BIOREMEDIATION OF SOIL CONTAINING DIOXINS BY USING BACTERIA IN CRUDE OIL

Toyohisa Fujita[1*], Gjergj Dodbiba[1], Atsushi Shibayama[2], Vo Dinh Long[3]

[1]*The University of Tokyo, Graduate School of Engineering, Japan*

[2]*Akita University, Graduate School of International Sciences, Japan*

[3]*Industrial University of Ho Chi Minh City*

*Email: *tfujita@sys.t.u-tokyo.ac.jp*

Received: 3 August 2018; Accepted for publication: 5 September 2018

ABSTRACT

The mixed bacteria collected from the water phase in crude oil deposit shows the decomposition of dioxin by adding the soil, mixing and settling. High concentration 50 ppm of dioxin in soil could decompose without nutritional salts at the rate of 60% after 6 weeks. Low concentration 10 ppm of dioxin in soil could decompose with nutritional salts at the rate of 80% after 6 weeks. If the dioxins still remain in the soil in Vietnam, there would be a possibility to decompose by using these bacteria inhabiting water separated from crude oil.

Keywords: Bioremediation, soil, dioxin, decomposition, water phase in crude oil.

1. INTRODUCTION

Dioxins are called persistent organic pollutants (POPs), meaning it takes a long time to break down once they are in the environment; and dioxins are highly toxic and can cause cancer, reproductive and developmental problems, damage to the immune system, and can interfere with hormones [1].

Nowadays, the dioxins present in the environment by incineration, combustion, industrial and reservoir sources [2]. However, the production of dioxins has been reduced by heating and rapid cooling for incinerations. On the other hand, during the Vietnam War from 1961 to 1971, the Agent Orange [3] was used as herbicide which contains 2,4-dicholorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) contaminated with 2,3,7,8-tetrachlorodibenzodioxine (TCDD) as shown in Figure 1.

Figure 1. Chemical formula of herbicide used in Vietnam War [3]

It was reported that between 1962 and 1971, the United States military sprayed nearly 76,000 m^3 of various chemicals of the herbicides [2] in various areas of Vietnam as shown in Figure 2. If the dioxins still remain in the soil, the soil remediation is important to prevent the health hazard.

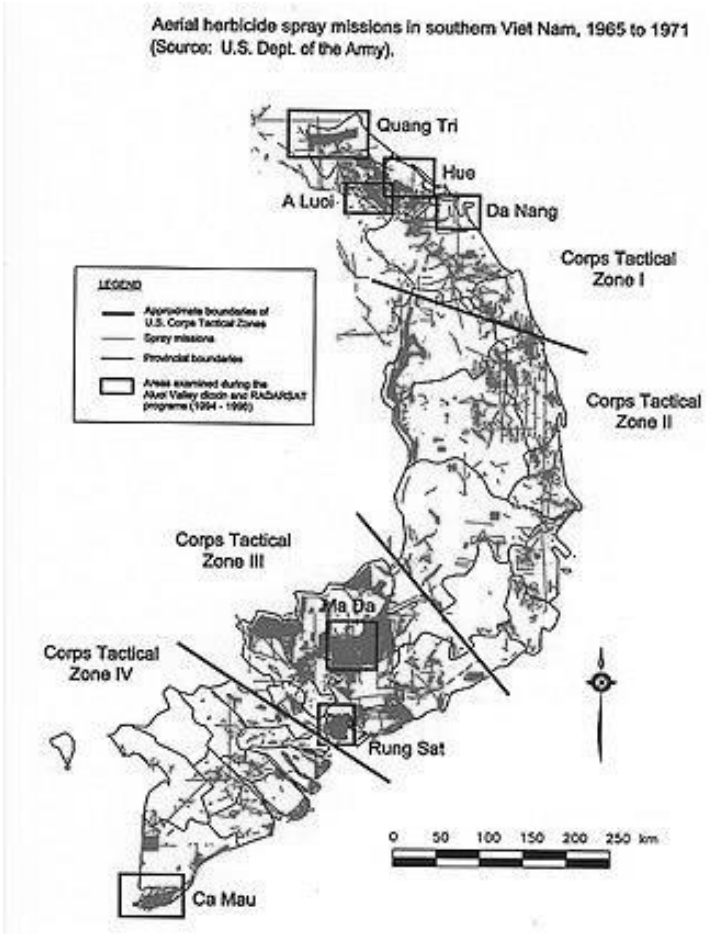


Figure 2. Map showing locations of U.S. Army aerial herbicide spray missions in South Vietnam taking place from 1965 to 1971 [3].

