EVALUATION OF ACCELERATED STABILIZATION OF LANDFILL BY MONITORING FUNCTIONAL MICROBES

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ABSTRACT

For the evaluation of the accelerated stabilization technology of landfill, functionally important microbes were monitored in two landfill sites using molecular biological techniques as follows: (i) the spatial distribution of nitrifying/denitrifying bacteria was monitored in coastal reclamation model reactors with or without leachate recirculation, and (ii) the time course behavior of nitrifying/denitrifying bacteria and change of microbial community structure was monitored in leachate from in-situ accelerated stabilization test cells under aerobic/anaerobic conditions with/without leachate recirculation. The more *amoA* genes were detected in test cells with leachate recirculation and all the *amoA*, *nirK*, and *nirS* genes were detected in upper layer of the model reactors with leachate recirculation. Thus, it could be considered that both ammonium oxidation and denitrification were occurred in this layer. The *amoA*, *nirK*, and *nirS* genes were detected continuously in aerobic cell, while they were only temporarily or intermittently detected in anaerobic or control cells. Venting and leachate recirculation would contribute to the activation of the bacterial growth, and to enhance the organic compounds decomposition potential as well as nitrification/denitrification potentials. It is also confirmed that venting and leachate recirculation contributed to form stable microbial community.

KEYWORDS

accelerated stabilization of landfill, functional microbes, monitoring

INTRODUCTION

Reduction of stabilization time of landfills is an important task to minimize the post-closure care, maintenance, and risk, and to promote post-closure land utilization. However, it is difficult to accelerate the decomposition of refuse and stabilization of the landfills so as to shorten the regulated post-closure monitoring period. This is often too heavy a burden on landfill owners and regulators to achieve the long-term management of landfills and treatment of leachate after closure. Thus the cost effective accelerated landfill stabilization technology is required. The stabilization of landfills is judged from various aspects such as physical, chemical, and biological status, and biological processes such as organic compounds decomposition in the refuse and nitrogen removal in the leachate are essential. Therefore, establishment of the technologies and strategies for the best utilization of the microbial functions deeply related to the above mentioned processes is one of the

important keys for the accelerated landfill stabilization. For this purpose, monitoring the functional microorganisms and the change of microbial community structure in landfills along with the measurement of physico-chemical indexes to elucidate the relationships between the biological status and stabilization process of the landfills is needed. The biological investigations in landfills will help to evaluate and optimize the previously proposed accelerated landfill stabilization technologies such as semi-aerobic landfills and leachate recirculation, and will lead to the establishment of biological indexes for the evaluation of the stabilization.

This study aims to elucidate the behavior of functionally important microorganisms, to develop the rational and effective accelerated landfill stabilization technologies, and to establish biological indexes for the evaluation of accelerated landfill stabilization technologies. For this purpose, microbial monitoring was performed in two landfill sites using molecular biological techniques especially polymerase chain reaction (PCR) techniques as follows: (i) the behavior of nitrifying/denitrifying bacteria in coastal reclamation model reactors settled at landfill site in K city was monitored focusing on the spatial distribution, and (ii) the behavior of nitrifying/denitrifying bacteria and change of microbial community structure in leachate from in-situ accelerated stabilization test cells settled at landfill site in S prefecture was also monitored focusing on the time course.

MATERIALS AND METHODS

Landfill sites and sampling

For monitoring spatial distribution of nitrifying/denitrifying bacteria in coastal reclamation model landfill reactors, the core samples (upper layer: 20-40 cm, middle layer: 140-160 cm, bottom layer: 260-280 cm) were collected on the day 1021 from the beginning of the operation from reactors constructed at landfill site in K city, in which municipal or industrial solid wastes were filled. The reactors were operated with or without leachate recirculation to construct 4 types of test cells (municipal solid wastes/recirculation; KR, industrial solid wastes/recirculation; HR, municipal solid wastes/non-recirculation; KA, and industrial solid wastes/non-recirculation; HA).

For monitoring the time course behavior of nitrifying/denitrifying bacteria and change of microbial community structure in the leachate of in-situ accelerated stabilization test cells, the leachate samples were collected on the days 34, 59, 124, 179, and 206 from the beginning of the operation from test cells constructed at landfill site in S prefecture, in which municipal solid wastes incineration ash and industrial solid waste were filled. The test cells were operated under aerobic/anaerobic conditions with/without leachate recirculation to construct 3 types of test cells (aerobic/leachate recirculation; AE, anaerobic/leachate recirculation; AN, and anaerobic/without leachate recirculation: control; CN).

