

## Mobilization and Biodegradation of 2-Methylnaphthalene by Amphiphilic Polyurethane Nano-Particle

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**Abstract** Amphiphilic polyurethane (APU) nano-particle enhanced the mobilization of 2-methylnaphthalene (2-MNPT) in soil. Significant increase in the solubility of 2-MNPT was achieved. The molar solubilization ratio was 0.4 (mole 2-MNPT/mole APU). Simple precipitation of APU particle by 2 N  $\text{CaCl}_2$  recovered 95% of APU particle and 92% of 2-MNPT simultaneously. Also, 2-MNPT, which was entrapped inside the APU particle, was directly degraded by *Acinetobacter* sp. as same efficiency as without APU particle. These results showed the potentials of APU particle in the mobilization and biodegradation of hydrophobic compounds from soil.

**Keywords** APU particle · 2-Methylnaphthalene · Soil washing · Polymeric surfactant · Biodegradation

### Introduction

Soil contamination caused by hydrophobic compounds such as petroleum products, solvents, heavily used industrial products, and insecticide is one of the worldwide environmental problems. At contaminated soil sites, those hydrophobic compounds also contaminate groundwater. Although those compounds have low solubility in water (ppm and below), they remain very toxic even at extremely low concentration [2, 4, 8, 9, 11, 13, 20, 22, 23]. Therefore, soil contamination caused by those compounds causes fatal problem to people using the groundwater as a source of drinking water. When confronted with the shortage of drinking water based on surface water in the near future, developing and applying groundwater as a source of drinking water is going to be one of the important alternatives.

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A common remediation process of soil and groundwater at contaminated sites is based on the extraction of groundwater by wells or drains, followed by the treatment of the extracted water, a method called pump-and-treat remediation [21]. Unfortunately, many hydrophobic organic liquids that are common groundwater pollutants cannot be efficiently extracted by the pump-and-treat method because of their low solubility in water and high interfacial tensions. Recently, surfactant-enhanced remediation techniques have been proposed for the potential use in *in situ* soil remediation and as an improvement in the pump-and-treat technology. Surfactants can increase extraction of hydrophobic materials through micelle solubilization and mobilization of such materials through a reduction in their interfacial tension.

Such an approach for the extraction of pollutants has been pursued extensively in recent years [1, 3, 6, 12, 14, 26, 27, 28, 31]. The use of surfactants presents, however, several drawbacks that led us to develop a different approach using amphiphilic nano-network particles. The main disadvantages of surfactant-enhanced soil washing are the breakage of micelle, loss of surfactant through sorption to soil, and the formation of hydrophobic pollutant emulsion in the soil pores during the remediation process [21, 25, 28, 30]. Large-scale studies of surfactant-enhanced remediation have shown few promising results because of the high cost of the process caused by surfactant loss [12, 17–19, 29]. Recent research has been directed towards the design of surfactants that minimizes their losses and the development of surfactant recovery and recycling techniques [10, 12, 19, 24, 29, 30].

Sorption and breakage of surfactant micelles during soil washing lead us to use amphiphilic nano-network polymer particles dispersed at water for removal of hydrophobic pollutants. These particles make up a micelle-like structure at water phase and their structures are permanently locked-in by chemical cross-linking reaction. These amphiphilic polymer particles can be synthesized by amphiphilic polyurethane precursor chains that have hydrophilic and hydrophobic segments at the same molecules like surfactant [15, 16]. On mixing with water, hydrophilic and hydrophobic segments at these chains are phase separated with each other and form a micelle-like structure at water phase. These structures are locked-in by the reaction between reactive vinyl end groups at the chains through soap-free emulsion polymerization. These micelle-like nano-network polymer particles made up very stable dispersion and could extract sorbed hydrophobic pollutant from the soil with extremely low sorption onto the soil. In addition, almost all of these particles could be recovered from soil, requiring relatively smaller number of washings.

In this study, we present the increase in the solubility of hydrophobic pollutant in water by the presence of amphiphilic polymer particles and almost 100% recovery of these particles as well as entrapped pollutant from water phase via aggregation process. We also demonstrate the direct biodegradation of entrapped pollutant within polymer particles.