There are various techniques to decompose dioxins. In Japan, Ministry of the Environment uses the following techniques: (1) Fusion method at high temperature around 1300 ºC, (2) Incineration method at 1100 ºC in an oxidizing atmosphere, (3) Reduction method by hydrogen at more than 850 ºC, (4) De-chlorination at 400 ºC in nitrogen atmosphere, (5) Decomposition by supercritical water, (6) Reaction with metal sodium, (7) Decomposition using UV and ozone [4]. On the other hand, the biological treatment was reported as one of dioxin treatment methods [5]. Many kinds of microbe such as fungi, aerobic and anaerobic bacteria were reported for decomposition of dioxins as listed in Table 1.

Table 1. Microbe reported for decomposition of dioxins [6]

Fungi	*Schizophyllum commune, Phanerochaete sordida, Phlebia brevispora, Phlebia lindtneri, Pleurotus pulmonarius, Flammulina velutipes, Pleurotus pulmonarius , Schizophyllum commune*, Aphyllophorales etc.
Aerobic bacteria	*Sphingomonas sp., Pseudomonas sp., Nocardioides sp. Rhdococcus sp., Burkholderia sp., Terrabacter sp*. etc.
Anaerobic bacteria	*Dehalococcoides sp*., etc.

The decomposition of oil using bacteria was first investigated [6]. Also, the dioxin removal in water by using bacteria has already been reported in our group [7]. In this report, the artificial dioxin removal in soil using bacteria inhabiting in water contact to crude oil in

Japan was investigated. Though the oil deposit inVietnam is located in the sea, there would be a possibility to decompose the dioxin in soil by using bacteria inhabiting water separated from crude oil.

2. EXPERIMENTAL METHOD

2.1. Bacteria

Bacteria were collected from the water phase in the crude oil separated tank at Amarume oil well of Yamagata prefecture in Japan. The photo of sample collection is shown in Figure 3. As the oil is lighter than water, the water is discharged from the bottom of tank. The water phase contans various bacteria.

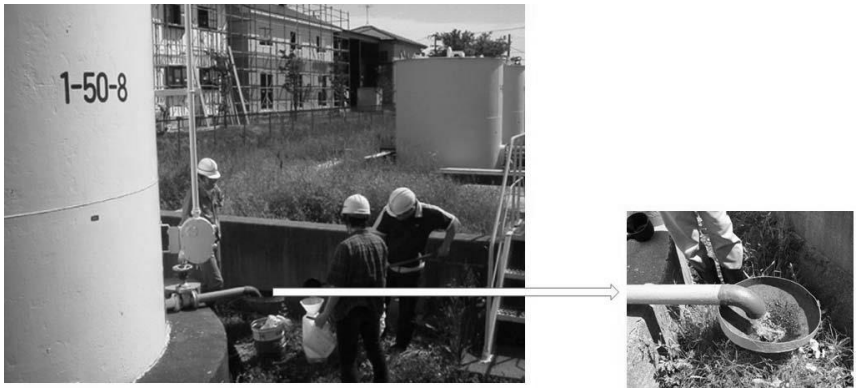


Figure 3. The collection of water phase inhabiting bacteria in the crude oil separated tank (Amarume oil well, Yamagata Pref., Japan).

The collected water phase was dropped on the agar medium followed by incubating the agar at 30 ºC for 3 to 5 days to produce colony. Next, it was cultivated in other agar medium for 1 to 2 days for the colony production which is shown in the Figure 4. The colony was analyzed by the company [8] to measure 16SrDNA-Full nucleotide sequence.

