AN INTRODUCTION OF BIODEGRADATION SYSTEM OF DIOXINS IN CONTAMINATED WATER AND SOIL

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Introduction

Dioxins emitted from incinerators penetrate into the environment by (1) uncontrolled leakage from the facility, (2) ash drifting through the air and soil, (3) burnt ash buried in the ground into seepage water and the river sediment.

Due to the Dioxin Control Act enacted in January 15, 1999, dioxin emissions due to (1) and (2) above are being reduced in Japan[1)]. However, most of the dioxins previously emitted have still been accumulated in high concentrations in soil and the river sediment[2)]. Dioxins accumulate in human organs by food chain at about 2.1 pg-TEQ per day, and the half life of the excretion outside of the body is seven years[2)]. We have to establish a systematic remediation technology against dioxins contaminated in water and soil as soon as possible.

Bioremediation involves a complex enzymatic reaction pathway. Though there are examples of white rot fungus[3)] and the CA10 strain[4)], which requires a long time duration to destroy dioxin[5)]. Most of the microorganisms used in the research remain inert and ineffective, because they lost their ability to degrade 2,3,7,8-TCDD, due to a steric hindrance of the structure of the chlorinated dioxins. This paper covers biodegradation of polychlorinated dioxin and shows utility of the thermophilic *Bacillus midousuji* (SH2B-J2) strain, which is currently predicted a destruction effect. This SH2B-J2 strain was discovered by Hoshina[6),7)] in a compost from the tree lining the roads in the city of Osaka. This organism is aerobic with optimal growth temperature of 65C. Mutations are also easily prone to occur in this bacteria, and after the lag phase has continued for appropriate time, suddenly, it starts to grow during the log phase, with a mean generation time is 7 to 8 minutes.

This paper describes that the SH2B-J2 strain has the capability to cleave the oxygen link or ether linkage to break down dioxins. This paper further evaluates the characteristics of a bioreactor and growth conditions in degradation systems with bacterial, which is verified to act on dioxins.

Methods and Results

1. Condition of dioxin degradation of SH2B-J2 strain, and breakdown mechanism

A fluorescence substrate[8)] (synthesized from chlorinated monobenzene and esculetin) was used in a test to verify performance in breaking down dioxins, in which a high luminance fluorescent light is observed when the dioxin oxygen link or ether linkage is opened. This test performed at 65C for 18 hours, the fluorescence assay substrate was broken down by the SH2B-J2 strain when cultivated with 1 g/L of fly ash of dioxins. Cleavage reaction was detected in the oxygen link or ether linkage of the dioxin to observe an esculetin spot formation on TLC plate. The location of this enzyme generated by this induction was confirmed in the cell membrane fraction by the fluorescence assay on TLC plate also (65C, 18 hours). An enzyme in this cell membrane was showen to break down the 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7

-TCDD) under the 65C, 18 hours growth conditions by enzyme linked immunosorbent assay also.
Membrane fraction was prepared by an ultra centrifugation (50,000xG) of sonicated J2 cells by fresh culture induced by fly ash. Vigorous shaking culture was performed for 3 hours in Tripticase Soy Broth (BBL) with Yeast Extract (DIFCO).
Both the fluorescence assay and braking 2,3,7 –TCDD by ELISA using the membrane fraction suggest the enzyme within the membrane broke down the dioxin.

On the other hand, when fly ash added with dioxin contaminated water was cultivated in a sealed Erlenmeyer's flask with SH2B-J2 strain for 24 hours at 65C, polychlorinated dioxins were broken down and the dioxins toxic equivalency quantity base was confirmed to be degraded by approximately 34 %. [10)]

Also, in the 48 hour cultivation at 65C performed, which was utilized as 0.01 m^3 bioreactor, TEQ degradation level was confirmed by approximately 70 percent.

