Examination of Dioxin Degradation Conditions for Geobacillus midousuji SH2B-J2.

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Introduction
Cellular membrane degradation capability of SH2B-J2 strains[1)] for dioxin mixtures that are greater than tetra-chlorinated have been examined. Optimal temperature, reaction time, optimal pH, and heavy metal resistance of cellular membrane enzyme were investigated, to examine dioxin degradation characteristics of SH2B-J2 strains. For chlorinated dioxins, a mixture (PCDD/PCDF mix) containing 7 species of dibenzo-p-dioxins greater than tetra-chlorinated, as well as 10 isomers of dibenzofurans, was used. Using GC/MS, decrease of 17 species of dioxin isomer/congener was measured.

1. Materials and Methods
(1) Preparation of Cellular Membrane Enzyme [2)] from SH2B-J2
SH2B-J2 was cultured in an agar with 3% Tripticase Soy Broth (BBL)(TSB) and 0.3% Yeast Extract (TSB+YE). For induction of dioxin degrading enzymes, PCDD/PCDF mixture containing 1000 pg of isomer/congener was added to 500 ml broth. Broth was cultured for 3 hours at 65 . Cultured bacteria was sonicated, and centrifuged, to obtain membrane. Membrane obtained was used as cellular member enzyme.
(2) Temperature conditions
500 ml of 1/4 TSB/YE (broth diluted 4x containing 500 pg of PCDD/PCDF isomer/congener mix) was prepared, and then the entire cellular membrane was added. Mixture was divided equally to five 100-ml aliquots. Reaction was conducted at temperatures of 4 , 20 , and 65 along with control. Reaction took place in static sealed glass flasks. Reaction time was120 hours. During reaction, hydrogen ion concentration was maintained at pH 7. Control was broth reacted for 72 hours at 65 after adding concentrated hydrochloric acid. PCDD/PCDF mix from enzyme induction culturing was also used for degradation reaction.
(3) Reaction Time
Reaction was started for control, 1 Day, 2 Days, and 7 Days. Reaction took place in static sealed glass flasks, with reaction temperature of 65 . pH during reaction was maintained at 7. PCDD/PCDF mix from enzyme induction culturing was also used for degradation reaction.

(4) pH Conditions

Reaction was started for control, pH 3, pH 11, and pH 13. Reaction took place for 168 hours in static sealed glass flasks, with reaction temperature of 65 . In this case cellular membrane enzyme was prepared by culturing at 65 for 3 hours without adding any substances that induce dioxin degradation enzyme.

(5) Heavy Metal Resistance of Cellular Membrane Enzyme

Reaction was started for control, and heavy metal kit concentration of 1 ppm, 10 ppm, and 100 ppm. Reaction took place for 168 hours in static sealed glass flasks, with reaction temperature of 65 . pH during reaction was maintained at 7. Cellular membrane enzyme was prepared by culturing at 65 for 3 hours without adding any substances that induce dioxin degradation enzyme.

2. Results

(1) Temperature Conditions

Dioxin concentration for control was initially 19400 pg (Toxic Equivalent: 3816 pg-TEQ). After 72 hours of reaction at 65 , dioxin concentration decreased to 13010 pg (Toxic Equivalent: 1680 pg-TEQ). This meant that dioxin concentration decreased by 33%, and toxic equivalent dropped 56% after 72 hours of reaction. Decrease is more prominent in lesser-chlorinated dioxins. On the other hand, no dioxin degradation was observed at 4 and 20 . These results show that optimal temperature of cellular membrane enzyme activity in case of SH2B-J2 strain is 65 .At 65 , no evaporation of Dioxin was observed in this experimental systems.

(2) Reaction Time

Dioxin concentration for control was initially 18710 pg (Toxic Equivalent: 4160 pg-TEQ). After 1 day of reaction at 65 , dioxin concentration decreased to 14360 pg (Toxic Equivalent: 2380 pg-TEQ). After 2 days of reaction, dioxin concentration decreased to 12240 pg (Toxic Equivalent: 1729 pg-TEQ). After 7 days of reaction, dioxin concentration decreased to 7305 pg (Toxic Equivalent: 561 pg-TEQ). Dioxin concentration decreased by 34%, and toxic equivalent dropped 58% after 2 days of reaction. These results are close to 3 reaction day temperature-based dioxin degradation. For 7-day reaction period, dioxin concentration decreased by 61%, and toxic equivalent dropped 85%. In long-term reaction, it seems that cellular membrane enzyme is heat resistant, since continuation of degradation activity was observed.

