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EFFECT OF ADDITION OF BACTERIA ON THE REMOVAL OF RADIOACTIVE CESIUM FROM OCEAN SLUDGE IN A CIRCULATION TYPE PURIFICATION SYSTEM

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ABSTRACT: Following the Fukushima nuclear accident of March 11, 2011, soil and water were contaminated by radioactive cesium. Moreover, radioactive cesium was found in the ocean sludge in Tokyo Bay, carried by rivers flowing into the bay. The cesium adsorbed in the sludge cannot easily be removed. The objective of this study was to investigate the effect of the addition of bacteria to the micro-bubble circulation system on the efficient removal of radioactive cesium from ocean sludge. One of the authors has developed an ocean sludge decomposition system employing circulation of micro-bubbles. Model sludge was prepared using seawater, sea sludge, and cesium chloride. Bacteria were added to the system after 24 h. Dried tangle extract was added as a nutrient at 24 h and 36 h. The decomposition experiment was carried out for 120 h. The circulation of micro-bubbles created an aerobic state that activated aerobic bacteria, facilitating decomposition and purification of the sludge. Thus, decomposition of the deposited sludge using our system renders the elution of the radioactive cesium possible. If the cesium is eluted in the water, we can fix it using existing technology such as zeolites. We identified and isolated the most useful bacteria for sludge decomposition. Effects on purification seem to be greatest when additional bacteria are added directly to the process. The methodology proposed is expected to facilitate decomposition of sludge and removal of radioactive cesium from the environment.

Keywords: Decontamination, Radioactive Cesium, Ocean Sludge, Micro-bubble, Bacteria

1. INTRODUCTION

The 2011 accident at the Fukushima Daiichi nuclear power station has resulted in radioactive cesium contamination of soil and water. Radioactive cesium has been detected in the sludge in Tokyo Bay [1], carried by rivers flowing into the bay.

It has been reported that cesium easily adsorbs to the microscopic particles that constitute soil [1], [2]. Most cesium adsorbed by soil is difficult to remove by external factors, and remains present over long timescales. Moreover, closed water areas such as river and bay systems make it difficult to decompose piling organic sludge. Therefore, radioactive cesium is predicted to be deposited in the sediment of the seabed over time, expanding contamination into the ocean. It is important to decontaminate this sediment.

One of the authors developed a decomposition system for ocean sludge that employs the circulation of micro-bubbles. These create aerobic conditions that activate aerobic bacteria, facilitating decomposition and purification of the sludge [3]. Accordingly, it is considered that radioactive cesium can be eluted, after decomposition of the deposited sludge by our system. If the cesium can be eluted in water, it can then be fixed using existing technology such as zeolites [4]. We would thus be able to decontaminate the sediment.

To remove radioactive cesium effectively, we carried out isolation and identification of bacteria to break down sludge in the micro-bubble circulation system, and investigated the effect of the addition of microorganisms to the decomposition system on the efficient removal of radioactive cesium from ocean sludge.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Bacteria to Break Down Sludge in the Micro-Bubble Circulation System

The micro-bubble circulation system consists of two parts, shown in Fig. 1 (without zeolite). The water circulates through two tanks. In one tank (length 40 × width 28 × height 28 cm), micro-bubbles are generated. The micro-bubbles have micro-size diameter and high solubility. This means that water with a high concentration of dissolved oxygen (DO) circulates through these

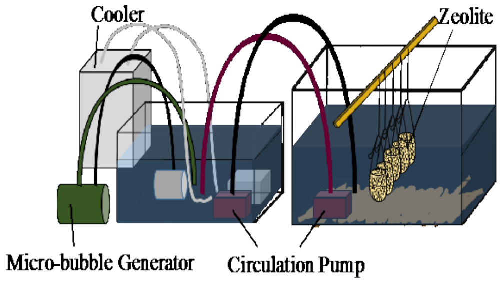

Fig. 1 Circulation purification system

tanks. The experimental tank is 60 × 29 × 35 cm. We used 30 L of seawater and 1 kg of sludge. The micro-bubble generator was based on [6] and the flow rate was 900 L/h. The flow rate of the water pumps connected to each tank was 300 L/h. A cooler for the tank that generates the micro-bubbles was set at 30 °C.

