Menefee Humate와 혼성균체의 고정화를 이용한 토양내 탄화수소물의 Bioremediation

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Bioremediation of Hydrocarbons in Soil by Immobilized MIxed Cells on Menefee Humate

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Introduction

Granular Menefee humate is a naturally occurring carbonaceous material with high humic acid. The physical and chemical characteristics of humate is fairly unique in that the organic and mineral constituents of the humate is known to stimulate microbial activity in soil by supplying a carbon energy source for growth and metabolic activity to the soil microorganisms. For this reason, the material has commercially been applied to soil to improve soil structure and moisture retention.

The use of humate with soils may offer a substantial advantage to bioremediation systems compared to conventional systems solely depending on soils. Compared to previous soil systems, the humate-soil system provides (1) higher nutrient level (2) higher microbial activity and population, and (3) better soil structure. In addition, the humate seems to play a major role as a source of microbial supply to the soil where the microorganisms are immobilized and nurtured until they eventually propagate.

In the past, the use of humate has been investigated mainly in the area of the agriculture and mining. *Gosz et. al.* evaluated the effect of humate on mine spoils and plant growth [8]. The extent of the research includes the application in which microbial growth was promoted under sea [9]. However, no efforts have been made to use the humate and mixed culture to bioremediate hydrocarbon contaminants.

Brown and other researchers found that mixed cultures of hydrocarbon-degrading bacteria were more effective in degradation than were individual isolates [3,6,12]. However, seeding hydrocarbon contaminants to effectively promote degradation will have to be accompanied by additional nutrients in most cases[3].

Humate itself is not capable of carrying living cells with high potency for a considerable period despite the fact that some applications have been used with inorganic carriers such as sands [2,11]. Certain improved techniques should be followed for a better immobilization of living microbial cells. Currently, the use of immobilized cells have been under extensive exploration on enzyme immobilization to overcome many limitations[1,4,5,7,10].

Current research has considered the immobilization of mixed cells on humate using binder and the application of immobilized biocatalysts to the bioremediation of hydrocarbons in soil [Fig. 1]. The efficiency of the biosystem was found followed by a comparison between the use of immobilized cells by noble means and the conventional use of microorganisms.

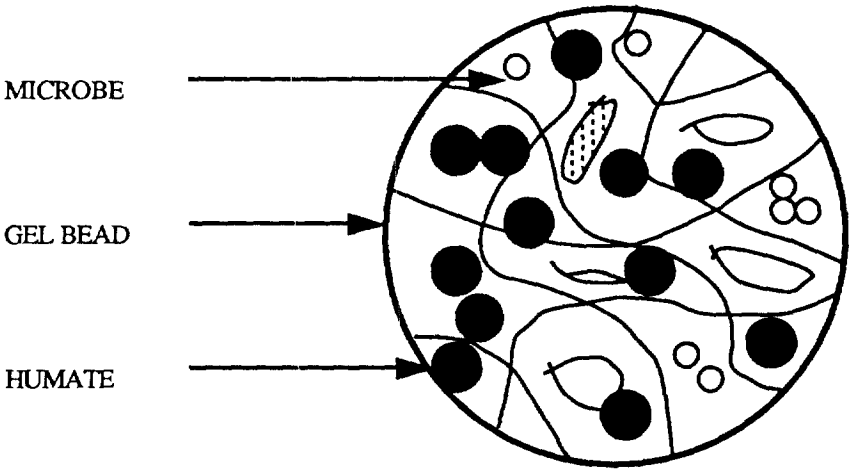

Figure 1 Schematic Diagram of Immobilized Cells

Material and Method

The bioreactors were developed for batch bioreaction. One such bioreactor was composed of an Erlnmeyer flask on a shaker and another consisted of a mixture of contaminated soil and humate in a reactor column with ventilation tubes. In the latter case, an air feed was used when the saturated oxygen was required. In addition, BOD test tubes were adopted as batch reactors when the remediation rate was measured with BOD. The reactor performance was evaluated for the biodegradation of hydrocarbons by microorganisms under the influence of humate over a range of time. The operating conditions are near room temperature (28C) and atmospheric pressure.

Analytical- The reduction of sugar in the soil and slurry samples was determined using dinitrosalicylic acid. The residual hydrocarbon in the soil was determined followed by the solvent extraction. Biodegradation of glucose and hydrocarbons in slurry was measured by biological and chemical oxygen demands. The microbial population was estimated by measuring the dehydrogenase activity of the mixed culture in the soil.

Experiments

The kinetic behavior of an aerobically mixed culture was studied in a well stirred batch reactor and soil column reactor with glucose and several hydrocarbons as then sole source and limiting factor, respectively. The growth rates with glucose and hydrocarbons were obtained experimentally at various conditions. The effect of humate, cell carrier, seed, recycling of immobilized cells, and bead size were investigated in terms of pH, biodegradation and microbial activity.

The performance of immobilized mixed cells on biodegradation of hydrocarbons in soil was investigated in both slurry and soil bed. A mathematical model using Monod kinetics was chosen to fit the experimental data and the Lineweaver-Burk method was employed for plotting the data to obtain the specific growth rates and saturation constants. The biodegradation rates in BOD tests were obtained from the Thomas graphical method.

Results

The immobilization of mixed culture was made in alginate gel with an addition of Menefee humate. The beads sizing about 3 mm in diameter were produced and applied to both glucose and hydrocarbons in biodegradation experiments with satisfactory results.

Co-immobilizing humate in gel with mixed culture resulted in a better biodegradation performance than other applications.

The results from the glucose consumption by microbes showed the effects of parameters and variables such as humate content, solute concentration, bead size and cell recycling on biodegradation.

The specific growth rate and rate constants (calculated from the bioreaction of the slurry batch reactor) were similar to those of the reported values in other applications.

The internal diffusional limitation was negligible when the gel bead size was less than 3 mm and the glucose concentration was larger than 1.5 g/l.

Discussion

The primary goal of this work was to understand a co-immobilized system, humate and mixed culture in alginate gel and to use the information to evaluate the performance of a novel immobilized system on the biodegradation of hydrocarbons. A series of experiments was conducted to study the effects of parameters such as humate content, solute concentration, cell recycling, bead size, and cell carrier influencing the system performance in terms of K_m, V_m and k.

Although some favorable features of humate on biological effect were reported widely and also expected in this research, the acidic nature of humate made it difficult to use Menefee humate as a free powder in a slurry. However, the results of performance by co-immobilized humate-mixed culture clearly showed that the inclusion and immobilization of humate inside gel did not affect pH significantly and boosted biological growth and activity.

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