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Simultaneous methanogenesis and phototrophic bacterial growth in relatively dry sewage sludge under light

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Anaerobically digested sewage sludge with a variety of moisture content, namely 81%, 86%, 90% and 98%, were anaerobically cultured at 35°C under light. Phototrophic bacteria grew in the 86% moisture sludge (bacteriochlorophyll *a*, 0.46 g/L), 90% sludge (bacteriochlorophyll *a*, 0.36 g/L) and 98% sludge (bacteriochlorophyll *a*, 0.04 g/L) with methane production. Phototrophic bacteria could not grow in the 81% moisture sludge (bacteriochlorophyll *a* 0.004 g/L). Phototrophic bacteria could assimilate about 46% of the extracellular ammonium in the 90% moisture sludge. Phototrophic bacteria utilized organic compounds competing with methanogens; therefore, methane yield from the 90% moisture sludge under the light conditions was lower than that under the dark conditions. Phototrophic bacteria could grow in anaerobically digested sludge with relatively low moisture content, and assimilated extracellular ammonium in the sludge. The quality of digested sludge with phototrophic bacterial biomass for fertilizer could be improved compared with that without phototrophic bacterial biomass.

Key Words——ammonia; anaerobic digestion; phototrophic bacteria; sewage sludge

Introduction

The recycling of organic wastes is required from the viewpoint of environmental issues. Sewage sludge makes up about 30% of the organic wastes in Japan, and at present, the ratio is increasing (Study group on bio-waste recycling, 1999); therefore, it is important to treat and utilize the sewage sludge. Conventional treatment methods such as land filling and ocean dumping have been regulated to protect environmental water.

Anaerobic digestion produces energy in the form of biogas and fertilizers; therefore, it is one of the most effective treatment systems for wet organic wastes. There are lots of reports concerning the anaerobic digestion of organic wastes with relatively high moisture contents (Salminen and Rintala, 2002). Organic wastes from municipal waste have been evaluated using anaerobic digestion by a number of researchers (Held et al., 2002; Mata-Alvarez et al., 2000). In general, anaerobic digestion at a relatively higher organic concentration has an advantage compared with that of a lower concentration. Dry anaerobic digestion has been found to produce a larger volume of methane than wet digestion without wastewater treatment (Lissens et al., 2001). Dewatered sewage sludge contains a high concentration of nitrogen, and ammonium was released during anaerobic digestion. A high concentration of ammonium in degrading dewatered sewage sludge is thought to inhibit the methanogenesis (Fujishima et al., 2000). Lay et al. reported that methanogenic activity dropped from 100% to 53%

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Table 1. Characteristics of methanogenic sludge with different moisture contents.

	Moisture content (%)	Volatile solid content (g/wet g)	Ammonium concentration(mg N/L)	DOC concentration (mg/L)
MC81 sludge	80.5	0.132	2,568	832
MC86 sludge	87.2	0.098	1,836	549
MC90 sludge	89.1	0.087	1,520	217
MC98 sludge	98.3	0.011	591	40

when the moisture content decreased from 96% to 90% (Lay et al., 1997). There are a few reports about the anaerobic digestion of dewatered sewage sludge (Fujishima et al., 2000; Poggi-Varaldo et al., 1997). Although ammonium is a nutrient for bacteria and archaea involved in the anaerobic digestion process, it inhibits methanogenesis at concentrations exceeding approximately 50 mmol (Koster and Lettinga, 1984). If protein is the substrate, a high ammonium concentration suppresses the biological reactions of methanogenesis. This may lead to a failure of methane production and to an accumulation of fatty acids and hydrogen (Bryant, 1979; Winter, 1984). To effectively digest a high concentration of sewage sludge, we have to control ammonium inhibition against methanogenesis.

Phototrophic bacteria have been studied for use in organic wastewater treatment (Sawada and Rogers, 1977). Phototrophic bacteria can be grown under anaerobic conditions and can utilize ammonium as the nitrogen source for their growth (Johanson and Gest, 1976). Sawayama et al. (1999) reported that phototrophic bacteria successfully grew and reduced ammonium levels under light conditions during the upflow anaerobic sludge blanket treatment of wastewater. There is a possibility of removing ammonium from dewatered sewage sludge under anaerobic-light conditions; however, there are no reports about the anaerobic digestion of dewatered sewage sludge under light conditions.

In this study, we investigated the growth of phototrophic bacteria, the concentration of ammonium and the production of methane gas during the anaerobic digestion of dewatered sewage sludge under light and dark conditions.

