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Short Communication

Relation of microbial biomass to counting units for *Pseudomonas aeruginosa*

Dong-ju Kim[1], Seung-gun Chung[1,2], Sang-hyup Lee[2] and Jae-woo Choi[2]*

[1]Department of Earth and Environmental Sciences, Korea University, Seoul 136-701, Republic of Korea.
[2]Center for Water Resource Cycle Research, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea.

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In this study, we investigated the relationship between cell counting units and biomass for *Pseudomonas aeruginosa* since microbial parameters often need to be defined as mass rather than colony forming unit (CFU) or optical density (OD) which is easily measurable using plate counting method or spectrophotometer especially for any attempt in modeling of contaminant transport and biodegradation in aquifer systems. Results showed that 1.0 OD corresponded to 2.04×10^8 CFU/ml and 2.085 mg/ml with well defined linear relationships of Y (CFU/ml) = 2.0×10^8 X (OD_{600}) + 4.0×10^6 and Y (mg/ml) =2.0087 × (OD_{600}) + 0.0764.

Key words: Cell counting unit, colony forming unit, optical density, *Pseudomonas aeruginosa.*

INTRODUCTION

Contaminant transport coupled with microbial degradation in the subsurface environment has attracted a considerable attention because of its importance in bioremediation of contaminated groundwater. Numerous studies have been performed through a theoretical modeling or experimental approach to predict the degradation potentials of indigenous microbial populations in aquifer systems. In order to understand and quantify the biodegradation capabilities, numerical modeling have been attempted under various conditions assumed (Abriola and Chen, 1995; Schirmer et al., 2000). The most well-known numerical code is BIO3D which solves for the advective-convective transport and biodegradation of multiple organic substrates with various bacterial growth kinetics. The main components of the code consist of governing equations for the substrates, electron acceptor, and the microbial populations. In order to accurately perform the modeling task, it is impending to obtain correctly estimated physicochemical and biological parameters. Physicochemical parameters include porosity (L^3/L^3), permeability (L/T), dispersivity (L) and distribution coefficients (L^3/M) while biological parameters consist of mainly initial biomass (M/L^3), maximum specific growth rate (1/T), half-saturation constant of substrate and oxygen (M/L^3), and yield coefficients (M/L^3) of bacterial cells. Among the microbial parameters, determination of initial biomass and yield coefficients requires expression of biomass in the unit of weight. However, most widely available methods for measurements of bacterial populations are direct cell counts using either DAPI staining solution (Yu et al., 1995) or AODC (direct counting acridine orange) (Ghiorse and balkwill, 1983), plate counting method and method of measuring optical density using a spectrophotometer. For instance, Schirmer et al. (1999) used DAPI cell count method to determine yield coefficient of an undefined aquifer microbial population. Since the cell count should be converted to biomass gram or milligram, they used a conversion factor of 1.72×10^{-10} mg/cell (Balkwill et al., 1988) which was derived from application of the AODC method to coccobacillary bacteria present in subsurface aquifer sediment. According to Balkwill et al. (1988), the conversion factor was dependent not only measurement

*Corresponding author. E-mail: plead36@kist.re.kr. Tel: +82 2 958 5820.

