatography [10].

Methane-oxidizing consortia were obtained from lab-scale methanotrophic biofilters with tobermolite and perlite as filter bed materials for immobilizing the methanotrophs. The biofilters were operated with an inlet methane concentration of 5% for 50 days. Tobermolite and perlite were collected from the biofilters. A 10 g sample was added to a 50 ml sterile conical tube containing 20 ml of sterile saline solution (0.9%). Ultrasonic pulse was applied for 5 min with 10 W

**Corresponding author*
Phone: +82-2-32772393; Fax: +82-2-32773275;
E-mail: kscho@ewha.ac.kr

at a fixed frequency of 20 kHz using a Q500 ultrasonic processor (Qsonica, Newton, USA). The samples were washed twice with NMS medium. Methane-oxidizing consortia were designated as TC and PC, obtained from tobermolite and perlite, respectively.

The three methane-oxidizing cultures (258, 5,625, and 5,850 mg-DCW·l^{-1}, M6, PC, and TC, respectively) were serially diluted in NMS from 1/1 to 1/25. Four milliliters of the dilution series was added to 120 ml serum bottles (n = 3), capped with butyl rubber septa and sealed with parafilm. Methane (99.9%) was added to a final concentration of 5%, then the bottles were incubated at 30°C with agitation of 180 rpm. Methane concentrations were monitored as mentioned above. Biomass was measured as follows: M6 biomass was spectrophotometrically determined, using a standard curve between the optical densities measured at 600 nm and g-dry cell weights (DCW). For cell weights of the consortia, dry cell weights were measured.

Methane SBR and BCU were quantified in each dilution series. First of all, the apparent methane removal rate (AMRR) is calculated by the following equation: AMRR = $(M_{t=0} - M_t)/\Delta t$ (M, methane concentrations at the headspace). Methane SBR is expressed as follows: SBR=AMRR·$biomass^{-1}$. BCU is a ratio of AMRR to the theoretically maximum methane removal rate (TMMRR) for a given time (BCU=AMRR/TMMRR×100). TMMRR is calculated by the following equation: TMMRR = $(M_{t=o} - C_g)/\Delta t$ (C_g, the theoretical concentration of remained methane at the headspace after a given time). C_g is calculated using the equation [14]

$$\frac{1}{C_g} \cdot \frac{dC_g}{d_t} = -K_L a \cdot \left(R \cdot \frac{T}{H}\right) \cdot \frac{V_L}{V_g}$$

where C_g is the methane concentration in the gas phase (ppm), $-K_La$ is the volumetric mass transfer coefficient of methane·h^{-1} [9], R is 0.082 (atm·l·mol^{-1}·K^{-1}), T is the temperature in kelvin of the experimental system, H is the Henry's law constant for methane (637 atm-l·mol^{-1}) [3], V_L is the volume of the liquid phase (L), and V_g is the volume of the gas phase (L).

Methane removal was monitored over a 70 h period (Fig. 1). All cultures revealed that reduction of inoculum biomass by dilution decreased the speed of methane oxidation, and complete methane oxidation was observed in 1/1 and 1/5 dilutions. Insufficient methanotrophic biomass did not complete the removal of CH_4 gas at the headspace within the experimental time in dilution series more than 1/5. SBR was calculated for the first 15 h, during which the undiluted cultures showed the highest SBR (Fig. 2). The SBR of the undiluted M6 culture was 11.53 ± 0.61 mmol·g-DCW^{-1}·h^{-1} with 95.69 ± 3.84 BCU. This SBR differed from our previous report, up to 4.93 mmol·g-DCW^{-1}·h^{-1} [11], suggesting that methane SBR cannot be compared with each other unless experimental conditions (*i.e.*, biomass and gas concentrations) are identical. The undiluted PC and TC showed 0.54 ± 0.03 and 0.58 ± 0.01 mmol·g-DCW^{-1}·h^{-1} with 100.75 ± 2.83 and 108.26 ± 1.32 BCU, respectively. Since the undiluted consortia reached the full capacity, biomass accumulation would no longer increase their SBR and change their BCU.

