오염토양으로부터 Toluene 분해 미생물의 분리 및 Toluene 분해 특성

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Isolation and Characterization of Toluene Degradation of Microorganisms Degrading Toluene from Contaminated Soil

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Introduction

The hydrocarbon has contaminated frequently groundwater and soil, because of the extensive use and unsuitable treatment of organic compounds and oil products in the world. Most of soil pollution unnoticed because large portion of soil is hidden from view and the effect is often slow[1].

Major material of soil pollution is known oil, oil compounds, and toxic inorganic · organic compounds. these pollutants have been increasingly diversified in species and toxicity. Specially, toluene has been classified as priority pollutants by the EPA(Environmental Protection Agency)[2]. Toluene is present in gasoline at 5~7%(wt/wt) and has a solubility of 515 mg/L in water 20℃. The EPA has recommended that drink water contain not more than 2 mg/L of toluene for lifetime exposure. and found toluene in 54% of groundwater samples near chemical waste sites, in 1988[3]. Bioremediation is the act of adding materials to contaminated environments to cause an acceleration of natural biodegradation process and biodegradation is known to be the principal natural process for the removal of the nonvolatile fraction of organic compounds and oil from the environment[4].

Bioremediation involves the use of naturally occurring microorganisms to degrade and detoxify hazardous constituents in the soil at a contaminated site to protect public health and the environment[5]. Hence, one possible approach to bioremediation is to prepare a pure culture of microorganisms and to use context, and the screening of potential hydrocarbon degrading organisms is one of the key steps[6].

The present study purpose to isolate of microorganisms degrading toluene as the sole source of carbon and energy from contaminated soil and to confirm their characterization by enrichment culture, with the intention of bioremediation of contaminated soil by isolated microorganisms degrading toluene.

Materials and Methods

Media : The minimal medium for isolation of bacteria utilizing aromatic compounds was composed of 1g NH_4Cl, 4.35g K_2HPO_4, 3.9g $NaH_2PO_4 \cdot 2H_2O$, 0.48g $MgSO_4 \cdot 7H_2O$,

0.03 $CaCl_2$, 5 mL of a trace elements solution in 1L distilled water. The trace elements solution contained 0.6g H_3BO_3, 2g $MnCl_2 \cdot 4H_2O$, 0.4g $ZnSO_4 \cdot 7H_2O$, 0.2g $Na_2MoO_4 \cdot 2H_2O$, 0.2g $CuSO_4 \cdot 5H_2O$ and 0.2g $Co(NO_3)_2 \cdot 6H2O$ in 1L distilled water. Toluene as a sole carbon and energy source was added sterilized minimal medium. The LB medium for isolation and preservation of microorganisms degrading toluene was composed of Trypton 10g/L, Yeast extract 5g/L, NaCl 8g/L. LB agar plate medium added agar 1.5 %(w/v) to LB medium. A initial pH was adjusted to 7.0.

Isolation and culture of microorganisms : The soil samples, which obtained from a depth of 10 ㎝ of contaminated soil around the gas station in April, in Suwon, were suspended in 250 mL of sterilized distilled water and shaken for 1 hour on shaking incubator. Then 10 mL of the supernatant of the suspension were incubated in a minimal medium containing toluene as a sole source of carbon for enrichment. Cultures were repeatedly transferred to fresh minimal medium for three times at 24 hours intervals.

From the enriched cultures, a one drop of culture was spread on LB agar plate. Isolated colonies were transferred to fresh minimal medium and repeated until a pure culture was obtained. Each isolates was transferred to LB agar plate and was tested their biodegradable activity of toluene in minimal medium.

All isolates were maintained in agar plate medium and preserved in refrigerator at 4 ℃ and liquid nitrogen.

The seed culture was carried out in a 250 mL flask containing toluene of 1000 ppm and sterilized minimal medium of 50 mL at 30 ℃, 150 rpm for 20 hours, and mixed PY seed culture were inoculated two colonies of P and Y strain, respectively. The cultured seed was harvested by centrifugation at 8000 rpm for 20 min and washed twice with KCl buffer of 15 mL, and suspended in the KCl buffer.

The Suspended seed (O.D=1.0) was inoculated 2 % of minimal medium. The culture for determination of biodegradable activity of toluene by temperature, rpm, and initial pH was carried out in 1000 mL flask containing the sterilized minimal medium of 250 mL and toluene of 1000 ppm.

Analycal methods : Determination of cell growth was accomplished by UV spectrophotometer(Duksan OPTIZEN) at 640 nm. The optical density was indicated to compare with dry cell weight. Dry cell weight was measured as follows; 10 mL of culture broth was centrifuged at 8000rpm for 10 min. The packed cell was washed twice with distilled water. The washed cell was dried in a vacuum oven at 80 ℃ for 12 hrs, and then weighted.

The concentration of toluene were determined using Gas Chromatography equipped with a flame ionization detector(FID). The sample of batch-culture broth was filtered through membrane filter (pore size: 0.45 ㎛) and the sample were extracted for 10 minutes using dichlorobenzene. Calcium chloride was added to remove water in samples. A Column fused silica capillary was used. The temperature of injector and detector were 200 ℃ and 280 ℃, respectively. The column temperature was initially maintained at 110 ℃ for 2 min., raised 10 ℃/min and finally maintained at 220 ℃ for 2 min. The flow rate of nitrogen as carrier gas was 15 mL/min. The pH was

determined using pH meter(Orion 410A). Initial pH was adjusted by 2N NaOH and HCl.