## Materials and Methods

### Materials

Amphiphilic urethane acrylate anionomer (UAA) precursor chains were synthesized through an established three-step process. Synthesis of UAA chains was carried out using a 500 ml four-neck vessel with a stirrer, a thermometer, a reflux condenser, and an inlet system for nitrogen gas. The preparation method is illustrated in previous papers in detail [15]. For the synthesis of APU nano-network particles, UAA chains were first emulsified through the phase inversion emulsification process without using any external surfactant,

because a surfactant may affect the adsorption of APU nano-network particles onto soil. DDI water (90 g) is slowly dropped into the homogeneous mixture of UAA, triethylamine (TEA) as neutralizing reagents, and AIBN. After the formation of UAA nanoparticles dispersed in water, cross-linking polymerization of UAA nanoparticles was carried out at 80 °C for 5 h with stirring at 200 rpm to obtain APU nano-network particles [15]. Figure 1 shows the chemical structure of urethane acrylate anionomers (UAA), a precursor of APU particle. In Fig. 2, the schematic diagram of APU solution preparation was shown. 2-Methylnaphthalene (2-MNPT) was used as a model contaminant chemical and was purchased from Sigma.

## Analysis

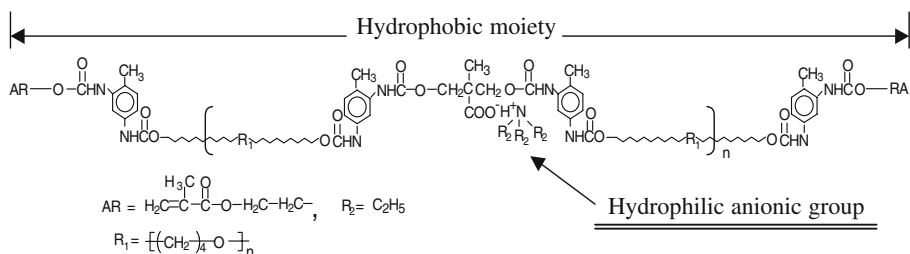
The concentration of the APU nano-particle in the aqueous phase was determined from the standard curve between the dry weight and the optical density at 550 nm. 2-MNPT was determined by GC-14B (Shimadzu, Japan) equipped with FID detector. Operating conditions of GC were as follows: column, silicon OV-101 (2 cm SUS); initial temp, 200 °C; final temp, 240 °C; program rate, 5 °C/min; injection temp, 200 °C; detection temp, 240 °C. The surface tension was measured by Fisher Surface Tensionmat 21 (Fisher, USA).

### Solubilization and Recovery of 2-MNPT

2-MNPT was solubilized in the water by mixing the sufficient amount of 2-MNPT in 10 ml of various concentrations of APU solution in a 20-ml vial, followed by shaking for 24 h at 25 °C, 200 rpm. After additional 48 h of equilibrium, 2-MNPT in the supernatant was measured by gas chromatography. For recovery test, APU solution (10 g/l, pH 6.5) containing various concentrations of 2-MNPT was stirred for 20 h at 25 °C in a 30-ml vial with Teflon cap. To this solution, 30 ml 0.5 N  $\text{CaCl}_2$  was added for precipitation. Precipitate was filtered through No.1 Whatman filter paper and 2-MNPT was analyzed by gas chromatography.

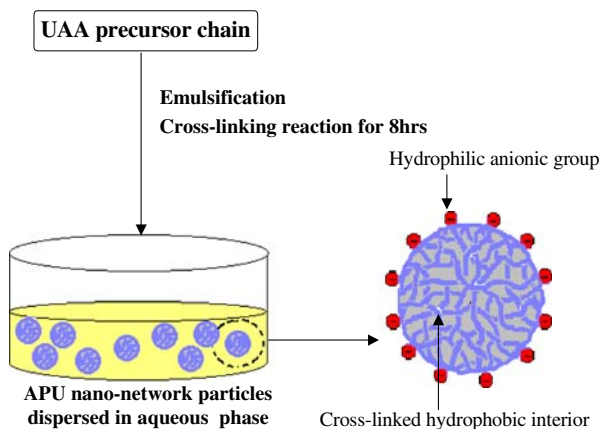
### Biodegradation of 2-MNPT in APU Solution

Sterilized solution containing the various amounts of APU and 2-MNPT was stirred in the 30-ml vial for 20 h for adsorption of 2-MNPT in APU particle. To this 10-ml solution, 10 ml of twofold mineral salt medium (MS) was added. The composition of MS was  $\text{KH}_2\text{PO}_4$  0.5 g/l,  $\text{K}_2\text{HPO}_4$  1 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/l,  $\text{CaCl}_2$  0.01 g/l,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/l,  $\text{NaNO}_3$  2 g/l, and  $\text{KCl}$  0.01 g/l. *Acinetobacter* sp. K2-2 (a gift from Korea Ocean Research Institute) was inoculated in 20 ml MS medium and was cultured for 3 days at 37 °C.