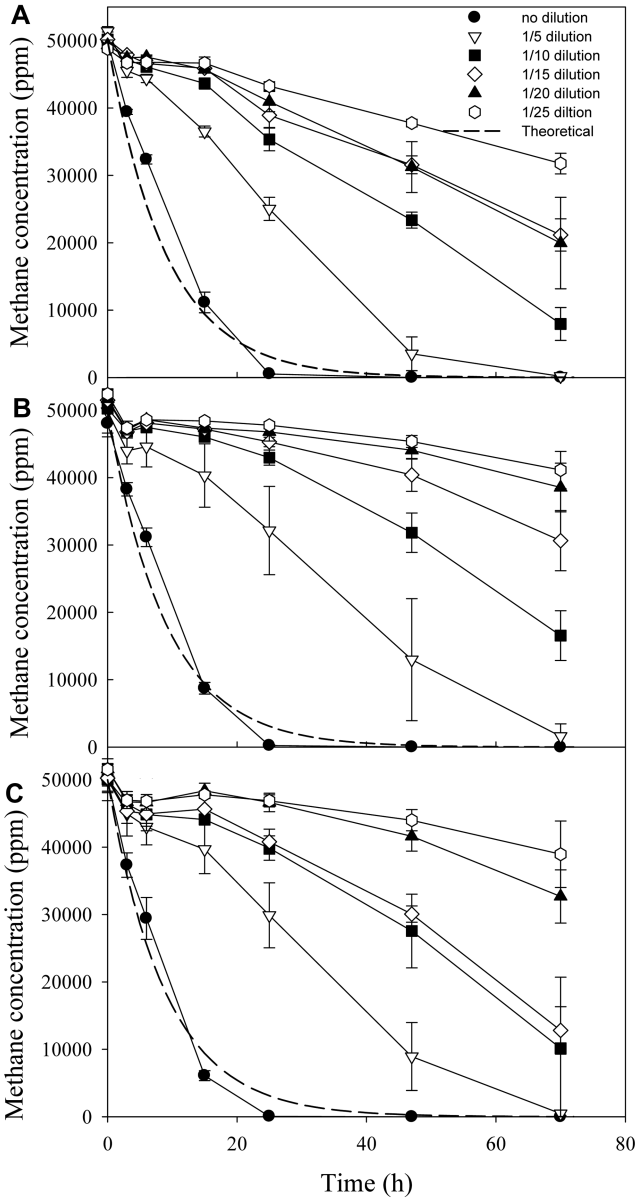

Fig. 1. Effects of biomass on methane oxidation.
(**A**) Methane oxidation by *Methylocystis* sp. M6; (**B**) PC consortium; and (**C**) TC consortium. Bars indicate the standard deviation of the mean (n = 3).

As expected, all methanotrophic cultures showed that the biomass reduction increased the SBR while it reduced the BCU. There was a significant linear relationship ($p < 0.05$ and $r > 0.97$) between the biomass and BCU. However, BCU below 10 did not reflect the biomass