The following bacteria were mainly detected: *Pseudomonas* sp., *Gammaproteobacteria* class, and *Shewanella* sp.

The mixed solution was used in the dioxin decomposition experiment. The cultivated colony was stirred and mixed with distilled water, and then was agitated for 2 hours to receive a mixture of bacteria used in the experiment. There is a possibility to decompose the herbicide contaminated soil by mixing bacteria in water phase under the collected crude oil.

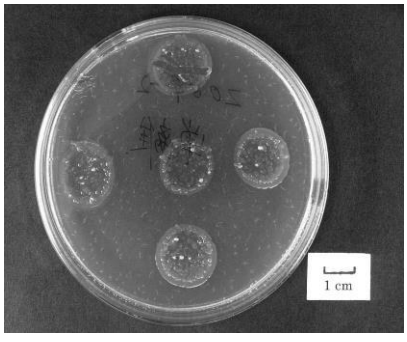


Figure 4. The colony of bacteria collected from water phase in the crude oil.

2.2. Synthesized dioxin

The synthesized dioxin is obtained from Environmental Research Center, Akita University. The dioxin has been produced by thermal chlorination reaction with 2,4,5-TCP (Trichlorophenol) and copper chloride through 12% oxygen flow at 400 ºC. The synthesized dioxin is listed in Table 2, including PCDDs (polychlorinated dibenzodioxins) and PCDFs (polychlorinated dibenzofurans). As the prepared concentration of dioxin is 1370 ppm, the dioxin sample is used as the diluted concentration.

2.3. Experimental method

The flowsheet of experiment for dioxin decomposition in soil is shown in Figure 5.

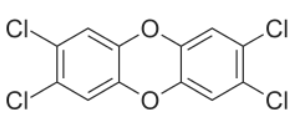
The soil sample was prepared with 5% of wood (Lauan) powder which was washed and dried with 50% hexane and 50% benzene mixture liquid and 95% of soil (max. particle size: 100 mesh) (Kanuma soil) which was heated at 250 ºC, washed with benzene followed by washing distilled water and dried at 120 ºC. 10 g of the soil was put into a dish and 5 mL of bacteria water was added and mixed. In this experiment, 5 mL water with bacteria was used by two kinds of water. One was only distilled water and another was included nutritional salts such as KH_2PO_4, Na_2HPO_4, $MgSO_4$, $FeSO_4$, $CaCl_2$, NH_4NO_3. Next, synthesized dioxin is added to be 50 ppm dioxin concentration and stirred. They were put in the incubator at 30 ºC for 6 weeks. Three parts from one petri dish were collected and analyzed for dioxin concentration every week.

The samples were set in the dry oven at 60 to 65 ºC for 2 hours to evaporate the water.

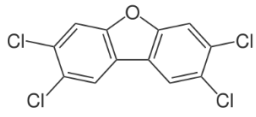
The samples in the filter were set in the Soxhlet extractor and extracted by benzene for 24 hours. The extracted sample was concentrated by rotary evaporator and put into test tube. Next, the tube was heated in the water bath at 90 ºC by blowing N_2 gas and the liquid was concentrated at 200 mL. The dioxin in the liquid was analyzed by GC-MS (SIM mode).

Table 2. Synthesized dioxin

PCDDs	Concentration (ppm)	PCDFs	Concentration (ppm)
T4CDDs	86	T4CDFs	0,14
P5CDDs	210	P5CDFs	1,6
H6CDDs	670	H6CDFs	60
H7CDDs	160	H7CDFs	99
O8CDDs	1,7	O8CDFs	82
Total PCDDs	1,128	Total PCDFs	242
Total (PCDDs + PCDFs)		1,370 (ppm)	
TEQ		180 (μgTEQ/mL)	