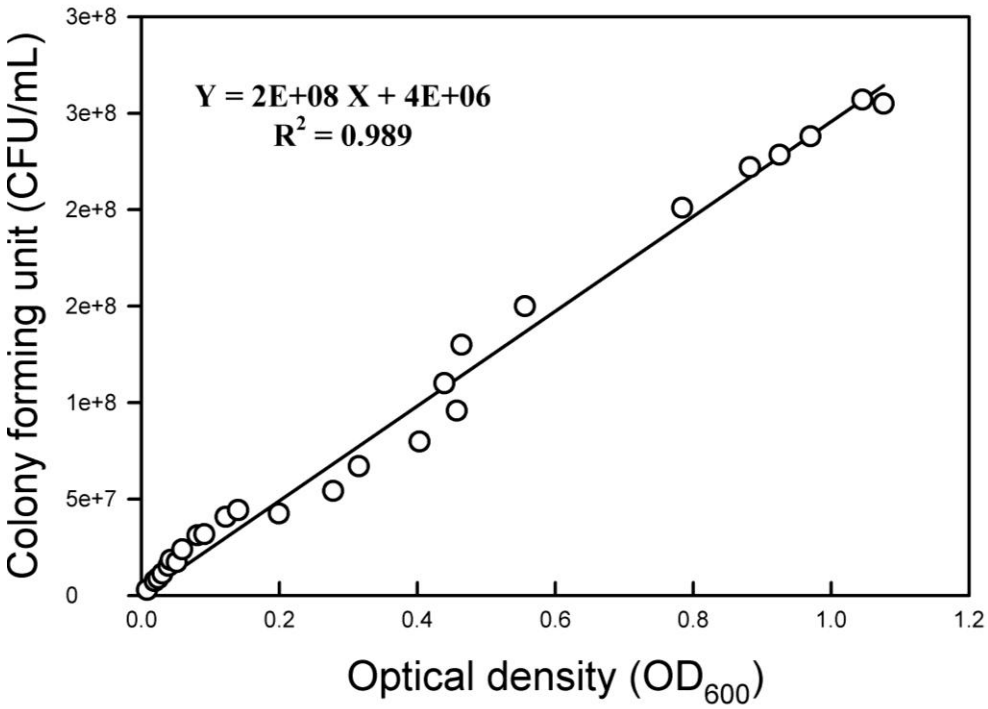

Figure 1. Relationship between CFU and optical density of *Pseudomonas aeruginosa.*

methods used but also bacterial species.

Therefore in this study we attempted to investigate a relationship between different bacterial population units such as colony forming unit (CFU), optical density and biomass for a given bacterial strain of *Pseudomonas aeruginosa* which is known as a benzene and toluene degrading microorganism.

MATERIALS AND METHODS

Bacterial strains *P. aeruginosa* KCCM-40269 was obtained from Korea Culture Center for Microorganisms, Seoul, Korea. The strain have been known as benzene-degrading bacteria (Kim et al., 2005) and cultured on Luria-Bertani (LB) medium in 1 L flask. To eliminate the bacterial adaptation period in the biodegradation experiments, *Pseudomonas* spp. were pre-adapted to aqueous benzene concentrations ranging from 100 to 600 mg/L. The adapted cells in the late exponential growth phase were harvested by centrifugation at 10,000 rpm, 4°C for 15 min and then resuspended in fresh MSM and washed twice. The composition of minimal salt medium (MSM) described in our earlier study was used as the culture medium (Kim et al., 2005). After this, the cells resuspended in MSM and serially diluted in the range of 10^{-1} to 10^{-10}. Bacterial concentrations for one part of the duplicate of the diluted cells were determined by UV-Visible spectrophotometer (Heyios ß, Thermo–Electron Corporation) at 600 nm by measuring the absorbance of the bacterial solutions and used for determination of the colony forming unit (CFU) by serial dilutions of bacterial culture while the second part of the duplicate cells was used for determination of biomass by centrifuge at 10,000 rpm for 15 min, collection of pellet, and resuspension in distilled water. After the pellet was centrifuged again 10,000 rpm for 15 min, biomass was collected and kept in a hot air oven to get dry weight.