Materials and Methods

Anaerobic digestion under light and dark conditions. Anaerobically digested and dewatered sewage sludge was collected from the sewage treatment plant in Ibaraki, Japan. The dewatered sewage sludge which was collected contains 81% moisture. To change the moisture content of the sludge, the dewatered sludge was diluted using distilled water. The characteristics of the diluted sludge, moisture content, volatile solid (VS), ammonium and dissolved organic carbon (DOC) were determined for different dilution conditions (Table 1). In each reactor, 20 g of diluted sludge was placed in a 100 ml glass vial (diameter 3.5 cm, height 12.5 cm, columned vial), and then sealed with a rubber septum retained with a thin aluminum cover-cap for the anaerobic conditions and a plastic syringe was injected into the vial for gas yield measurement. Nitrogen gas was added to remove the air from the glass vial. The vials were then placed in a thermostated incubator at 35°C. The vials were kept at a light intensity of 1,200 lux (which was measured at the bottom of the reactor) using incandescent lamps for 2 weeks. The vials which were kept in the dark were covered with aluminum foil, then placed in a thermostated incubator. The vials were agitated once a day.

Isolation and determination of phototrophic bacteria. The sludge was laid over the PE plates for isolation of phototrophic bacteria. The PE plates contained the following chemicals per liter: sodium glutamate 0.5 g, sodium succinate 0.5 g, sodium acetate 0.5 g, yeast extract 0.5 g, casamino acids 0.5 g, $Na_2S_2O_3 \cdot 5H_2O$ 0.5 g, KH_2PO_4 0.38 g, K_2HPO_4 0.39 g, $(NH_4)_2SO_4$ 0.5 g, vitamin mixture 1 ml, basal salt solution 5 ml and agar. The plates were placed in 35°C incubator under light and anaerobic conditions. After incubation, a red and round colony was picked up, and transferred to another plate several times. Pure culture was grown with a PE medium which did not contain agar. The isolated bacterial cells were collected by centrifugation, and DNA extraction was done by using a FastPrep kit (Bio 101, Vista, CA, USA) according to the manufacturer's instructions. Specific DNA fragments of the 16S rRNA

gene were amplified by the polymerase chain reaction (PCR) with the primer set, Euba0010S20 (5′-AGAGTTTGATCCTGGCTCAG-3′) and Univ1500A19 (5′-GGTTACCTTGTTACGACTT-3′) (Alm et al., 1996; Lane, 1985). The PCR program consisted of 15 cycles each of 1 min at 95°C, 1 min at 50°C and 2 min at 72°C. The PCR amplification and cloning were performed as described by Sekiguchi et al. (1998). The PCR product was then used for cloning according to the manufacturer's protocol (TA cloning kit, Invitrogen, Carlsbad, CA, USA).

Sequence (1,448 bp) was determined with a dRhodamin Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), and an automated sequence analyzer (model 310; Applied Biosystems). The sequence was compared with known 16S rRNA gene sequences by a nucleotide-nucleotide BLAST search of a DNA database.

Sequence data was aligned using the CLUSTAL W package for phylogenic analysis (Thompson et al., 1994). A phylogenetic tree was constructed by the UPGMA method by the MEGA V2.1 package (Kumar et al., 1994).

Analytical methods. The concentration of ammonium was determined using an ion chromatograph (DX120; Dionex Corp., Sunnyvale, CA, USA) and an IonPac CS12A column (Dionex) with methane-sulfonic acid solution (18 mmol) as the mobile phase for NH_4^+. The DOC concentration was determined using a TOC meter (TOC-5000A, Shimadzu, Kyoto, Japan). The cell density was measured by bacteriochlorophyll *a* (BChl *a*). BChl *a* was extracted with acetone–methanol (7 : 2), for 30 min at 80°C, and its concentration was measured using a Spectrophotometer 120A (Shimadzu) with 775 nm (Cohen-Bazire et al., 1957). The chlorophyll content in milligrams per 100 ml of culture is obtained from the expression $O.D._{775} \times 2.19$. The composition of the biogas was determined using a gas chromatograph (GC-8A, Shimadzu) with a thermal conductivity detector equipped with a steel column packed with Porapak Q (Shinwakakou, Kyoto, Japan) at 90°C.