PCDD



PCDF

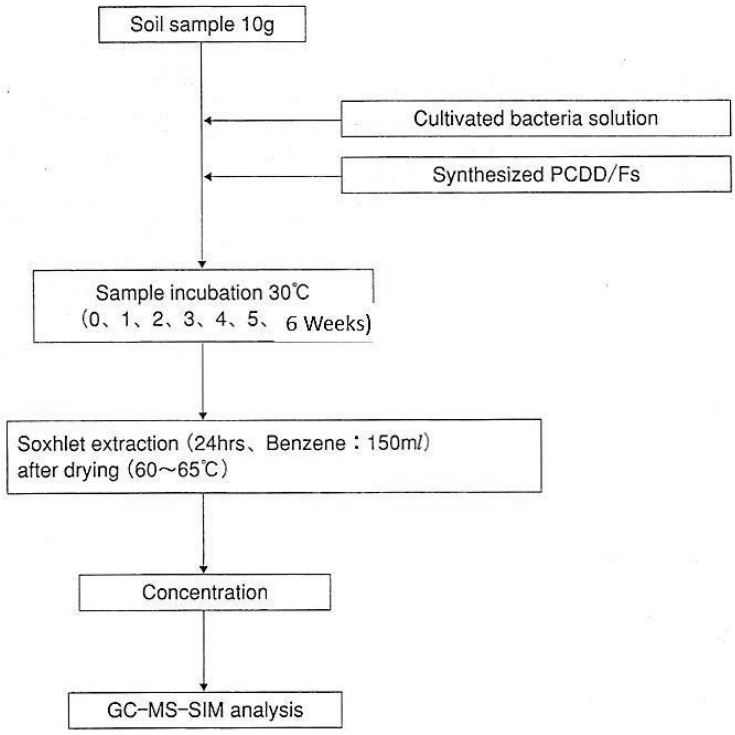


Figure 5. Flowsheet of experiment for dioxin decomposition in soil.

3. EXPERIMENTAL RESULTS

The decomposition of high concentration 50 ppm of dioxin in soil depending on time without nutritional salts is shown in Figure 6. The vertical axis indicates the ratio of dioxin concentration and time when PCDD/Fs is one of the initial concentrations. The change of PCDDs and PCDFs is shown in (a) and (b), respectively and any kinds of dioxin concentration reduced depending on time. Figure 7 shows that, after 3 weeks the dioxin concentration decreases gradually and after 4 weeks the dioxin concentration is almost the same. It means total dioxin concentration changes depending on time. The results also shows 60% of dioxin reduced after 6 weeks.

The decomposition of low concentration 10 ppm of dioxin in soil depending on time with nutritional salts is shown in Figure 8. The vertical axis indicates the same as Figures 6 and 7. The change of PCDDs and PCDFs is shown in (a) and (b), respectively and any kinds of dioxin concentration reduced depending on time. Figure 9 shows that, after 1 weeks the dioxin concentration decreases largely and after 2 weeks the dioxin concentration decreases gradually. It means total dioxin concentration changes depending on time. The result also shows 80% of dioxin reduced after 6 weeks.

From the results, bacteria could reduce the dioxin nevertheless of chlorine number of PCDD and PCDF. Also, the addition of nutritional salts increased the dioxin decomposition speed.