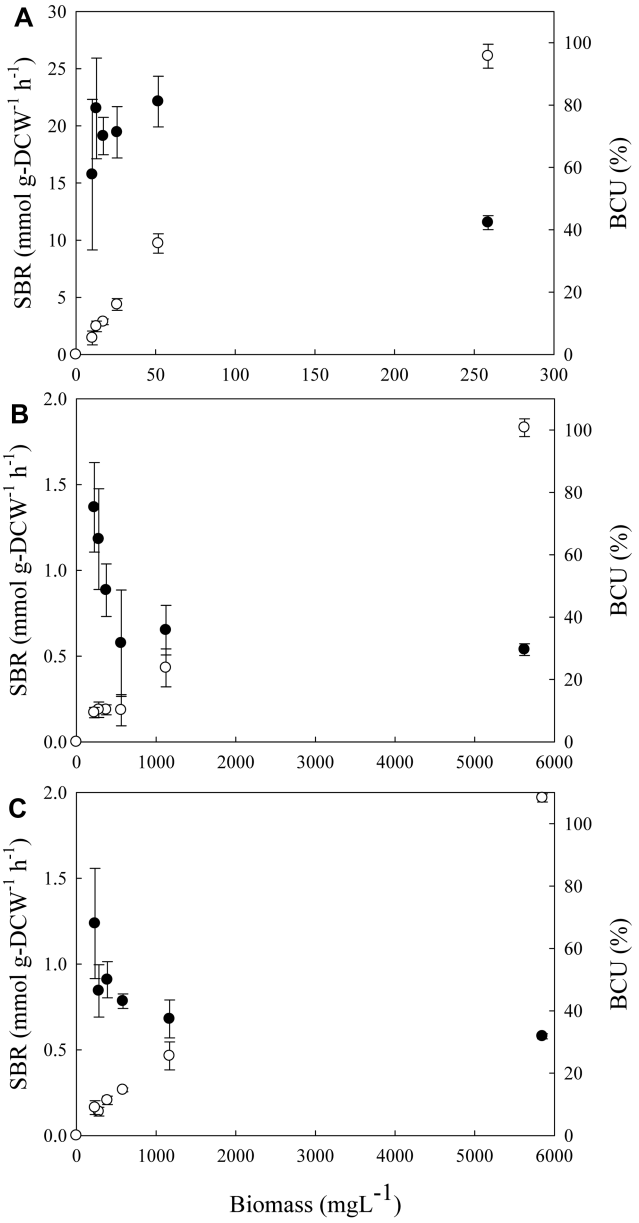

Fig. 2. Effects of biomass on specific biodegradation rate (SBR) and biodegradation capacity utilization (BCU).
(**A**) *Methylocystis* sp. M6; (**B**) PC consortium; and (**C**) TC consortium. Bars indicate the standard deviation of the mean (n = 3). ●, SBR; ○, BCU.

change in TC. These results indicate the importance of inoculum density in SBR measurement. The SBR peaked at 22.12 ± 2.21 mmol·g-DCW^{-1}·h^{-1} (21.74 mg-DCW·l^{-1}, 1/5 dilution), with 35.62 BCU, which was the maximum SBR (SBR_{max}), and then did not change with dilution in M6. The SBR_{max} values of PC and TC were 1.36 ± 0.26 and 1.24 ± 0.32 mmol·g-DCW^{-1}·h^{-1} (225 and 234 mg-DCW·l^{-1}, 1/25 dilution) with 9.38 and 8.96 BCU, respectively. The patterns of the BCU vs. SBR of TC and PC were highly similar to each other. The SBR of the undiluted PC and the SBR_{max} did not differ from those of TC, suggesting similar methane-oxidizing characteristics of the consortia depending on the biomass.

The SBR_{max} values were observed from the three cultures at below 36 BCU. However, the biomass reduction showed an increase in variance of SBR. For instance, the variance of SBR increased from 0.37 to 43 as the BCU decreased from 96.69 to 5.33 in M6. It is noteworthy that the BCU reduction by dilution increases the chance of experimental error occurrence. A exponential decay function ($Y = a*e^{(-b*x)}$) was used to determine the relationship between the BCU and SBR when BCU was more than 10, since BCU below 10 could not reflect the biomass reduction. The model equation was fit to the data using SigmaPlot (Systat Software, San Jose, USA). Correlation coefficients were 0.78 ($p < 0.05$), 0.68 ($p < 0.05$), and 0.64 ($p = 0.07$) for M6, PC, and TC, respectively, indicating the SBR reduction with BCU. Therefore, inoculum cell density should satisfy less than 100 but be at least more than 10 BCU for proper SBR measurement. In conclusion, BCU is a novel concept for estimating the biodegradation capacity of low-soluble gaseous compounds, which is useful for determining SBR with appropriate biomass.

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