유류오염토양에서의 Gasoline분해균의 분리 및 Gasoline 분해 특성

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Isolation of Gasoline degradable from petroleum pollution soil and their degradation Characteristics of Gasoline

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Introduction

Demand in petrochemicals increased as the heavy and the chemical industry developed according to the fast growth of industry nowadays, as a result, the amount of consumption of gasoline has dramatically increased. Pollution from underground gasoline storage or gasoline station and that from mistreated soil and industrial complex does not only pollute the soil but also contaminate subsurface water, river, and atmosphere. Consequently, it is demanded to control and manage various pollutants by understanding the condition[1].

Soil environment maintenance law revised in 2001 designates, excluded animal and oil, BTEX(benzene, toluene, ethylbenzene, xylene), and TPH(total petroleum hydrocarbons) as standards for petroleum pollution soil, pollution petroleum basis in BTEX is 80 mg/kg and soil pollution measures basis is 200mg/kg. In TPH's case, concern basis is 2,000 mg/kg and measures basis is 5,000 mg/kg[2]. According to a controlling policy of soil pollution occurrence equipment in ministry of environment of 1999, soil which has been contaminated by oil is 800 mg/kg[3]. The need to analyse petroleum component in soil is being increased in soil environment relation reseach and industry by the fact that 12,007 gasoline stations occupy 61% of reported soil pollution occurrence equipment among 19,625 which include 16,411 gasoline storage facilities like gasoline stations according to ministry of environment research in 1999[4].

Especially most pollutants which transfer through soil has hydrophobic property which means they are hard to be ressoluted in water. Consequently soil pollutants can not move easily and their transfer routes are complicated. as a result, it has been hard to develop the technology even though the damage from them has been more standed out than that from water and air[5]. The main causes of soil pollution is known as oil, oil compounds, and toxic inorganic · organic compounds. As the ill effects of these materials on natural environment and human have been known and

been concerned about, methods to restoration technology have been developed continuously. Among those bioremediation which applied decomposition by biological method is being payed a lot attention[6].

In this research we are analysing the senergy effect of gasoline degradable ability by picking and selection or combining microorganisms which has the most degradable ability on gasoline and the soil pollutant from contaminated soil.

Materials and Methods

Media : The composition of a minimal medium for isolation of bacteria utilizing gasoline was composed 1g NH_4Cl, 4.35g K_2HPO_4, 3.9g $NaH_2PO_4 \cdot 2H_2O$, 0.48g $MgSO_4 \cdot 7H_2O$, 0.03g $CaCl_2$, 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 $CuSO_4 \cdot 5H_2O$, 0.2g $Co(NO_3)_2 \cdot 6H_2O$, NaCl in 1L distilled water. Gasoline as a sole carbon and energy source is added to sterilized minimal medium. The medium for isolation and preservation of bacteria degrading gasoline was composed of Trypton 10 g/L, Yeast extract 5 g/L, NaCl 8 g/L. LB agar plate medium added agar 1.5 %(w/v) to LB medium. 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 gasoline station in May, 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 gasoline 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 gasoline 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 gasoline of 1000 ppm and sterilized minimal medium of 50 mL at 30℃, 125 rpm for 36 hours.

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

Results and disscussion

Microorganisms degrading gasoline were finally isolated six different strains from

contaminated soil by enrichment culture. The dry cell weight of the isolated six different strains G1, G2, G3, G4, G5, G6 was indicated 1.726 g/L, 1.586 g/L, 1.9 g/L, 0.972 g/L, 1.359 g/L, 2.716 g/L, respectively. Two strains(G3 and G6) of them were selected as experimental strains because of their high degradable ability and cell growth. To investigate the optimal conditions for biodegradation of gasoline by G3 and G6 in batch culture, the temperature 30℃ and initial pH 6 changes from to pH 8.

The effect of pH on the growth of G3, G6, and mixed G3 and G6 strains in a minimal medium containing gasoline of 1000 ppm at 125 rpm, temperature 30℃ was indicated in fig 2, fig 3, and fig 4, respectively. On the change of cell growth, G3 and G6 were better as follows pH 6.0, pH 7.0, pH 8.0, and mixed G3 and G6 strains were better as follows pH6.0, pH 7.0, pH 8.0, respectively.

Gasoline is made of many different kinds of hydrocarbon. Aliphatic hydrocarbons are more easily than aromatic hydrocarbons resolved generally. If early microbe growth is high, the microbe is thought of as the microbe which can resolve aliphatic hydrocarbon well. A case of the microbe which is high by growth later, decomposition rate of aromatic hydrocarbons will be high.