Accession numbers. The sequences determined in the present study have been deposited in the DDBJ/EMBL/GenBank databanks (SR1; AB127985). The organisms whose 16S rRNA seaquences were used for the phylogenic analysis (and their accession numbers) were as follows: *Rhodopseudomonas palustris* (AB079680), *R. rhenobacensis* (AB087719), *R. cryptolactis* (AB087718), *R. julia* (AY428572), *Blastochloris sulfoviridis* (D86514), *Afipia genosp* (U87769), *Bradyrhizobium japonicum* (AF530466), *Rhodoplanes roseus* (D25313), *Rhodomicrobium vannielii* (M34127), *Phaeospirillum fulvum* (M59065).

Results

Phototrophic bacterial growth

Figure 1 shows the growth of phototrophic bacteria in sludges under light or dark conditions. Phototrophic bacteria grew in the 86% moisture sludge (BChl *a*, 0.46 g/L), 90% moisture sludge (BChl *a*, 0.36 g/L) and 98% moisture sludge (BChl *a*, 0.04 g/L) with simulta-

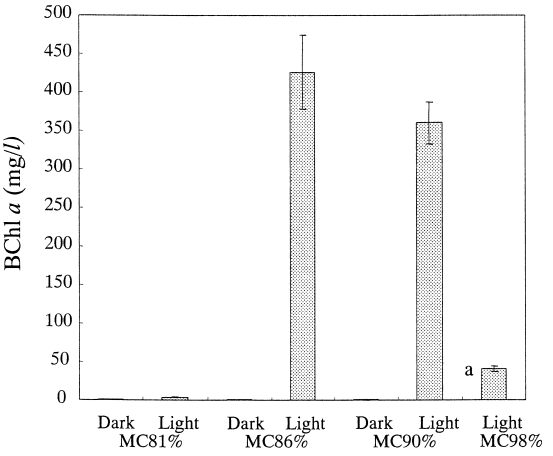

Fig. 1. Concentration of bacteriochlorophyll *a* in methanogenic sludges with different moisture contents after 14 days of incubation under dark or light conditions.

The bars represent standard deviations. [a] The growth of phototrophic bacteria under dark conditions was not tested.

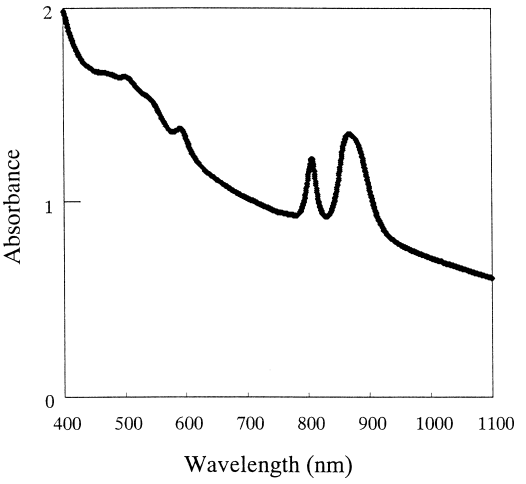

Fig. 2. Absorption spectra of cultures of isolated strains from reactor under light conditions.

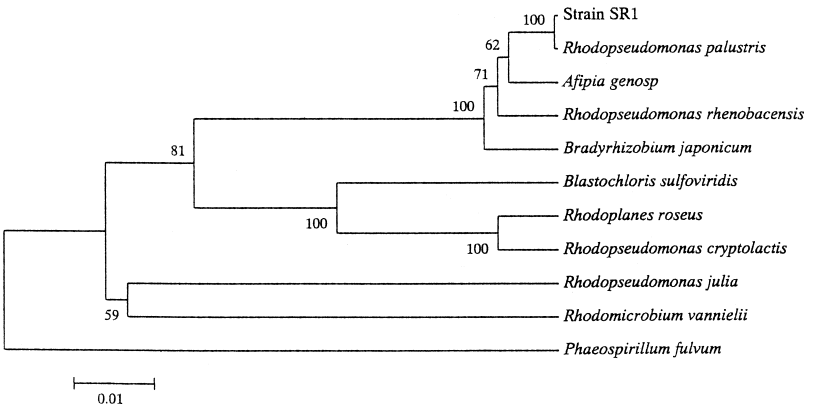

Fig. 3. 16S rRNA-based phylogenetic relationship between the isolated and previously reported phototrophic bacteria.
Numbers at nodes represent bootstrap values (100 replicates). The phylogenetic tree was constructed using the neighbor joining method.

neous methane production. The BChl *a* concentrations in the 86% and 90% moisture sludges under light conditions were about 9 times higher than that in 98% moisture sludge. Phototrophic bacteria could not grow in the 81% moisture sludge (BChl *a* 0.004 g/L). The concentration of BChl *a* did not increase in any moisture condition for sludges under dark conditions. Figure 2 shows the absorbance spectrum of isolated bacteria. There are two absorption peaks at 804 and 862 nm. The DNA sequence of isolated phototrophic bacteria strain SR1 had a 99% similarity to *Rhodopseudomonas palustris* (Fig. 3).