Monitoring the behavior of microbes and change of microbial community structures

The DNA templates were extracted from samples following the method previously described (Sei *et al.*, 2000), and the extracted DNA was purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the samples were enumerated by MPN-PCR (Picard *et al.*, 1992). The extracted DNA were serially diluted by 10-fold and subjected to PCR in triplicate at each dilution ratio. The primer sets used were EUB-f933 and EUB-r1387 (Iwamoto *et al.*, 2000) for 16S rDNA of all the eubacteria, *amoA*-1F and *amoA*-2R (Rotthauwe *et al.*, 1997) for ammonia monooxygenase genes of ammonia oxidizing bacteria, NIRK-F and NIRK-R (Sei *et al.*, unpublished data) for copper containing nitrite reductase genes (*nirK*) of nitrite reducing bacteria, and NIRS-F3 and NIRS-R3 (Sei *et al.*, unpublished data) for nitrite reductase genes with heme *c* and *d1* (*nirS*) of nitrite reducing bacteria. The obtained amplified fragments were analyzed by electrophoresis on a 1.5% agarose gel stained with 0.5 μg/mL of ethidium bromide solution. According to the MPN technique, the number of amplifiable target DNA was determined. For the PCR-DGGE analysis the primer set EUB-f933GC and EUB-r1387 was used. The obtained DGGE banding pattern was analyzed by calculating the Shannon-Weaver index of diversity (*H'*)

(Shannon and Weaver, 1963), performing principal component analysis (PCA), and constructing dendrogram based on Dice's coefficient of similarity (S_D) (Eichner *et al.*, 1999) with the clustering algorithm of Ward (1963).

RESULTS AND DISCUSSION

Spatial distribution of nitrifying/denitrifying bacteria in coastal reclamation model reactors

The more *amoA* genes were detected in test cells with leachate recirculation (i.e., KR and HR cells) than in those without leachate recirculation (i.e., KA and HA cells) (Figs. 1 and 2). Especially, the number of *amoA* genes occupied 5.8% of that of 16S rDNA in KR140-160. Thus the leachate recirculation would affect to the growth of the ammonia oxidizing bacteria. About 10^2 MPN-DNA/g-dry waste of *amoA* genes were detected even in the bottom layer of the test cells with recirculation (KR260-280 and HR260-280) (Fig. 1), however, because the bottom layer was an anaerobic condition, they were considered to be inactive.

The *nirK* and *nirS* genes carried by nitrite reducing bacteria were detected in the upper and middle layers but not in bottom layer in all the test cells (Figs. 1 and 2). The upper and middle layers were considered to be the suitable condition for nitrite reducing bacteria.

All the *amoA*, *nirK*, and *nirS* genes were detected in upper layer of the test cell with leachate recirculation (HR20-40). It can be considered that both ammonium oxidation and denitrification were occurred in this layer. Indeed, Tachifuji *et al.* reported that both ammonia oxidizing and denitrifying bacteria were unevenly distributed at the semi-aerobic upper layer (0-30 cm from the surface) in model landfill reactor and suggested that simultaneous ammonium oxidation and denitrification occurred at this layer (Tachifuji *et al.*, 2001).

As the more ammonium oxidizing bacteria existed in KR and HR cells, it is suggested that leachate recirculation will enhance the ammonia oxidizing potential. As for the denitrifying bacteria, they intended to exist in semi-aerobic layer rather than anaerobic layer. As the rapid decrease of the total nitrogen was observed in KR and HR reactors, further elucidation on the relationship between the nitrogen removal and nitrifying/denitrifying bacteria or their activity is needed.

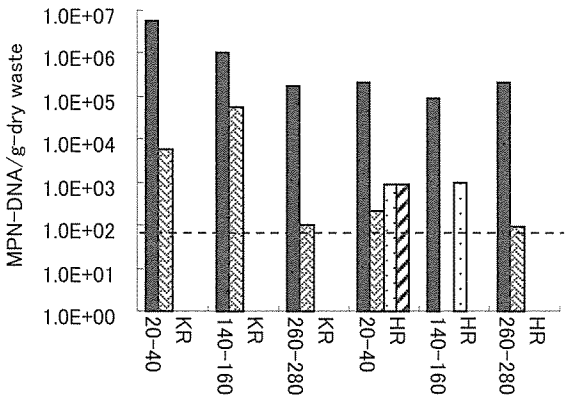

Fig. 1 The number of 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the test cells with leachate recirculation. The dotted line indicates the detection limit (8.0×10^1 MPN-DNA/g-dry waste). The number with the sample name indicates the depth (cm).