**Fig. 1** Structure of amphiphilic polyurethane (APU) precursor, urethane acrylate anionomers (UAA)

**Fig. 2** Schematic diagram of preparation and microstructure of APU nano-particle



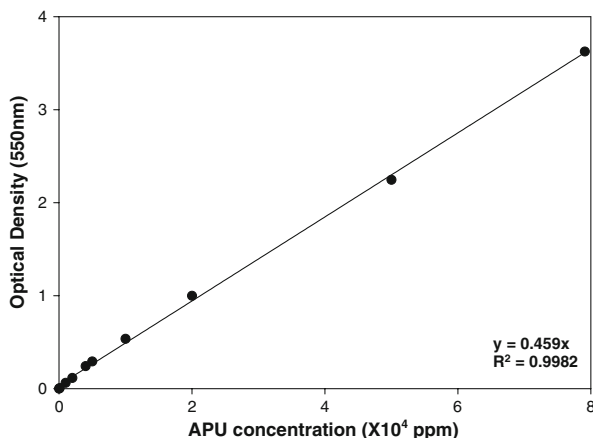
200 rpm in a shaking incubator. The vial was sealed with Teflon tape to prevent evaporation loss. Control experiment was also conducted without cells or without APU particle. Same volume of hexane was added to culture broth to extract 2-MNPT for further quantification. All measurements were expressed as the mean of two measurements.

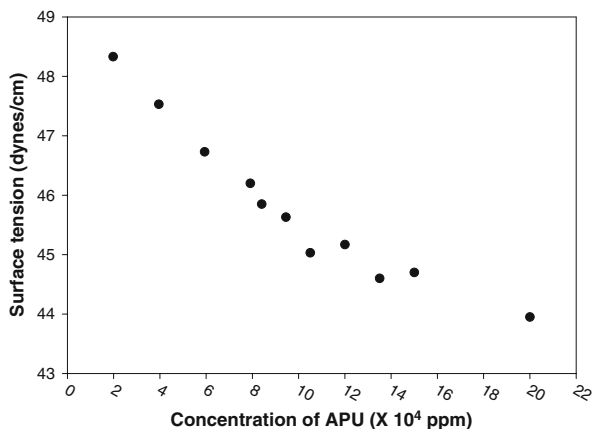
## Results and Discussion

The concentration of the APU particle was difficult to measure, since the APU particle was in situ synthesized in the APU precursor solution. However, correct quantification of APU was possible by using the standard curve between the dry weight and optical density (Fig. 3). One optical density corresponded to 20 g/l of APU in the water solution.

When the surface tension of the APU solution was measured, it decreased continuously as the concentration of the APU increased (Fig. 4). In the monomer surfactant, as the surfactant concentration increases, the surface tension decreased to some point and remained constant at the so-called critical micelle concentration (CMC). At CMC, the surfactant begins to form a micelle and shows various characteristics of surfactant. However, in the case of polymeric surfactant, i.e., APU, no CMC was observed. Monomer