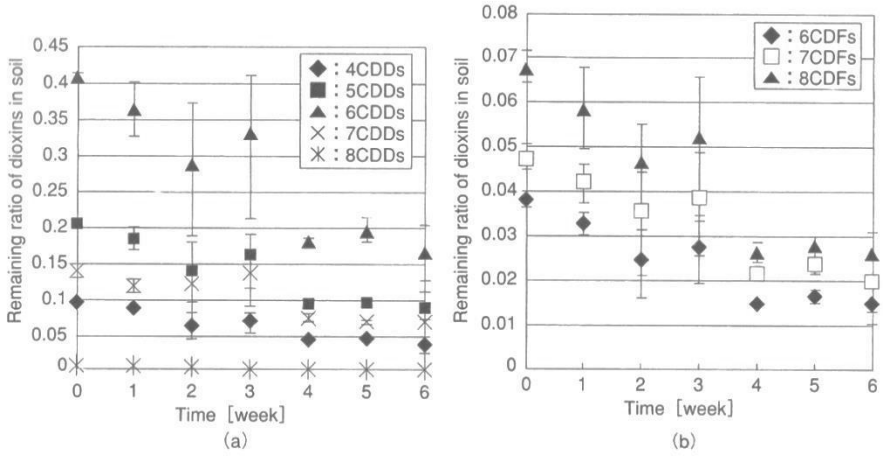


Figure 6. Change of decomposition rate of PCDDs and PCDFs after adding bacteria in the soil. (High concentration 50 ppm of dioxin in soil and no nutritional salts with bacteria)

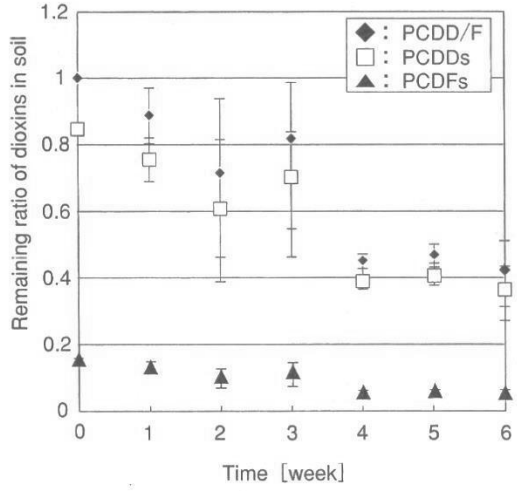


Figure 7. Total dioxin concentration change depending on time. (High concentration 50 ppm of dioxin in soil and no nutritional salts with bacteria)

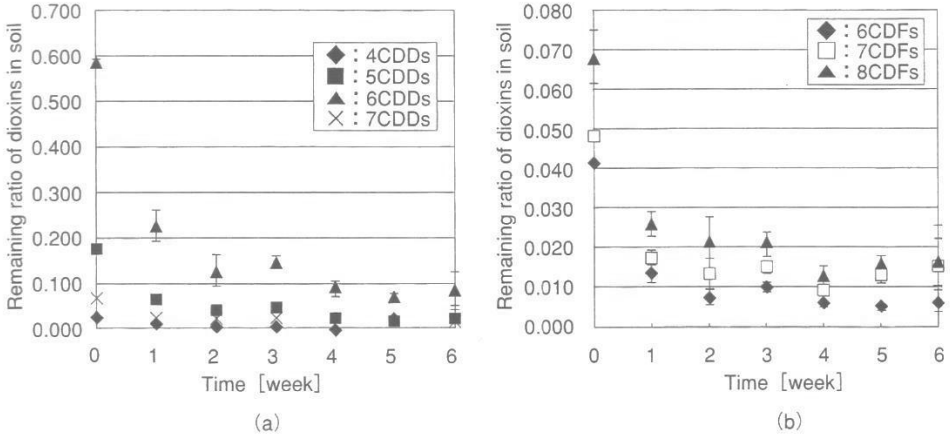


Figure 8. Change of decomposition rate of PCDDs and PCDFs after adding bacteria in the soil. (Low concentration 10 ppm of dioxin in soil and nutritional salts with bacteria)

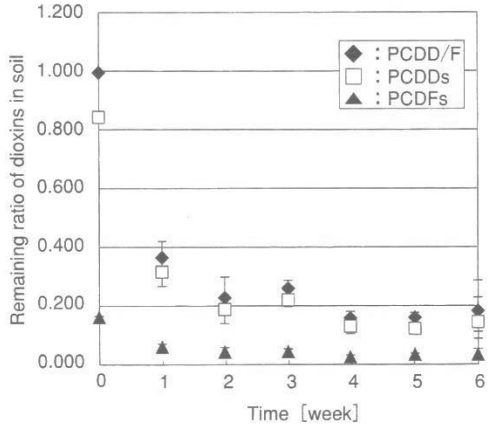


Figure 9. Total dioxin concentration change depending on time.
(Low concentration 10 ppm of dioxin in soil and nutritional salts with bacteria)