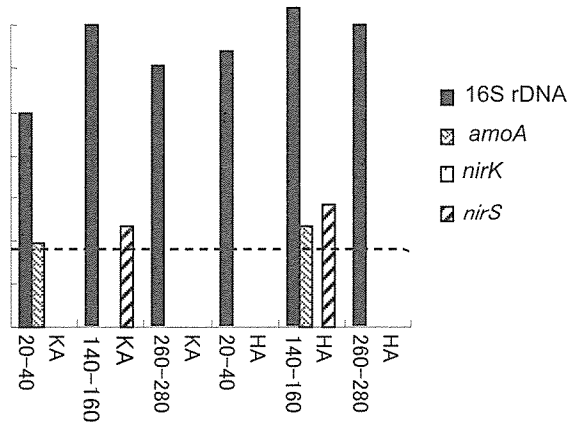

Fig. 2 The number of 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the test cells without leachate recirculation. The dotted line indicates the detection limit (8.0×10^1 MPN-DNA/g-dry waste). The number with the sample name indicates the depth (cm).

Time course behavior of nitrifying/denitrifying bacteria and change of microbial community structure in the leachate of in-situ accelerated stabilization test cells

The number of 16S rDNA was 10^5-10^7 MPN-DNA/mL in AE and CN cells (Figs. 3 and 5) while that in AN cell was less in 1-4 order of magnitude than AE and CN cells except for the sample on

the day 179 (Fig. 4). Thus, it is suggested that the decomposition potential of organic compounds in AE and CN cells was higher than in AN cell. The result of Biolog-MPN performed by Tanaka revealed that the bacterial population and activity in AE cell were higher than in other 2 cells (i.e., AN and CN cells) (Tanaka, unpublished data). This could partially support the result obtained in the present study. Although in AN cell, the equal or more TOC decreasing rate was observed in comparison to CN cell (Figs. 4 and 5), Inanc *et al.* suggested that this is because of the dilution by the leachate recirculation rather than biodegradation (Inanc *et al.*, unpublished data). From these results and related report, the aerobic condition in AE cells accelerated the decomposition of organic compounds and the leachate recirculation decreased TOC by dilution.

The *amoA*, *nirK*, and *nirS* genes were continuously detected after days 59, 179, and 124, respectively, in AE cells (Fig. 3). This is attributable to the activation of the bacterial growth by venting and leachate recirculation. On the other hand, the *amoA*, *nirK*, and *nirS* genes were only temporarily or intermittently detected in AN and CN cells (Figs. 4 and 5). It can be considered that the high pH (10-11) inhibited the growth of nitrifying and denitrifying bacteria in those cells. However, because the number of 16S rDNA in AN and CN cells was over 10^3 and 10^6 MPN-DNA/mL, respectively (Figs. 4 and 5), it seems that bacteria existed even in a certain population though some kinds of alkalophiles which are not responsible for nitrification/denitrification dominated in AN and CN cells.

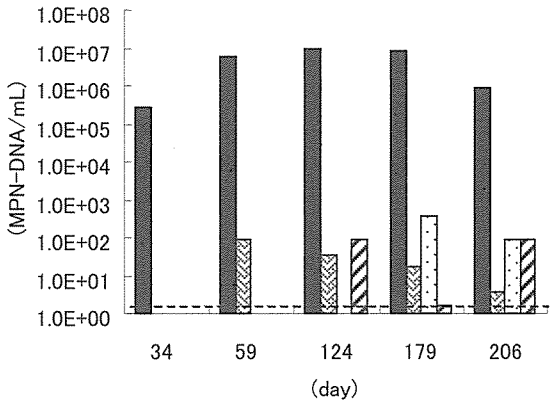

Fig. 3 The number of 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the leachate from AE cell. The dotted line indicates the detection limit (1.6×10^0 MPN-DNA/mL).