(3) pH Conditions

Dioxin concentration for control was initially 8350 pg (Toxic Equivalent: 1713 pg-TEQ). At pH 3, dioxin concentration decreased to 2295 pg (Toxic Equivalent: 111 pg-TEQ). At pH 11, dioxin concentration decreased to 1446 pg (Toxic Equivalent: 46 pg-TEQ). At pH 13, dioxin concentration decreased to 927 pg (Toxic Equivalent: 28 pg-TEQ). It meant that at pH 3, dioxin concentration decreased by 73%, and toxic equivalent dropped 94%, and at pH 13, dioxin concentration decreased by 89% and toxic equivalent dropped 98%. These results show that dioxin degradation activity is dependent on initial dioxin concentration. Cellular membrane enzyme activity increase as broth pH increase, meaning that better results can be obtained under alkali conditions. On the other hand, as level of dioxin chlorination decrease, dependence on pH level decreases as well.

(4) Heavy Metal Resistance of Cellular Membrane Enzyme

Dioxin concentration for control was initially 10730 pg (Toxic Equivalent: 2127 pg-TEQ). For sample with 1 ppm of heavy metal added, dioxin concentration decreased to 7420 pg (Toxic Equivalent: 1087 pg-TEQ). This meant that dioxin concentration decreased by 31%, and toxic equivalent dropped 49%. The result is comparable to reaction temperature dependent (65 for 3

days) and reaction time dependent (65 , 2 days) test results. It means that heavy metal does not disrupt enzyme reaction at around 1 ppm. However, when more than 10 ppm of heavy metal is added, dioxin concentration became no different from that of control, signifying enzyme disruption.

3. Discussions

In the temperature-dependent test, decrease of 13010 pg (Toxic Equivalent: 1680 pg=TEQ) was observed after 3 days at 65 for control that had initial concentration of 19400 pg (Toxic Equivalent: 3816 pg-TEQ). Decrease, when compared to initial dioxin concentration, is 56%, at rate of 29.7 pg-TEQ/h.

When 1 ppm of heavy metal is added, decrease of 7420 pg (Toxic Equivalent: 1087 pg=TEQ) is observed after 3 days at 65 for control that had initial concentration of 10730 pg (Toxic Equivalent: 2127 pg-TEQ). Decrease, when compared to initial dioxin concentration, is 49%, at rate of 14.4 pg-TEQ/h.

Decrease in toxic equivalent from initial dioxin concentration is similar for all samples, but rate of decrease is higher for those with higher initial concentration. This is most likely because collision frequency of degrading enzyme and dioxins is controlled, and after such collision, reaction of ether bonds and cleavage of enzyme-linkage immediately takes place.

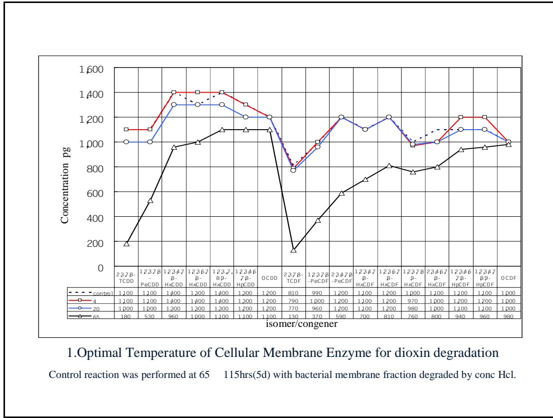
For concentration dependency, there is a need to elucidatereaction parameter and limiting concentration for dioxin degradation. It seems that for dioxin-contaminated water and soil treatment system, a pretreatment system to either detoxify or reduce/concentrate contaminated water/soil, is required.

