II －92 PROCEDURES FOR MONITORING METHANE PRODUCTION RATE OF METHANOGENS IN WET LAND SEDIMENTS

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Abstract

Analyses are presented for measuring the biodegradability (Methane Production Rate-MPR) to wetland sediments. These relatively simple procedure can be conducted in most research laboratories without the need for sophisticated equipment. MRP is a measure of substrate biodegradability determined by monitoring cumulative methane production from a sample (wet land sediment) which is anaerobically incubated in a chemically defined medium. The MPN of samples of Izunuma lake.

Introduction

Atmospheric methane concentrations are increasing globally. This temporal increase begane *150-200* years ago, and the rate of increase has accelerated in recent decades(Bingemer and Crutzen, 1987). It is important to find out the reason for these global changes through accurate measurement and predictions of methane concentration while taking into consideration chemical effects and climatic conditions. In addition, atmospheric methane concentrations have been rising over the past three decades at an average rate of approximately $1\sim1.2\%\ yr^{-1}$ (Rasmussen et al., 1984). Recently, reserchers have begun to estimate the strength of various sources and sinks of methane to the global atmosphere, which has a total burden of about *4,900 Tg*(Watson et al., 1990); but there are a large range of error (*10% - 100%*) in current estimates. For the aim of estimating methane production rate with accuracy, the method of anaerobic microbiology will be applied in this study.

Materials and Methods

Sediments sampling

Sediments are taken by a plexiglas tube (length *50cm*; ID *31mm*) from lake. After thrown the tube into *30-cm* depth sediment, the top of tube is sealed with a silicon cap. The sediments will be sampled out while the tube are thrown out sediments. The other top of tube is also sealed a cap to prevent the sediments in contact with air.

Preparation of assay bottles

The MPR test is conducted with *1,000mL* reagent vials ($1{,}012\pm3\ mL$ actural volume with serum cap in place). Vials are gassed with a miture of *20%* CO_2 and *80* N_2 at a flow rate of approxiately $0.2\ min^{-1}$ for 5 min.

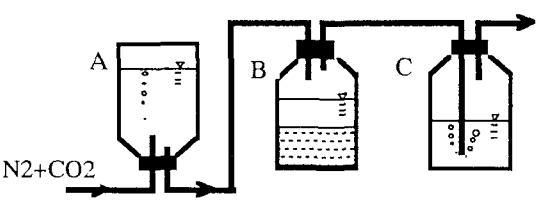
Composition of media

Concentrated stock solutions are used for preparing the media and are stored at *4°C*. The media contains nutrients and vitamins for mixed anaerobic cultures and the procedure for preparing 1 liter of media as adopted from(Li, 1989).

Anaerobic transfers

The media is equilibrated to assay temperature, inoculated, and transferred into vials. For the MRP assay, inoculation is accomplished anaerobically by inserting a gas flushing needle into the media vial.

The anaerobic transfer of media is shown in Fig. 1. Assay vials, gas tubing, and transfer tubing are initially gassed, and samples are added to vial (B) and equilibrated to the inocubation temperature. Media is anaerobically pipetted by opening and closing the guage of cylinder at *1.2 atm* with *20:80* volume ratio of CO_2:N_2. When the assay vial is filled to the appropriate volume, removing the media transfering and gas needles. After equilibration for *30 min* at the



Note: A, Nutrition and substrate, 1-Lvial.
B, Sediment and substrate what want to culture, 1-L vial.
C, Water seal.

Fig. 1 Anaerobic transfer apparatus for batch culture. Each vials was one liter. A was containted nutrition and substrate; B was sediment and the solution what A had transfered; C was water seal to prevent the air feedback to vial of B.

incubation temperature, gas volumes are "zeroed" with a syring and the vials are ready for inocubation and sampling.

Gas measurement

Gas-volume sampling and removal during inocubation is performed with glass syringes (*5-50 mL* depending on gas volume) equipped with 20-gauge needles. The sample syringe is initially flushed with the CO_2:N_2 gas mixture and lubricated with deionized water. Readings are taken at the inocubation temperature and the syringe is held horizontal for measurement. Volume determinations are made by allowing the syringe plunger to move and equilibrate between the vial and atmopheric pressures. Readings are verified by drawing the plunger past the equilibrium point and releasing; the plunger should return to the original equilibration volume. In order to continue the assay, gas can be removed for wasting.

Gas analyses

CH_4 and CO_2 are separated on a gas chromatograph (Shimadzu 8A) with Porapak T and Porapak Q 50/80 as a column material. The operating temperatures for the injection port, the oven and FID detecter are: *120, 80, and 120 °C*. The carrier gas was pure N_2 at a flow rate of *30 mL/min.*

After separation, CH_4 and CO_2 are analyzed with a flame ionization detector via a preceding conversion with a methanizer (Shimadzu MTN-1). The chromatogram are analyzed with a Shimadzu computing integrator. The detection limit was *1 ppm* for CH_4 and CO_2. High CH_4 and CO_2 gas concentration above *2,000 ppm* are determined on a Shimadzu 8A gaschromatograph with a thermal conductivity dector and Activated Carbon 60/80 as the column material the temperature is *120°C*, and the carrier gas is *He.*

Water analyses

Soluable substrates and fermentation products are acidified with H_3PO_4 (*1N* final) subseguently analyzed on a Shimadzu 8A gas chromatograph equipped with a KOCL-FM (60/80 mesh) column and a flame ionization detector. The column are operated at *140°C*. The detection limit are approximately *5 ppm* for acetate, propionate, and butyrate. COD is determined by a modification of standard method. The dectection limit is approximately *5 ppm.*

Results and Discussion

The methane production test for places of Izunuma p1, p2, and p3 are presented in Figure 2. These three places all followed the same trend of a rapid increase in methane production rate. The decomposition of COD in the samples also is inlustrated in the Figure 2. As observed here, cumulative methane production are 38 mL, 28 mL, and 27 mL as well as COD decomposition, from135 mg/L to 25mg/L, 59 mg/L, and 60 mg/L, need about one month. Therefore, there are 81% to 89% recovery appeared in this study. That is to say, there are 11% to 19% of error in the procedureof monitoring methane production rate of methanogens in wetland sediments. In addition, the relationship of Monod, -ds/dt=Rm*S/Ks+S, is also applied to this study and summarized in Table1. Since the COD concentration of water body is very low (less than 5 mgCOD/L), the activity of methanogenic bacteria also shows in a low level (1.7 *10^{-4} to 6.0*10^{-4} h^{-1}). Ks values are 158, 466, and 394 mgCOD/L, show in a reasonable range.

Table 1 The results of kinetic parameters of wetland sedments

Place	Rm, 1/h (10^{-4})	Ks, mgCOD/L
p1	1.7	158
p2	4.8	466
p3	6.0	394

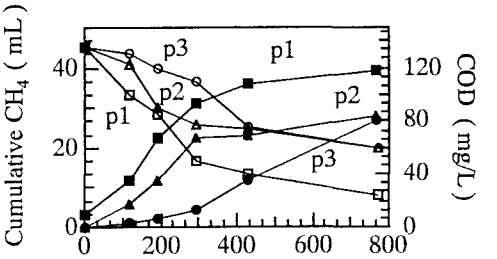


Fig. 2 The results of MPR test

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