**Fig. 3** Quantification of APU particles by optical density

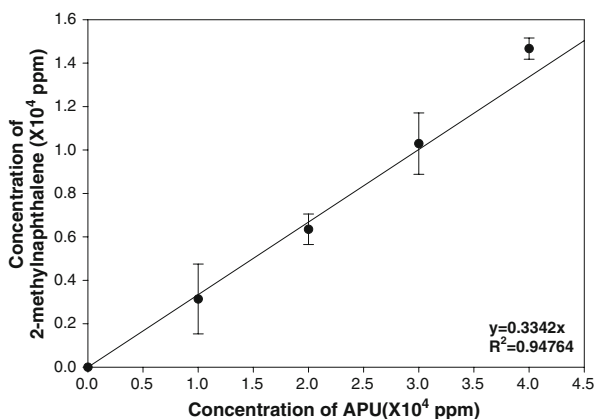


**Fig. 4** Surface tension of APU particles

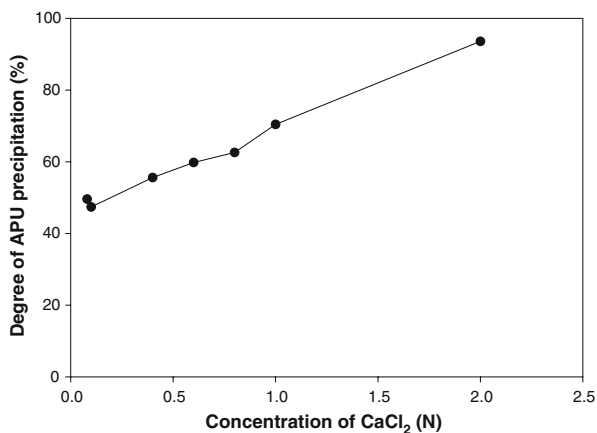
surfactant has a disadvantage that it adsorbs to the surface below CMC concentration, resulting in the loss of surfactant and inefficient recovery of hydrophobic contaminant from the soil. Therefore, APU particle can be more favorable for in situ soil washing.

The solubility of hydrophobic contaminant increased with the presence of APU particle. As shown in Fig. 5, the solubility of 2-MNPT increased proportionally to the APU concentration. The molar solubilization ratio (MSR) was 0.4 (mole 2-MNPT/mole APU) and 10 g/l of APU particle could increase the solubility of 2-MNPT by 100 folds. This enhanced solubilization of 2-MNPT by APU particle could be due to the preferential location of 2-MNPT inside the non-polar region of APU particle. The mechanism of solubilizing the non-polar material by APU is similar to that of the micelle. This increased solubilization can be used in the enhanced soil washing by surfactant [1–7].

The unique advantage of APU particle was demonstrated in the recovery of contaminant and APU particle from the washing solution after soil washing. The repulsion force in the APU particle made the dispersion of APU stable, prevented the adsorption of APU particle in the soil and inhibited the self-aggregation. However, addition of salt or shift of pH could reduce the repulsion force, resulting in the precipitation of the APU particle. As shown in Fig. 6, addition of CaCl<sub>2</sub> induced the precipitation of the APU particle, resulting in the recovery of the APU particle. At 2 N CaCl<sub>2</sub>, more than 95% of the APU particle was

**Fig. 5** Increased solubility of 2-methyl naphthalene by APU particles

**Fig. 6** Effect of  $\text{CaCl}_2$  concentration on APU particle precipitation for recovery (50 ml of 10 g/l APU solution)

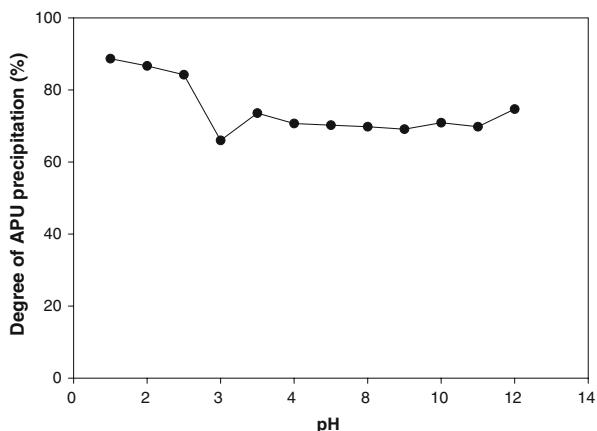


recovered. No significant difference in recovery ratio was observed at various temperatures between 17 and 40 °C (data not shown).

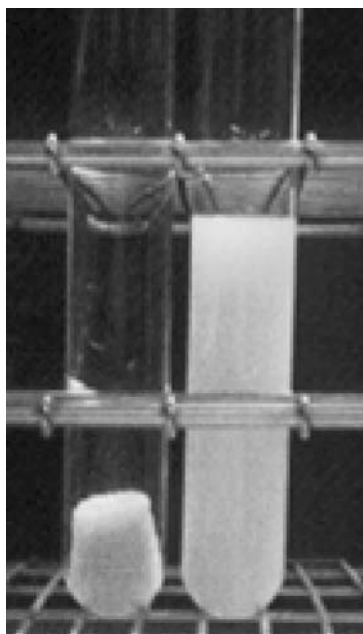
When the effect of pH on the precipitation was investigated, acidic pH was more favorable than alkaline (Fig. 7). The repulsion force would be decreased since the increase in proton, i.e., decrease in pH could reduce the ionic repulsion in the carboxylic group. However, at alkaline pH, the precipitation was comparable to acidic pH, indicating that there was no significant dependency on pH in precipitation reaction was observed. This result showed that this precipitation reaction would be applied to the actual field with ambient temperature and pH. The precipitation reaction occurred in less than a minute and the volume of the precipitate was less than 20% of the solution, making the physical separation of the precipitate easy (Fig. 8).