4. Acknowledgement

We greatly thank to Mr. Hiroshi Miyata and Mr. Akira Uchibo of Kurita Water Industries LTD. to analyse the sample materials. We also very thank to the research group entitled "Research for the construct of Bio-reactor to degrade dioxins in contaminated soil and sludge"(K1513) by Ministry of Environment in Japan.

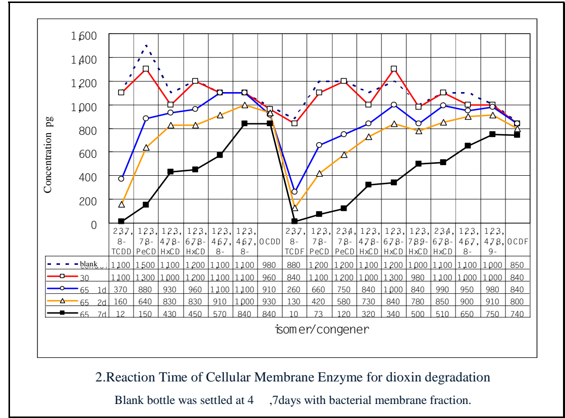
5. References

1) Nazina T.N, Tourova T.P, Poltaraus A.B, Novikova E.V, Grigoryan A.A, Ivanova A.E, Lysenko A.M, Petrunyaka V.V, Osipov G.A, Belyaev S.S. Ivanov M.V.: Taxonomic study of aerobic thermophilic bacilli: descriptions of Geobacillussubterraneus gen, nov., sp. nov. and Geobacillus uzenensis sp. nov. from petroleum reservoirs and transfer of Bacillus stearothermophilus, Bacillus thermocatenulans, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermodenitrificans to Geobacillus as the new combinations G. stearothermophilus, G. th. Int. J. Syst Evol Microbiol. 51, 433-446 (2001)
2) Favaloro B, Tamburro A, Trofino M.A, Bologna L, Rotilio D, Heipieper H.J: Modulation of the glutathione-S-transferases in Ochrobachtrum anthrop; function of xenobiotic substrates and other forms of stress, Biochem.J. (2000)



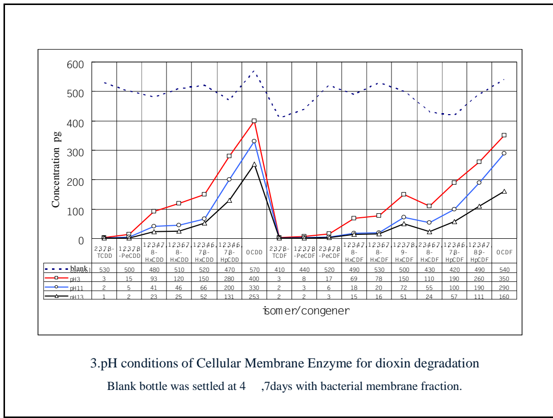
1.Optimal Temperature of Cellular Membrane Enzyme for dioxin degradation

Control reaction was performed at 65 115hrs(5d) with bacterial membrane fraction degraded by conc Hcl.



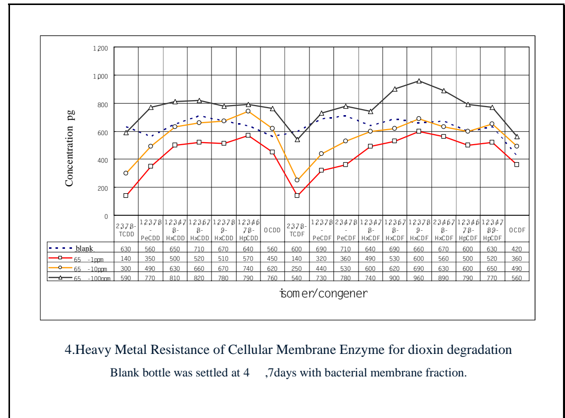
2.Reaction Time of Cellular Membrane Enzyme for dioxin degradation

Blank bottle was settled at 4 ,7days with bacterial membrane fraction.



3.pH conditions of Cellular Membrane Enzyme for dioxin degradation

Blank bottle was settled at 4 ,7days with bacterial membrane fraction.



4.Heavy Metal Resistance of Cellular Membrane Enzyme for dioxin degradation

Blank bottle was settled at 4 ,7days with bacterial membrane fraction.