4. CONCLUSION

The mixed bacteria were collected from the water phase in crude oil deposit by adding of 5 mL of bacteria water into 10 g soil containing dioxin, then mixed and settled. High concentration 50 ppm of dioxin in soil could decompose without nutritional salts at the rate of 60% in 6 weeks. While, low concentration 10 ppm of dioxin in soil could decompose with nutritional salts at the rate of 80% in 6 weeks. In Vietnam, if the dioxins still remain in the soil, the soil remediation is important to prevent the health hazard. Though the oil deposit in Vietnam is located in the sea, there would be a possibility to decompose the dioxin in soil by using these bacteria inhabiting water separated from crude oil.

Acknowledgement: We appreciate the dioxin preparation and analysis by Mr. Hajime Muto at Environmental Research Center in Akita University, the discussion of result by Prof. Chihiro Inoue at Tohoku University and bacteria supply by JAPEX.

REFERENCES

1. EPA, Dioxin, https://www.epa.gov/dioxin
2. Kulkarni P.S., Crespo J.G., Afonso C.A.M. - Dioxins sources and current remediation technologies - A review, Environment International **34** (1) (2008) 139-153.
3. https://en.wikipedia.org/wiki/Agent_Orange
4. Ministry of the Environment, Japan, http://www.env.go.jp/recycle/dioxin/index.html
5. Strandberg J., Odén H., Nieto R. M., Björk A. - Treatment of dioxin contaminated soils, B1993, November (2011), IVL Swedish Environmental Research Institute Ltd, http://www.ivl.se/download/18.343dc99d14e8bb0f58b7600/1454339558127/B1993.pdf
6. Wu F., Kamiya O., Fujita T. and Ohyoshi T. - Experimental study on bioremediation of contaminated soil, J. Soc. of Materials Engineering for Resources of Japan **15** (2) (2002) 59-65.
7. Fujita T., Sano H., Muto H., Shibayama A., Miyazaki T. and Inoue C. - Biodegradation of polychlorinated dibenzo-p-dioxins and dibenzofurans by using bacteria inhabiting in crude oil, Shigen-to-Sozai **117** (2001) 43-48.
8. Tachyonish Holdings Co. Ltd., http://www.tachyonish.com/company/

TÓM TẮT

SỬ DỤNG VI KHUẨN TRONG DẦU THÔ ĐỂ XỬ LÝ ĐẤT Ô NHIỄM DIOXIN

Toyohisa Fujita[1*], Gjergj Dodbiba[1], Atsushi Shibayama[2], Vo Dinh Long[3]
[1]*Trường Kỹ thuật - Đại học Tokyo, Nhật Bản*
[2]*Trường Khoa học Quốc tế - Đại học Akita, Nhật Bản*
[3]*Trường Đại học Công nghiệp Thành phố Hồ Chí Minh*
*Email: *tfujita@sys.t.u-tokyo.ac.jp*

Tập hợp các vi khuẩn thu được từ pha nước của dầu thô khẳng định về sự phân hủy dioxin thông qua việc bổ sung đất, trộn và lắng. Ở nồng độ cao, 50 ppm dioxin trong đất, phân hủy diễn ra với hiệu quả đạt 60% trong 6 tuần mà không cần bổ sung muối dinh dưỡng. Nồng độ thấp, 10 ppm dioxin trong đất, phân hủy diễn ra với hiệu quả đạt 60% trong 6 tuần nhưng cần bổ sung thêm muối dinh dưỡng. Như vậy, nếu dioxin vẫn còn tồn tại trong đất ở Việt Nam, chúng ta có thể sử dụng những vi khuẩn sinh sống trong nước tách ra từ dầu thô để phân hủy.

Từ khóa: Bioremediation, đất, dioxin, phân hủy, pha nước trong dầu thô.