• **Original Article**

# Study on the biodegradation of alternatives (four species including $C_8H_8F_9KO_3S$) for perfluorooctane sulfonate

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**Objectives** The objective of this study was to evaluate the biodegradation potential of four perfluorooctane sulfonic acid (PFOS) alternatives that were developed at Chang-won National University. While PFOS has been used widely in industrial and consumer products, it is known to be a persistent organic pollutant. Therefore, greener alternatives are highly desirable.

**Methods** Biodegradation tests were run for 28 days using standard test protocols. The biochemical oxygen demand was measured daily throughout the experimental period, and the data were used to calculate the biodegradation rates. Microorganisms were isolated from the some of the tests that showed evidence of biodegradation.

**Results** $C_8H_8F_9KO_3S$, which has the same number of carbons as the parent compound PFOS but a reduced number of fluorines, showed the highest biodegradation rate followed by $C_{10}H_8F_{13}KO_3S$. Chemical alternatives with lower number of carbons did not biodegrade readily in the experiments.

**Conclusions** Together, these results suggest that it may be advantageous to develop PFOS alternatives with 8 carbons, the same as PFOS, but a reduced number of fluorines; as such, chemicals are more susceptible to biodegradation than the parent compound.

**Keywords** Biodegradation, Green chemistry technology, Perfluorooctane sulfonic acid

## Introduction

Perfluorooctane sulfonic acid (PFOS, $C_8F_{17}SO_3H$), a perfluorinated compound, is a synthetic material. PFOS possesses superior heat resistance, chemical resistance, water repellency, oil repellency, rub resistance, and surface activity; hence, it is widely utilized in fire blowing agents, in coating agents for carpets, clothing, paper, and leather, in commercial stain preventing agents, in metal cleaners, and in aircraft hydraulic fluids. While PFOS is highly valued as a chemical agent for industrial and consumer uses, it is not easily degraded and is known to remain in the environment and biological materials for long periods of time [1,2].

Cerebellum tests using rats have shown that PFOS can induce neurotoxicity, and it can also cause reproductive toxicity in pregnant rats as indicated by a decrease in the survival rate of newborns. A recent study reported that the average blood PFOS concentration for Koreans was 6.06 ng/mL [3].

PFOS was registered as a persistent organic pollutant (POP) at the 4th Stockholm Convention on POPs held at Geneva, Switzerland, in May 2009. In South Korea (hereafter Korea), PFOS was designated as a POP by amending the enforcement decree of the POPs Control Act in April 11, 2011, and consequently, its manufacturing, export, and utilization have been severely restricted.

The 3M company voluntarily stopped PFOS production in

2002 and began developing alternatives such as perfluorobutane sulfonate with a reduced number of carbon chains; but they have not yet succeeded in developing a substance that can completely replace PFOS to date (UNEP/POPS/POPRC.6/Add.3/Rev.1, 2011.10.14). In Korea, Shin's research team at Changwon National University (Department of Chemistry) are developing PFOS alternatives with a decreased or increased number of carbon atoms and a decreased number of fluorine atoms [4]. The objective of this study was to evaluate the biodegradation potential of five species four PFOS alternatives that were developed at Changwon National University. It is hoped that this research will aid in the identification of potentially useful PFOS alternatives.

## Materials and Methods

### Test Materials

This study used microorganisms that were collected from 15 sampling points at city sewage treatment plants, industrial waste water treatment plants, rivers, lakes, and the sea throughout Korea. The microorganisms were cultured at the good laboratory practice (GLP) culture room of the Korea Environment Corporation for more than 1 month prior to use in experiments.

PFOS alternatives that have been synthesized by Shin's laboratory at Changwon National University include $C_8F_{17}KO_3S$ (molecular weight [MW], 538.22, PFOS in a form of potassium salt), $C_{10}H_8F_{13}KO_3S$ (MW, 494.31), $C_8H_8F_9KO_3S$ (MW, 394.30), $C_6H_8F_5KO_3S$ (MW, 294.28), and $C_5H_8F_3KO_3S$ (MW, 244.27); all of these chemicals were used in this study.

Biodegradation tests were performed according to the Organization for Economic Cooperation and Development TG 301C protocol using basic culture medium prepared at the GLP laboratory of the Korea Environment Corporation. An OxiTop Control system (Control 100, WTW, Munich, Germany) was used in the experiments.