RESULTS AND DISCUSSION

The measured data on the relationship between CFU/ml and OD for *P. aeruginosa* is shown in Figure 1. The bacterial cells showed almost a linear relationship with a slope of 2.0×10^8 CFU/mL/OD indicating that 1.0 OD corresponds to 2.04×10^8 CFU/mL. The relationship between biomass and OD is shown in Figure 2a. This relation was also represented by a linear regression line with Y=2.0087 X + 0.0764. It can be inferred that 1.0 OD of bacterial solution approximately refers to 2.085 mg biomass/ml. This value is relatively higher than 0.281 mg/mL (Pequignot et al., 1998) for *Pseudomonas fluorescens* but similar to the results of 1.27 mg/mL (Joannis et al., 1998) for *P. aeruginosa*. Since the optical density of a bacterial solution can be easily determined using a commercially available spectrophotometer, the equation relating to OD to mg/ml would provide useful information on biomass which is essential for expression of bacterial concentrations in dry weight such as initial biomass and yield coefficients commonly used in BIO3D. Finally based on these two relationships, we derived a relationship between CFU/mL and biomass. As was shown in Figure 2b, a slope of 1.0×10^8 CFU/mg was obtained indicating that the number of dry cells per gram dry weight was 1.0×10^{11} cells/g or 1.0×10^{-11} g/cell. These values are slightly lower than the literature values of 6.4×10^{11} cells/g (Gray et al., 1974), 4.3×10^{12} cells/g (Holme, 1957), 2.5×10^{12} cells/g (Lund and Goksoyr, 1980), 2.08×10^{12} cells/g (Bratbak and dundas, 1984) and 1.72×10^{-13} g/cell (Balkwill et al., 1988). This value was slightly underestimated compared to the literature values. The lower estimation may be attributed to the difference in measuring

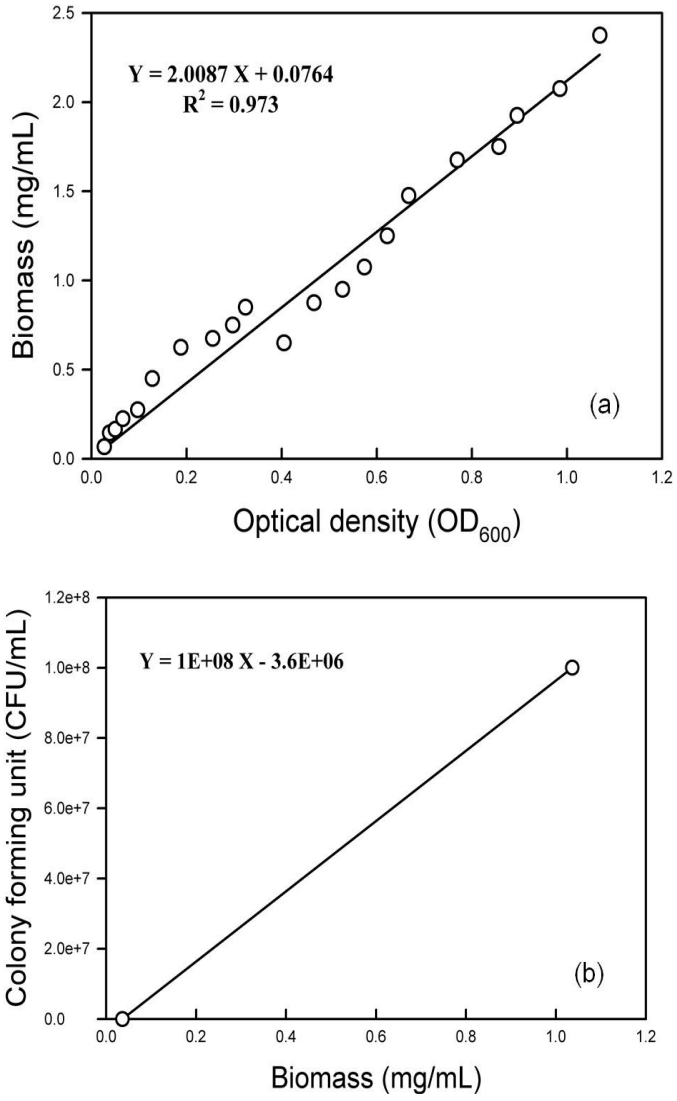

Figure 2. Relationship between (a) biomass and optical density; (b) CFU and biomass of *Pseudomonas aeruginosa.*

methods of cell counts and morphological nature of bacterial species. These relationships would be useful when modeling coupled transport and biodegradation of organic contaminants in aquifers because cell concentrations in water samples are easily determined by spectrophotometer or plate counting methods.

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