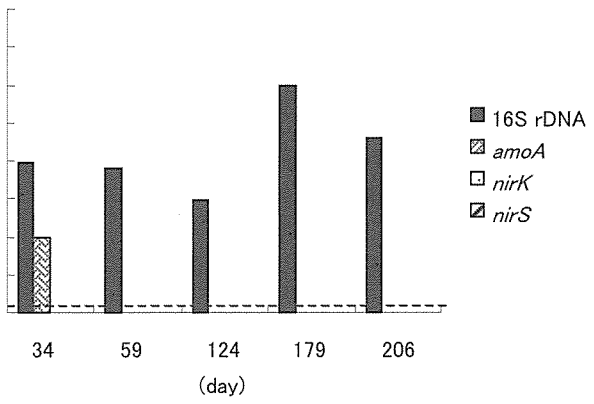

Fig. 4 The number of the 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the leachate from AN cell. The dotted line indicates the detection limit (1.6×10^0 MPN-DNA/mL).

Based on the PCR-DGGE banding pattern (Fig. 6), calculation of Shannon-Weaver index (*H'*) (Fig. 7), PCA analysis (Fig. 8), and cluster analysis (Fig. 9) were performed. The diversity gradually decreased through the experimental periods in AE and AN cells while drastic fluctuation was observed in CN cell (Fig. 7). On day 34 the diversity was in the order AE>AN>CN, while it was almost the same on day 206. Ishigaki reported the same tendency from the result of terminal restriction fragment length polymorphism (T-RFLP) analysis (Ishigaki, unpublished data), although there were some differences probably due to the characteristics of the methods used. The PCA and cluster analysis revealed that the bacterial community did not change so much in AE and AN cells while drastic change was observed in CN cell (Figs. 8 and 9). However, the change in CN cell might be attributable to the external factors such as seasonal change because CN cell was not artificially controlled. On the other hand, venting and leachate recirculation could be contributed to form the stable microbial community in AE and AN cells. Furthermore, microbial community in AE and AN cells tended to be similar by on day 206.

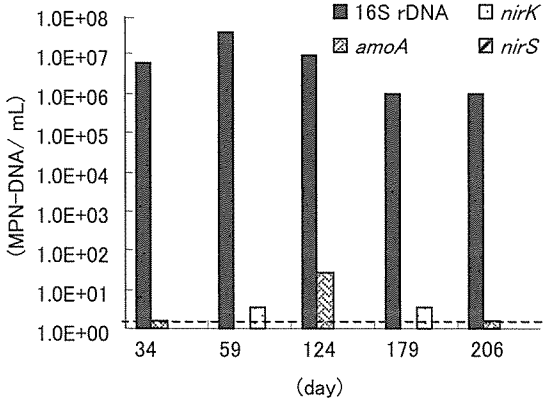

Fig. 5 The number of 16S rDNA, *amoA*, *nirK*, and *nirS* genes in the leachate from CN cell. The dotted line indicates the detection limit (1.6×10^0 MPN-DNA/mL).

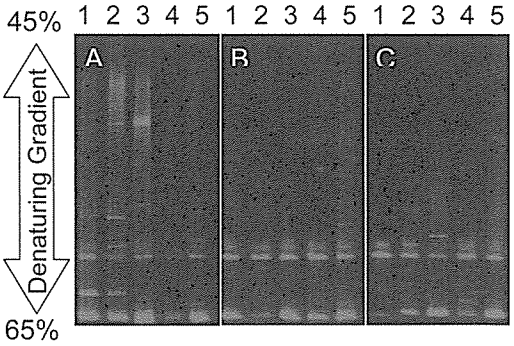

Fig. 6 PCR-DGGE banding pattern.
Lane 1 : day 34, lane 2 : day 59, lane 3 : day 124, lane 4 : day 179, lane 5 : day 206.
A : AE cell, B : AN cell, and C : CN cell

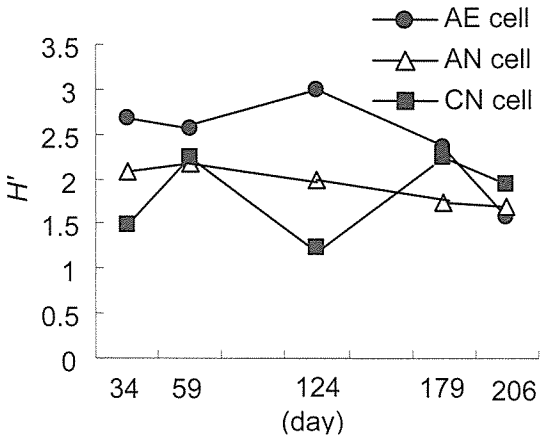

Fig. 7 Shannon-Weaver index (*H'*).