Results and discussion

Microorganisms degrading toluene were finally isolated seven different strains from contaminated soil by enrichment culture. The dry cell weight of the isolated seven different strains T_2, T_3(yellow), T_8, T_{11}, T_{12}(pink), T_{14}, T_{18} was indicated 0.289 g/L, 1.55 g/L, 0.883 g/L, 0.724 g/L, 2.376 g/L, 0.347 g/L, 0.132 g/L, respectively. Only two strains(Pink and Yellow) of them were selected as experimental strains because of their high degradable ability and cell growth.

To investigate the optimal conditions for biodegradation of toluene by P and Y strains in batch culture, the temperature changes from 25 ℃ to 35 ℃ and initial pH changes from pH 5 to pH 8.

The effect of temperature on the growth of P(pink), Y(yellow) strain and mixed PY strains in a minimal medium containing toluene of 1000 ppm at 125 rpm, initial pH 7.0 was indicated in fig. 1, fig. 2, and fig. 3, respectively. On the change of cell growth, P and Y strain were better as follows 30 ℃, 25 ℃, 35 ℃, and mixed PY strains were better as follows 30 ℃, 35 ℃, 25 ℃, respectively. The effect of initial pH on the growth was indicated the all maximum cell growth at initial pH 7.0.

Time courses of cell growth and toluene concentration by P strain and mixed PY strains at 30 ℃, 125 rpm and initial pH 7.0 were indicated fig. 4. The degradable ability of toluene was better P strain than mixed PY strains. Toluene of 1000ppm was perfectly degraded before 20 hours culture by P strain and mixed PY strains.

Therefore, The strain degrading toluene for bioremediation of contaminated soil was selected P strain. The optimal conditions of P strain was 30 ℃ and initial pH 7.0.

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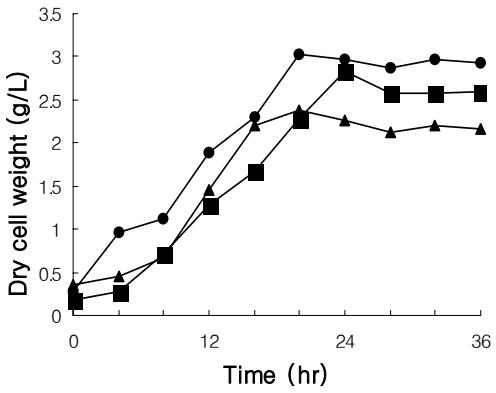

Fig. 1. Effect of temperature on the grow of P strain
(-■- : 25℃, -●- : 30℃, -▲- : 35℃)

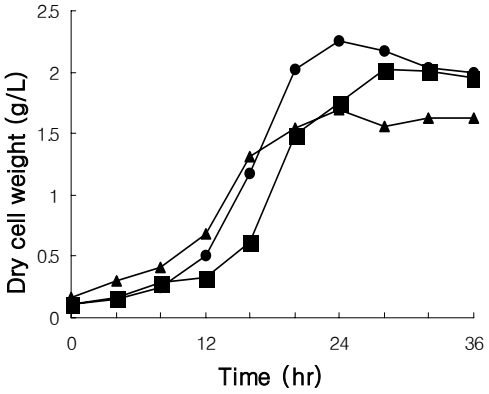

Fig. 2. Effect of temperature on the grow of Y strain
(-■- : 25℃, -●- : 30℃, -▲- : 35℃)

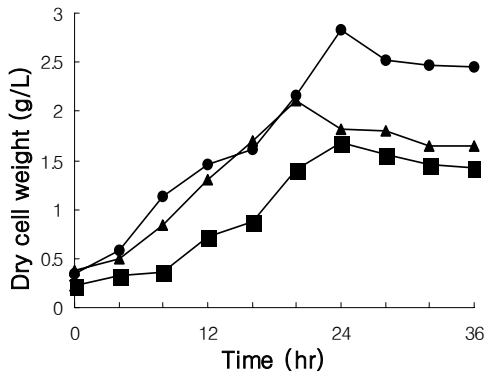

Fig. 3. Effect of temperature on the grow of mixed PY strains
(-■- : 25℃, -●- : 30℃, -▲- : 35℃)

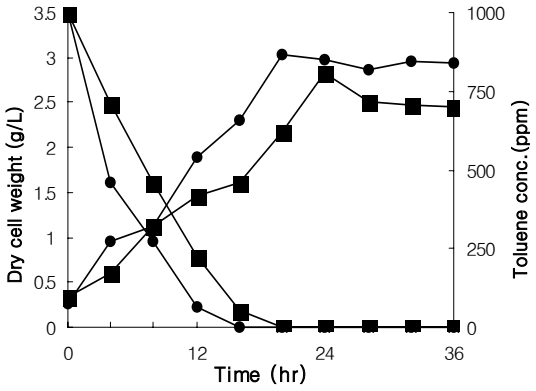

Fig. 4. Time courses of cell growth and toluene concentration by P strain and mixed PY strains.
(-●- : P strain, -■- : mixed PY strains)