We took samples of sludge and seawater at Funabashi Port in Chiba Prefecture in Japan. We removed the first 10 cm of sludge from the seabed before samples were taken.

After setting up the micro-bubble circulation decomposition system, the experiment started as the generation of micro-bubbles began. After 0, 36, 72, 108, and 120 h, we sampled the sludge in the experimental device.

Samples were diluted in distilled water and 10 μL of each sample was plated on standard agar plates (Nissui Pharmaceutical Co., Ltd., Japan) and cultured at 30 °C for 3–5 days. Thereafter, a bacterial colony was picked for isolation. The isolated bacteria were identified by 16S rRNA gene sequence analysis.

2.2 Experiments on Decomposition of Deposited Sludge for Cesium Removal

The system shown in Fig. 1 was used to experimentally remove cesium from ocean sludge. Model sludge containing cesium chloride was poured into the system. One kg of sea-sludge and 30 L of seawater and cesium chloride was mixed and stirred for 24 h. The final concentration of cesium was approximately 100 ppm.

Zeolites were placed in the tank (Fig. 2), micro-bubbles were generated, and the experiment began.

Fig. 2 Zeolites set in experiment tank

The micro-bubble generator again used a flow rate of 900 L/h. The flow rate of the water pumps connected to each tank was 2400 L/h. The cooler was set at 30 °C.

After 24 h, isolated bacteria (15 × 10^8 cell/mL) and an activator [7] were added. An extract of kelp was added to the experimental tank after 24 and 60 h. After 0, 12, 48, 60, 72, 96, and 120 h, DO, water temperature, and pH were measured by a multi-parameter water quality meter. A digital pack test (Kyoritsu Chemical-Check Lab. Corp., Japan) was used to measure ammonium ions (as ammonium nitrogen, NH_4-N), nitrite ions (as nitrite nitrogen, NO_2-N), nitrate ions (as nitrate nitrogen, NO_3-N), total nitrogen (T-N), and hydrogen sulfide (H_2S). The seawater was then filtered. We checked the shape of the sea sludge and the effect of cesium removal with SEM-EDX, after 0 and 120 h. Experimental conditions, including the amount of kelp used, are shown in Table 1.

Table 1 Experimental conditions

	Additive amount of bacteria	Activator	Extract of kelp
Case 1	1.5 × 10^8 cell	–	500 ppm
Case 2	1.5 × 10^8 cell	–	–
Case 3	–	100 ppm	–

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacteria

Water was sampled from the experimental device after 0, 24, 72, and 120 h. Fifty-three strains of bacteria were isolated from these samples and identified by 16S rRNA gene sequence analysis. Six species of bacteria were identified from samples at 0, 36 and 72 h: *Bacillus cereus*, *B. clausii*, *B. licheniformis*, *B. thuringiensis*, *B. subtilis*, and *Alcaligenes fecalis*.

B. clausii is alkaliphilic and produces high-alkaline proteases. *B. licheniformis* is commonly known to cause food poisoning and food spoilage. *B. subtilis* is non-pathogenic. It can contaminate food, however seldom results in food poisoning. *B. cereus* produces two types of toxins and is known to cause food poisoning. *B. thuringiensis* is a close relative of *B. cereus*. It produces insecticidal protein. Thus, *Bacillus* species are almost ubiquitous in nature, however, epidemiologically they are unsuitable to use for sludge decomposition.

Alcaligenes fecalis is commonly found in the environment in soil and in wastewater disposal apparatus. *A. fecalis* has heterotrophic nitrification and aerobic denitrification functions [8]. Thus, we assumed that *A. fecalis* would be useful for decomposition of deposited sludge. We carried out experiments of decomposition of the deposited sludge using our system (Fig. 1) with micro-bubbles and *A. fecalis*.