Ammonium removal by phototrophic bacteria

Figure 4 shows the concentrations of ammonium in the sludges under light and dark conditions. The ammonium concentrations of 81% moisture sludge under dark and light conditions were 4.6 g/L and 5.0 g/L, respectively. The ammonium concentrations in the 81% moisture sludge, 86% moisture sludge and 90% moisture sludge under dark conditions increased to about 1.5 times higher after anaerobic digestion of the sludge. Under light conditions, the concentration of ammonium in the 90% moisture sludge did not increase; on the other hand, those in the 81% and 86% moisture sludge under both light and dark conditions increased in the same manner.

The 90% moisture sludge incubated under light conditions contained about 40% less extracellular ammonium than that under dark conditions. Table 2 shows the composition of the sludge and phototrophic bacteria when the phototrophic bacteria increased to 350 mg/L of BChl *a* in the 90% moisture sludge under

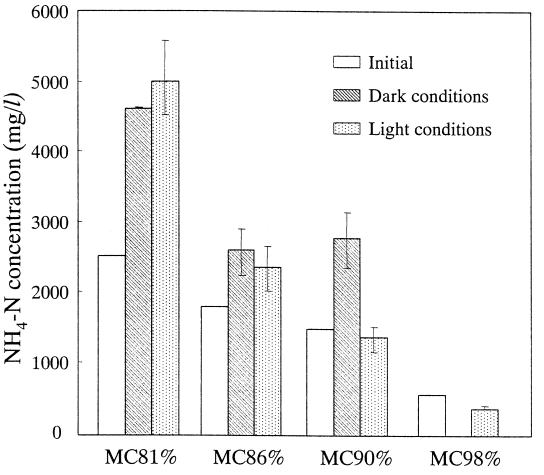

Fig. 4. Change in extracellular ammonium concentrations in methanogenic sludges with various moisture contents after 14 days of incubation under dark or light conditions.
The bars represent standard deviations.

light conditions. The extracellular ammonium concentration (NH_4-N) of the 90% moisture sludge after incubation under dark conditions was 1.0% of that of the dry sludge. Comparing both compositions, the nitrogen content of the phototrophic bacteria in the 90% moisture sludge under light conditions accounted for 10% of the sludge nitrogen, and about 46% of the extracellular ammonium in the 90% moisture sludge. The decreased quantity of extracellular ammonium in the 90% moisture sludge after incubation under light conditions compared with that under dark conditions was almost the same for the nitrogen content of the phototrophic bacterial biomass grown in the sludge.

Table 2. Elemental composition of original and digested sludge and phototrophic bacteria grown in the 90% moisture sludge under light conditions.

	Carbon (%)	Nitrogen (%)	Hydrogen (%)	NH_4-N (%)
Original sludge	32.9	4.8	5.0	1.06
Digested sludge incubated under light conditions	30.4	4.4	4.8	1.04
Phototrophic bacteria in the incubated sludge	1.93[a]	0.48	0.28	—

[a] This value was calculated using the elemental composition data of isolated phototrophic bacteria. Phototrophic bacteria grew in 90% moisture sludge at the Bchl *a* concentration of 350 mg/L under light conditions.

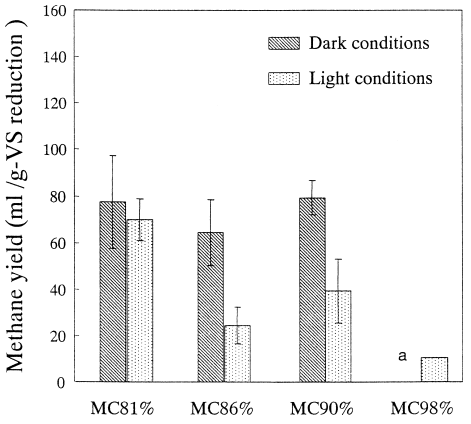

Fig. 5. Biogas yield from methanogenic sludges with different moisture contents during 14 days of incubation under dark or light conditions.

The bars represent standard deviations. [a] The growth of phototrophic bacteria under dark conditions was not tested.