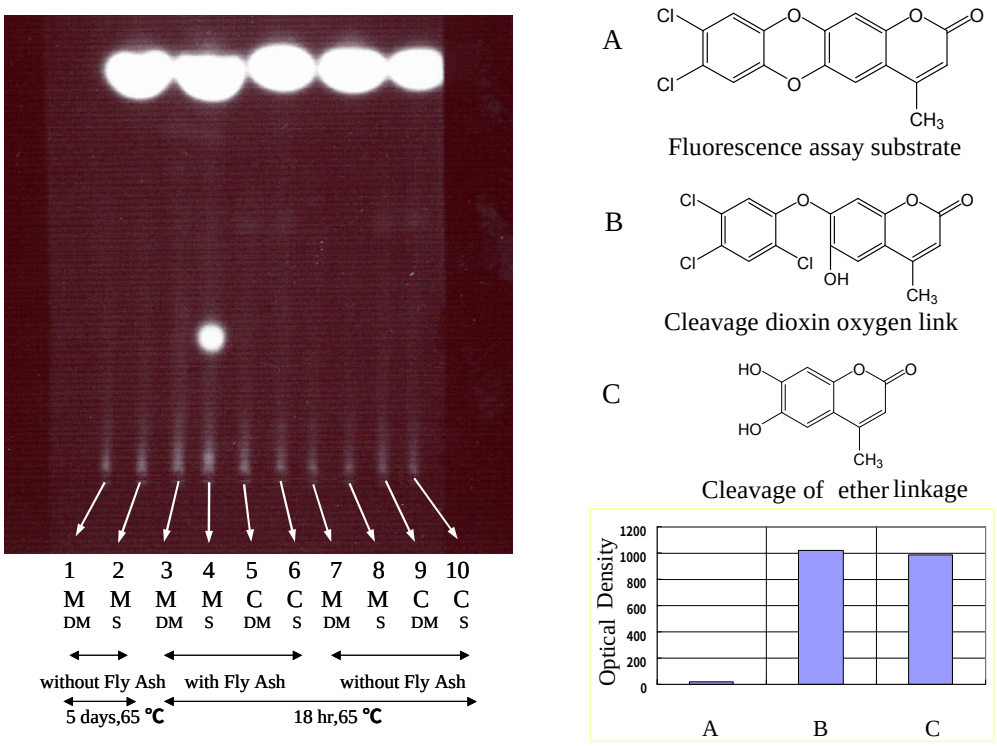

Figure 1: A spot of decomposition product from fluorescence assay substrate which is separated by Thin Layer Chromatography
Symbols ;
M : Membrane
C : Cytoplasm
DM : Dimethylsulfoxide (DMSO)
S : Fluorescence assay substrate including DMSO