3.2 Experiments of Decomposition of Deposited Sludge for Cesium Removal

3.2.1 Results of water temperature, pH, DO and H_2S in varying environmental conditions

Figures 3–5 show the water temperature, pH, and DO respectively, resulting from the environmental conditions of cases 1–3 (Table 1). Water temperature is almost constant at about 30 °C after 6 h, as a result of using a cooler for the experimental water tank. The pH of case 1 is constant at about 9.5, and that of cases 2 and 3 is constant at about 7.5 to 8.0: the pH of case 1 was higher than cases 2 and 3. The initial concentrations of DO are 1.9 mg/L, 5.3 mg/L and 5.6 mg/L in cases 1, 2, and 3 respectively. The DO of all cases is saturated at about 7.5 to 8.3 mg/L after 6 h, because the concentration of oxygen saturation is 8.11 mg/L-pure water.

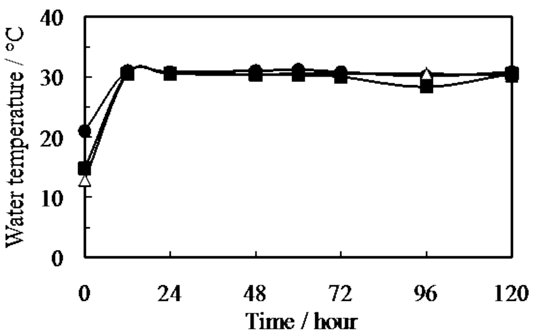

Fig. 3 Change in water temperature over time in case 1 (●), case 2 (Δ), and case 3 (■).

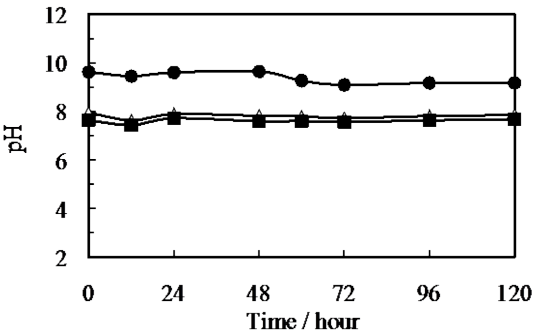

Fig. 4 Change in water pH over time in case 1 (●), case 2 (Δ), and case 3 (■).

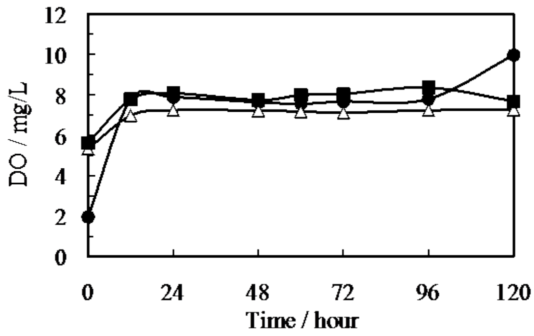

Fig. 5 Change in DO concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

H_2S concentration is shown in Fig. 6. In all cases, H_2S decreases up to 24 h, before the addition of *A. fecalis* to the experimental tank. *A. fecalis* is not inhibited by H_2S.

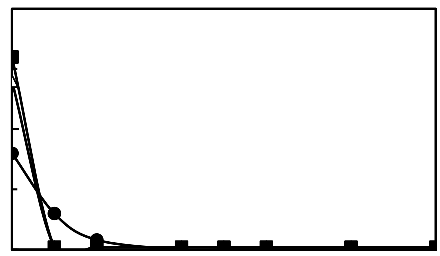

Fig. 6 Change in H_2S concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

3.2.2 Results of NH_4-N, NO_2-N, NO_3-N and T-N

Figure 7 shows the change in NH_4-N (ammonium nitrogen) concentration in the experimental tank over time. In all three cases (Table 1), initial concentrations of NH_4-N are approximately 2.0 mg/L. In case 1, NH_4-N decreases; at 24 h it is no longer detected. The effect of *A. fecalis* on NH_4-N concentration could not be ascertained, because *A. fecalis* was added at