VS reduction and methane yield

Methane was produced in all the sludge under both light and dark anaerobic conditions (Fig. 5). Methane production from 81% moisture sludge under dark conditions and light conditions was 77.5 ml/g VS reduction and 69.9 ml/g VS reduction, respectively. Methane production from 86% moisture sludge under dark conditions and light conditions was 64.4 ml/g VS reduction and 24.4 ml/g VS reduction, respectively. Methane production from 90% moisture sludge under dark and light conditions was 79.2 ml/g VS reduction and 39.2 ml/g VS reduction, respectively. Comparing the methane yield under dark and light conditions, the methane production in the all sludges under light conditions was lower than that under dark conditions. The VS reduction of 81% moisture sludge under dark conditions and light conditions was 0.04 g/g sludge and 0.04 g/g sludge, respectively. The VS reduction of 86% moisture sludge under dark and light conditions was 0.04 g/g sludge and 0.03 g/g sludge respectively. The VS reduction of 90% moisture sludge under dark and light conditions was 0.04 g/g sludge and 0.03 g/g sludge respectively.

Discussion

The present results indicated that phototrophic bacteria can grow in anaerobically digested sewage sludge with relatively low moisture content. The previous reports discussed anaerobic digestion of wastewater under light conditions (Sawayama et al., 1999, 2000a, b). The present results suggested the possibility of semidry anaerobic digestion of sewage sludge under light conditions. Low molecular organic matter is an important nutrient for the growth of phototrophic bacteria (Wiessner, 1970). The 98% moisture sludge might not contain enough organic matter for phototrophic bacterial growth.

It is well known that a high ammonium concentration is toxic for anaerobes (Hendriksen and Ahring, 1991; Sprott and Patel, 1986; Truper and Pfenning, 1981). It is reported that methane production was inhibited 50% by ammonium at 3,000 mg/L (Gallert and Winter, 1997). Hobson and Shaw (1976) reported that 235 mmol (3,290 mg N/L) ammonium completely prevented the growth of *Methanobacterium formicicum* in a pure culture. The present results indicated that phototrophic bacteria can grow in anaerobically digested sewage sludge by consuming extracellular ammonium. Extracellular ammonium in the anaerobically digested sludge was consumed by phototrophic bacteria under light conditions. The concentration of the BChl *a* in the 90% moisture sludge was higher under light conditions than under dark conditions. Comparing ammonium concentration under dark conditions and light condi-

tions in the 90% moisture sludge, the ammonium concentration under light conditions was lower than that under dark conditions. Sawayama et al. reported the similar result that the ammonium concentration decreased during the wastewater treatment for the growth of phototrophic bacteria under anaerobic and light conditions (Sawayama et al., 1999). This result showed that phototrophic bacteria could grow not only in wastewater but also in slurry with relatively low moisture content, and contribute to the decrease in the ammonium concentration. To keep a low ammonium concentration of digested sludge under light, further investigation is necessary to control the anaerobic digestion rate of organic matter and the removal rate of ammonium by phototrophic bacteria. Although the ammonium concentration in the 86% moisture sludge under light conditions was not less than that for dark conditions, phototrophic bacteria could grow to the same extent as that in the 90% moisture sludge. It is known that phototrophic bacterial cells have a beneficial effect on the development of grains (Haque et al., 1969). The cited reports and our results suggested that lighted anaerobic digestion of low moisture content sludge makes a good quality sludge for manure.

In our study, methane production under light conditions was less than that under dark conditions. It has been previously reported that decrease in methane production in the lighted UASB (LUASB) reactor compared with that in the UASB reactor under dark conditions exists (Sawayama et al., 1999) because of the competition to obtain organic compounds between the methanogens and phototrophic bacteria (Sawayama et al., 2000a). Harada et al. (2001) also reported that paddy soil slurries incubated under light conditions showed the growth of phototrophic purple bacteria and the reduction of CH_4 emission, indicating competition of purple bacteria with methanogens. Our results and these reports suggested that phototrophic bacteria and methanogens competed with low molecular weight organic acid in the anaerobic digestion of sewage sludge under light conditions.

Conclusions

Phototrophic bacteria could grow in relatively low moisture anaerobically digested sewage sludge with methane production, and assimilated extracellular ammonium in the sludge. The quality of digested sludge with phototrophic bacterial biomass for fertilizer could be improved compared with that without phototrophic bacterial biomass. The present results suggest the possibility of semidry anaerobic digestion under light conditions with controlled ammonium increase during methane fermentation.

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