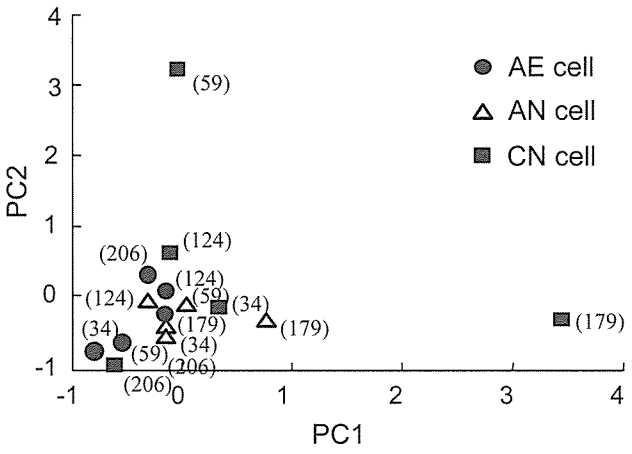

Fig. 8 Principal component analysis.
( ): The day from the beginning of the operation.

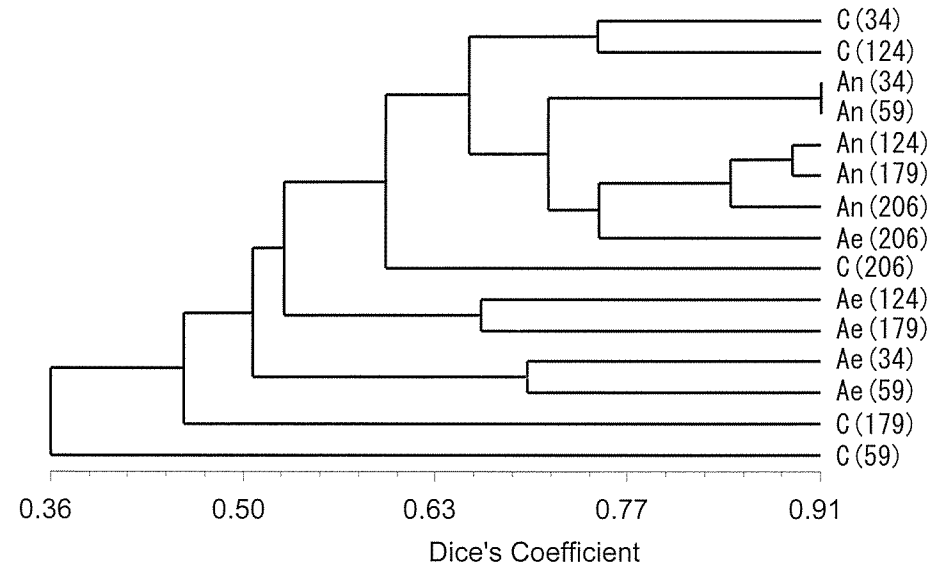

Fig. 9 Cluster analysis of the PCR-DGGE banding pattern.
Ae: AE cell, An: AN cell, C: CN cell. ( ): The day from the beginning of the operation.

CONCLUSIONS

Venting and leachate recirculation seemed to accelerate the formation of microbial community suitable for the decomposition of organic compounds and nitrogen removal.

From the experiments with coastal reclamation model reactors, it can be concluded that leachate recirculation would contribute to increase the nitrification potential in landfill. Further investigation on the leachate recirculation for the optimal control of *in situ* nitrification will give some solutions for the treatment and management of high NH_4-N concentration leachate over long periods of time.

From the experiments with leachate from in-situ accelerated stabilization test cell, it can be concluded that venting and leachate recirculation would contribute to enhance the organic compounds decomposition potential as well as nitrification/denitrification potential. It is also confirmed that venting and leachate recirculation contributed to form stable microbial community.

As there are only a few reports on the microbial community structure in landfills or leachate, further accumulation of the data is essential and will show the typical microbial community for the suitable organic compounds decomposition, nitrogen removal, and for the process control.

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