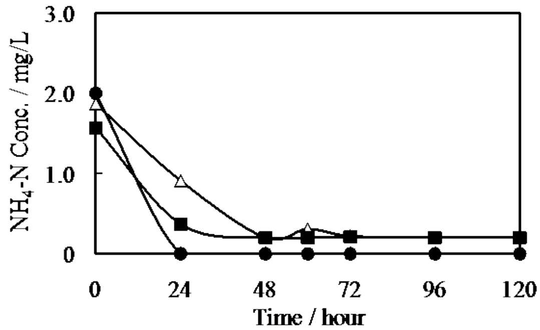

Fig. 7 Change in NH_4-N concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

24 h. In case 2, NH_4-N decreases up to 48 h and then remains constant at 0.2 mg/L. In case 3, NH_4-N decreases up to 24 h and then remains constant at 0.2 mg/L.

Figure 8 shows the change in NO_2-N (nitrite nitrogen) concentration in the experimental tank over time. In case 1, initial concentrations of NO_2-N are approximately 0.30 mg/L. Subsequently NO_2-N decreases up to 72 h, after which it is constant at 0.80 mg/L. In case 2, initial concentrations of NO_2-N are approximately 0.20 mg/L. NO_2-N increases to 0.4 mg/L at 24 h before decreasing up to 96 h, and then remains constant at 0.08 mg/L. In case 3, initial concentrations of NO_2-N are approximately 0.15 mg/L. Following this, NO_2-N increases to 0.25 mg/L at 24 h, then decreases up to 72 h after which it remains constant at 0.01 mg/L.

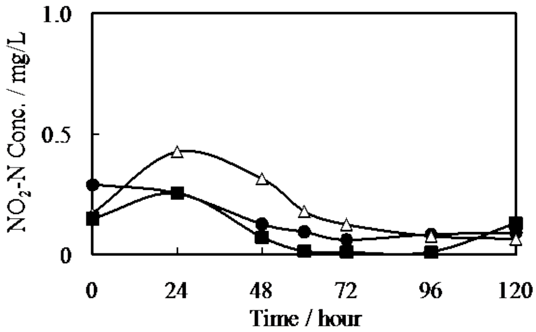

Fig. 8 Change in NO_2-N concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

Figure 9 shows the change in NO_3-N (nitrate nitrogen) concentration in the experimental tank over time. In case 1, initial concentrations of NO_3-N are approximately 4.3 mg/L. Subsequently NO_3-N decreases up to 96 h, after which it remains constant at 0.9 mg/L. In case 2, initial concentrations are approximately 2.0 mg/L.

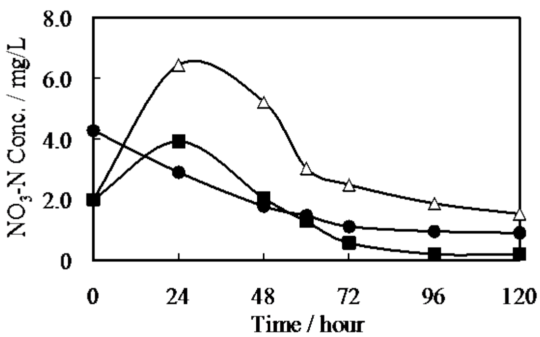

Fig. 9 Change in NO_3-N concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

Following this, NO_3-N increases to 6.4 mg/L at 24 h, then decreases to 3.0 mg/L at 60 h before continuing to gradually decrease to 1.5 mg/L at 120 h. In case 3, initial concentrations are approximately 2.0 mg/L. NO_3-N increases to 3.9 mg/L at 24 h, then decreases to 0.2 mg/L at 96 h. NO_2-N and NO_3-N showed similar trends.