After washing the contaminated soil by APU solution, there are two possible ways to remove the contaminant and reuse the APU particle for repeated use. One is to precipitate the APU particle as well as the contaminant, resulting in the concentration of the contaminant. The other is to biodegrade the contaminant directly and reuse the APU particle. As a first method, recovery of 2-MNPT from the APU solution was investigated at various 2-MNPT amounts. As shown in Table 1, more than 95% of 2-MNPT was recovered by the precipitation of the APU particle. At higher concentration of 2-MNPT (2,000 ppm),

**Fig. 7** Effect of pH on APU particle recovery (50 ml of 10 g/l APU solution, treated with 2 N  $\text{CaCl}_2$ )



**Fig. 8** Photograph showing the precipitation of APU particles (10 g/l APU solution; *left*, treated with 2 N  $\text{CaCl}_2$ ; *right*, no  $\text{CaCl}_2$ )



77% of 2-MNPT could be recovered. This result demonstrated that the contaminant (2-MNPT) could be concentrated from the washing solution by precipitating the APU particle, making the contaminant removal process very simple. As a second method, 2-MNPT in the APU solution was directly cultured with *Acinetobacter* sp. K2-2 for biodegradation as described in ‘[Materials and Methods](#)’. This strain was isolated based on its ability to degrade aromatic compounds (personal communication). The amount of 2-MNPT, which was degraded by “cells only”, increased in proportional to the initial amount (Table 1).

**Table 1** Recovery by precipitation and biodegradation of 2-MNPT entrapped in APU at various initial amounts (mg).

	Initial 2-MNPT (mg in 20 ml)		
	10 mg (500 ppm)	20 mg (1,000 ppm)	40 mg (2,000 ppm)
Recovery <sup>a</sup>			
	9.5 (95%)	16.6 (83%)	31 (77%)
Biodegradation <sup>b</sup>			
APU+cell	9.2 (92%)	10.4 (52%)	21 (55%)
Cell only	5.1 (51%)	10 (50%)	19.2 (48%)
APU only	2 (20%)	6 (30%)	12 (30%)
No cell, no APU	2 (20%)	4.2 (21%)	7.2 (18%)

<sup>a</sup> 2 N  $\text{CaCl}_2$  was added to the 15 g/l APU solution containing 2-MNPT. Precipitated 2-MNPT was determined

<sup>b</sup> Cells were cultured with 15 g/l APU solution containing 2-MNPT. Other conditions were described in ‘[Materials and Methods](#)’

These results show no inhibition of degradation by 2-MNPT. The degraded amount of 2-MNPT was almost same at 20 and 40 mg of initial amount with “cell only” and “APU+ Cell”. This outcome shows that 2-MNPT degraded at same efficiency even with the presence of APU. No inhibition of biodegradation by APU particle was observed. This indicates that 2-MNPT entrapped in APU can be possibly degraded in situ directly after recovery from soil washing. Higher biodegradation at 10 mg of 2-MNPT entrapped in APU needs further characterization. Kinetic study of 2-MNPT biodegradation entrapped in APU is under progress.

## Conclusions

Surfactant micelle-like structures of APU particles significantly increased the solubility of hydrophobic pollutant, 2-MNPT, in water. Enhanced solubility of 2-MNPT showed the potentials of applying APU particles for the enhanced extraction of hydrophobic pollutant from the soil. APU particles also could be easily separated from water phase by adding inorganic salt,  $\text{CaCl}_2$ , into water phase. No release of entrapped 2-MNPT within APU particles was observed in the course of aggregation and 2-MNPT could accordingly be recovered from APU sediments. Biodegradability of entrapped 2-MNPT within APU particles indicates the possibility that APU particles can be recycled and reused after the biodegradation of entrapped pollutant using an in situ bioreactor. Unlike a conventional surfactant showing high degree of sorption onto soil, sorption of APU particles onto soil could be extremely small, because micelle-like structure of APU particles is relatively strong and permanent due to chemical cross links. Therefore, application of APU particles as a new soil remediation process can provide a potential alternative because of extremely low degree of sorption, excellent recovery of APU particles, and biodegradability of entrapped pollutants.

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