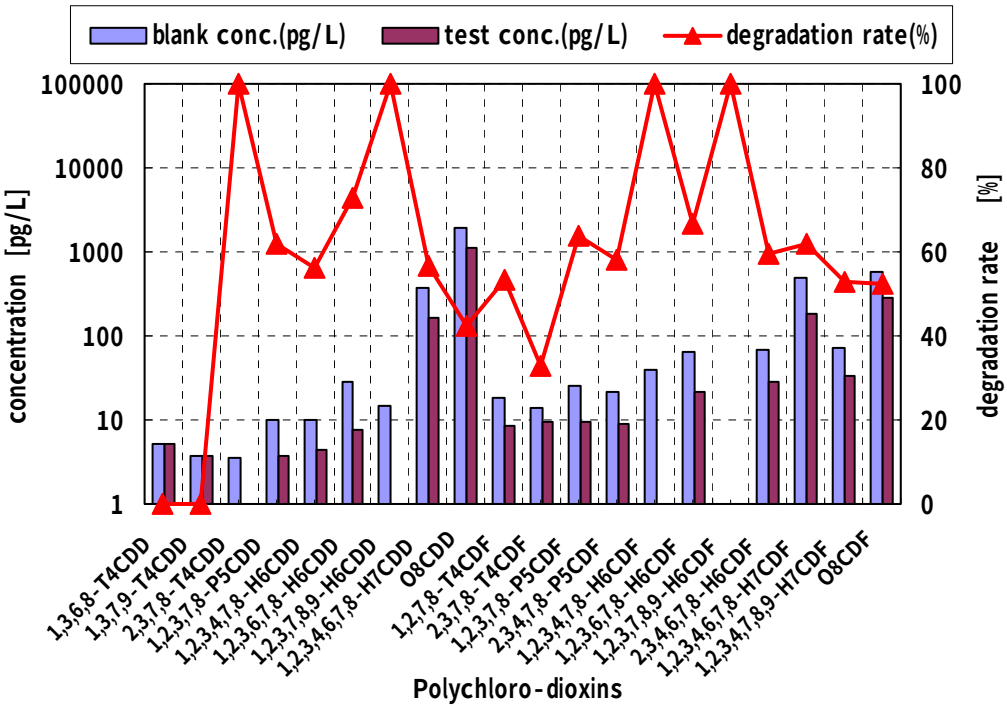

Figure 2: Each concentration on polychlorinated dioxins in the bioreactor and biodegradation rate by SH2B-J2 strain

2. Establishing technology for planning and implementing dioxin degradation

Research focused on technical condition for degradation of dioxin-contaminated water and soil lead to the following results.

(1) A bioreactor for the dioxin-contaminated water and soil with SH2B-J2 strain was designed and built. This bioreactor consisted of a batch type bioreactor on a scale of 0.01 m^3.

(2) To study growth factors for SH2B-J2 reactor, kinetic equation was calibrated. A dynamic characteristic analysis of growth was monitored from a sugar degradation of this bioreactor and confirmed that experimental results with bacteria concentration, oxygen potential and substrate concentration matched to the kinetic equation[9]. It was found that the oxygen consumption rate is a major factor on growth of the SH2B-J2 strain [10]. In the post-log phase, the analytical code was used to determine that the overall volumetric coefficient required for maintaining oxygen potential of approximately 1 ppm or more is 20 h^{-1}.

(3) The actual design specifications for the batch type bioreactor determined air permeability by this experimental equation.

(4) Oxygen potential is the primary element essential to growth of the SH2B-J2 strain. The additives were also analyzed to determine the optimal concentration of medium , calcium ions, dimethylsulfoxide (DMSO) and zeolite stone. Mainly, the minimum amounts required were determined for each additive

(except DMSO) from the point of the operation costs. It was found that there is threshold on DMSO concentration ,which if it is exceeded,DMSO interfered with the culture growth. These results will give a basic data for future evaluation of actual operation ofculture method.

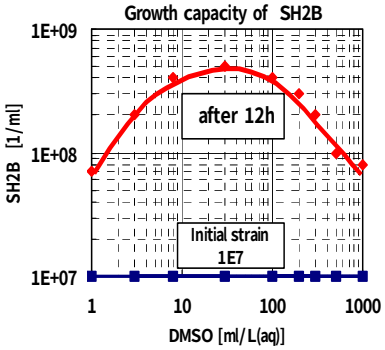

Figure 3: Growth of SH2B-J2 strain after 65C, 12 h on various added DMSO concentration.

Discussion

The breakdown of the dioxin and fluorescence assay with the SH2B-J2 strain indicated that the extremely minute decomposition product was a compound of a naphthalene-like sulfate group with a molecular weight near 420. This product suggests a metabolite with glutathione-S-transferase or arylsulfotransferase substituted into the sulfite-group after cleavage of a hydroxyl-group. With the emission of dioxin reduced, the amount of dioxins entering the environment decreased. However, there is still no technology for degradation of dioxin effectively and economically in the environment in the past. The study performed here for biodegradation of dioxins will surely prove a significant step towards a practical and low-cost technology.

Acknowledgement

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