In the initial 24 h, NH_4-N concentration decreased, and NO_2-N and NO_3-N concentrations increased. It is assumed that the metabolism of the bacteria switched from denitrification to nitrification because the experimental tank water changed from anaerobic to aerobic conditions (Fig. 5). Therefore we suppose that the nitrogen source shifts with $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ and that this trend of NO_3-N concentration was similar to NO_2-N.

Dissolved inorganic nitrogen (DIN; NH_4-N + NO_2-N + NO_3-N) shows 85 % and 90 % decreases in cases 1 and 3 respectively (Fig. 10).

The effect of the addition of *A. fecalis* on T-N (total nitrogen, and inorganic and organic nitrogen) concentration was investigated. After adding *A. fecalis* to the water, T-N decreased and was eventually no longer detected (Fig. 11).

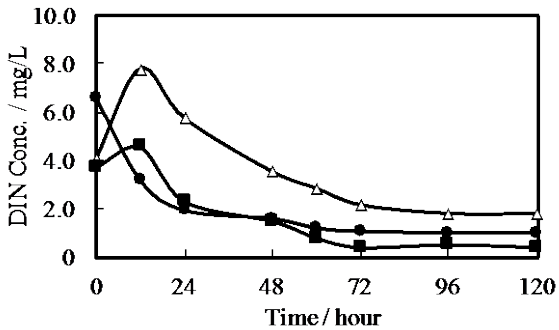

Fig. 10 Change in DIN concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

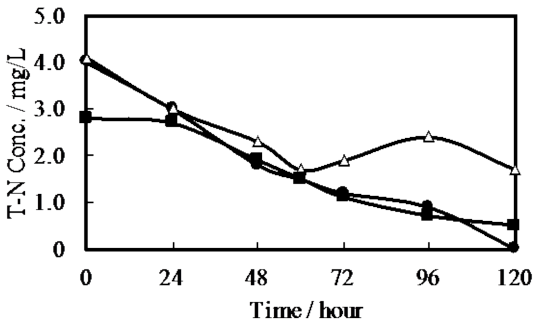

Fig. 11 Change in T-N concentration over time in case 1 (●), case 2 (Δ), and case 3 (■).

3.2.3 Observation of sludge shape

The shape of the sea sludge was observed using SEM at 0 and 120 h in case 1. Fig. 12 shows the SEM photomicrographs at 0 (a), and 120 (b) h. At 120 h after exposure to micro-bubbles and bacteria, the sludge particles had become smaller.

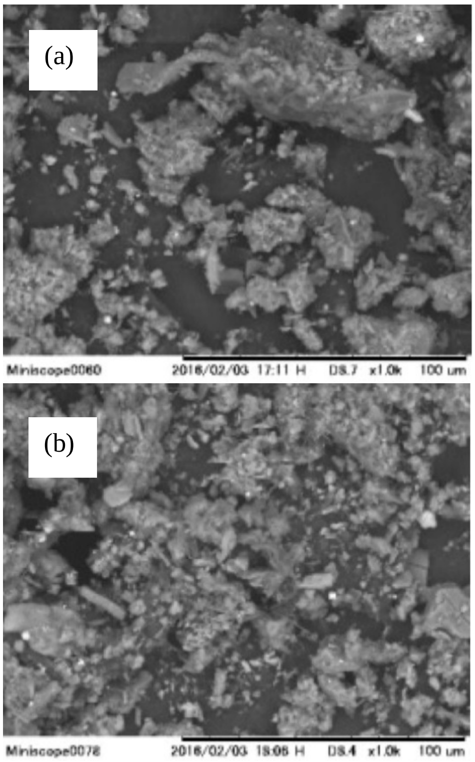

Fig. 12 SEM photomicrographs of sludge (a) before experiment and (b) after 120 h in case 1.