As for the growth of an initial microbe, G3 is high. And, as for the microbe growth, G3 is high 20 hours later.

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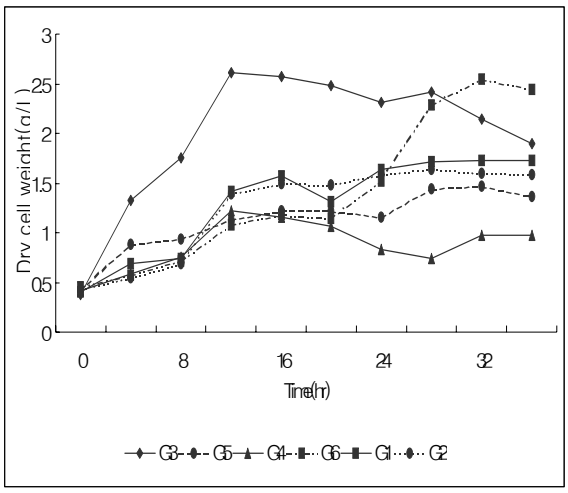

Fig 1. Effect of pH on the growth of microbes strain.

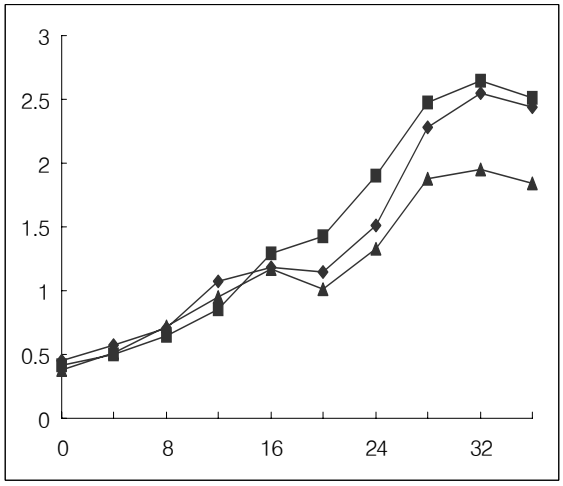

Fig 2. Effect of pH on the growth of G3 strain.
(-■-: 6.0, -◆-: 7.0, -▲-: 8.0)

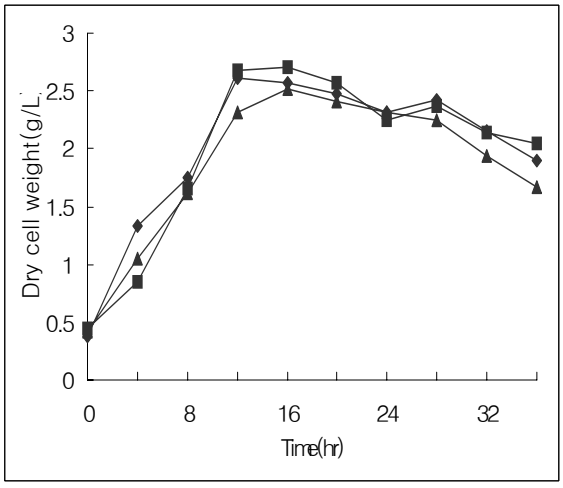

Fig 3. Effect of pH on the growth of G6 strain.
(-■-: 6.0, -◆-: 7.0, -▲-: 8.0)

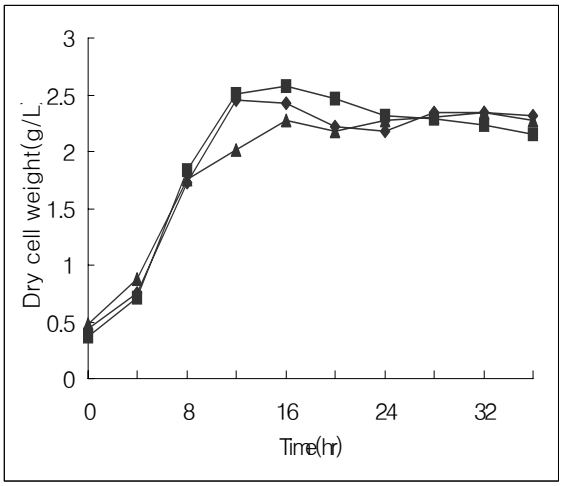

Fig 4. Effect of pH on the growth of G3G6 strain.
(-■-: 6.0, -◆-: 7.0, -▲-: 8.0)