3.2.4 Results of cesium concentration

We measured cesium and silica in the sludge using energy dispersive X-ray analysis (EDX). The weight ratios of cesium to silica (Cs/Si) in dry sludge are shown in Fig. 13. The cesium decontamination ratio was calculated from the ratios of cesium content at 0, 24, 72, and 120 h as measured by EDX, using standard values for silica after measuring the weight of the dried sludge. The decontamination ratio obtained in case 1 was about 50 %.

It has been reported that cesium concentration in water decreased abruptly after the beginning of similar experiments [9]. Therefore, eluted cesium from the sludge was adsorbed to zeolite, after the sludge was decomposed by micro-bubbles and *A. fecalis*.

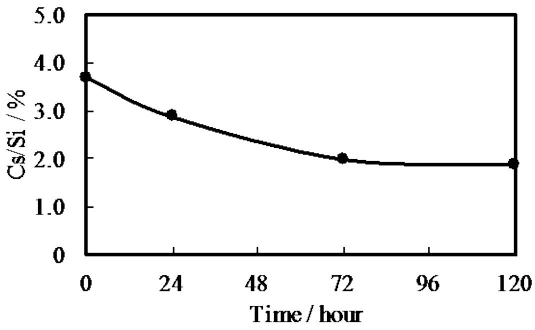

Fig. 13 Change in Cs/Si over time in case 1.

4. CONCLUSION

We carried out elution and fixing of cesium by micro-bubble and bacteria circulation decomposition system for ocean sludge. From water quality measurements, it can be concluded that after adding *A. fecalis*, T-N decreases and then is no longer detected. *A. fecalis* has a remarkable ability to treat samples through denitrification.

The sea-sludge particles decrease in size through treatment with micro-bubbles and bacteria for 120 h. We predict from the results shown here that the system proposed would greatly facilitate cesium removal from ocean sludge.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1] Soemori A, Shozugawa K, Nogawa N, Higaki S, Matsuo M, "A change in the concentrations of radioactive cesium in Tokyo-bay's sediments released by the Fukushima Dai-ichi Nuclear Power Station accident", Bunseki Kagaku, Vol. 62(12), 2013, pp. 1079–1086.

[2] He Q, Walling D E, "Interpreting particle size effects in the adsorption of ^{137}Cs and unsupported ^{210}Pb by mineral soils and sediments", J. Environ. Radioactivity, Vol. 30(2), 1996, pp. 117–137.

[3] Okamoto K, Hotta K, Toyama T, Kohno H, "Purification system of ocean sludge by activating microorganisms", Int. J. of GEOMATE, Vol. 6(1), 2014, pp. 791–795.

[4] Okamoto K, Toyama T, "Decontamination of radioactive cesium from ocean sludge by

micro-bubble and microorganisms", Int. J. of GEOMATE, Vol. 9(1), 2015, pp. 1390–1394.
[5] Tsukada H, Takeda A, Hisamatsu S, Inaba J, "Concentration and specific activity of fallout ^{137}Cs in extracted and particle-size fractions of cultivated soils", J. Environ. Radioactivity, Vol. 99(6), 2008, pp. 875–881.
[6] Matsuo K, Maeda K, Ohnari H, Tsunami Y, Ohnari H, "Water purification of a dam lake using micro bubble technology", Progress in Multiphase Flow Research, Vol. 1, 2006, pp. 279–286.
[7] Okamoto K, Hotta K, "Purification experiments on sedimentary sludge by microorganism activator", In Proc. of Pacific Congress on Marine Science and Technology (PACON), 2008.
[8] Inamori Y, Wu X-L, Kimochi Y, "Characteristics of N_2O production by *Alcaligenes faecalis* under aerobic conditions", Journal of Japan Society on Water Environment, Vol. 22, 1999, pp. 904–909.
[9] Okamoto K, Toyama T, "Ocean decontamination: Removal efficiency of radioactive cesium from ocean sludge by using micro bubbles and activating microorganisms", Int. J. of GEOMATE, Vol. 10(21), 2016, pp. 1924–1928.