### Biodegradation Test

A total of 12 biochemical oxygen demand (BOD) bottles were used in the biodegradation tests. In bottles 1 through 5, the five test materials ($C_8F_{17}KO_3S$, $C_{10}H_8F_{13}KO_3S$, $C_8H_8F_9KO_3S$, $C_6H_8F_5KO_3S$, $C_5H_8F_3KO_3S$) were added 0.015 g each, and 150 mL of deionized water (DW) was injected. In bottles 6 through 10, the five test materials were added 0.015 g each, and then 1.8 mL basic culture medium, 131.53 mL DW, and 16.67 mL activated sludge were injected. To investigate the condition of the activated sludge, 0.015 g of a control material (aniline, C6H7N) was added in bottle 11, and then 1.8 mL basic culture medium, 131.53 mL DW, and 16.67 mL activated sludge were injected. Bottle 12 contained 1.8 mL basic culture medium, 131.53 mL

DW, and 16.67 mL activated sludge, and it was used for comparison purposes with bottles 6 through 10. The experiment was conducted in an incubator for 28 days under aerobic conditions at 24.9 to 25.1°C and pH 6.70 to 7.92.

### Biodegradation Rate Calculation

The biodegradation rate (%) was calculated as follows:

$$\text{Biodegradation rate (\%)} = \% \text{ ThOD} = \frac{\text{BOD}}{\text{ThOD}} \times 100,$$

Test material:

$$\text{BOD} = \frac{(\text{bottle 6, 7, 8, 9, and 10 each}) - ([\text{bottle 1, 2, 3, 4, and 5 each}] + \text{bottle12})}{\text{concentration of each test material}}$$

Control material:

$$\text{BOD} = \frac{\text{bottle 11-bottle 12}}{\text{concentration of each test material}}$$

BOD (mg/L) of test materials (or control materials) that is measured by a BOD curve.

Theoretical oxygen demand (ThOD, mg/L) that is the total amount of oxgen required to oxidise test meterials (or control material) completely.

$$C_CH_HCl_{Cl}N_NNa_{Na}O_OP_PS_S$$

$$\text{ThOD} = \frac{16(2c+1/2(h-cl-3n)+3s+5/2s+1/2na-0) \text{ mg/mg}}{\text{MW}}$$

### Separation and Identification of Degrading Microorganisms

Mineral medium prepared in the biodegradation laboratory at Chonnam National University were used as the separation medium, and PFOS alternatives used in the test were the only carbon source. As for the microorganism separation, the growth of microorganisms was visibly confirmed after culturing at 35°C in incubator under aerobic conditions for 24 hours. These microorganisms were further subcultured five times to achieve pure separations. The separated microorganisms were identified by 16S rRNA sequencing analyses.

## Results

Biodegradation rates of aniline (i.e., the control material in bottle 11) were 60.5% and 72.1% on days 7 and 28, respectively, thus confirming that the activated sludge (microorganisms) used in this study are behaving normally. The BOD values for bottle 6 with $C_8F_{17}KO_3S$, PFOS in a form of potassium salt reached 116.1

mg/L on day 7, 129.3 mg/L on day 14, and 139.6 mg/L on day 28 (Figure 1A). On the contrary, the BOD values for bottle 12 without test materials reached 120.8 mg/L on day 7, 137.8 mg/L on day 14, and 150.9 mg/L on day 28. The BOD values for bottle 7 with $C_{10}H_8F_{13}KO_3S$ reached 126.0 mg/L on day 7, 151.0 mg/L on day 14, and 164.6 mg/L on day 28 (Figure 1B). The BOD values for bottle 8 with $C_8H_8F_9KO_3S$ reached 128.3 mg/L on day 7, 150.0 mg/L on day 14, and 170.6 mg/L on day 28 (Figure 1C). The BOD values for bottle 9 with $C_6H_8F_5KO_3S$ reached 122.5 mg/L on day 7, 141.0 mg/L on day 14, and 154.5 mg/L on day 28 (Figure 1D). The BOD values for bottle 10 with $C_5H_8F_3KO_3S$ reached 119.3 mg/L on day 7, 133.8 mg/L on day 14, and 144.2 mg/L on day 28 (Figure 1E).

After calculating the biodegradation rates, we found that bottle 6 with $C_8F_{17}KO_3S$, which is the PFOS in a form of potassium salt, and bottle 10 with $C_5H_8F_3KO_3S$ had negative values, thus indicating that biodegradation did not occur at all for 28 days. Biodegradation rates of bottle 7 with $C_{10}H_8F_{13}KO_3S$ were confirmed at 6.8% on day 7, 17.1% on day 14, and 17.7% on day 28. Biodegradation rates of bottle 8 with $C_8H_8F_9KO_3S$ were 9.3% on day 7, 15.1% on day 14, and 24.3% on day 28. Biodegradation rates of bottle 9 with $C_6H_8F_5KO_3S$ were 2.0% on day 7, 3.7% on day 14, and 4.1% on day 28. Considering that the biodegradation rate for highly degradable materials is greater than 60% in general, we cannot conclude that all four PFOS alternatives used in the present study were well degraded. However, the biodegrading rate of $C_8H_8F_9KO_3S$ was confirmed as the most superior (Table 1).
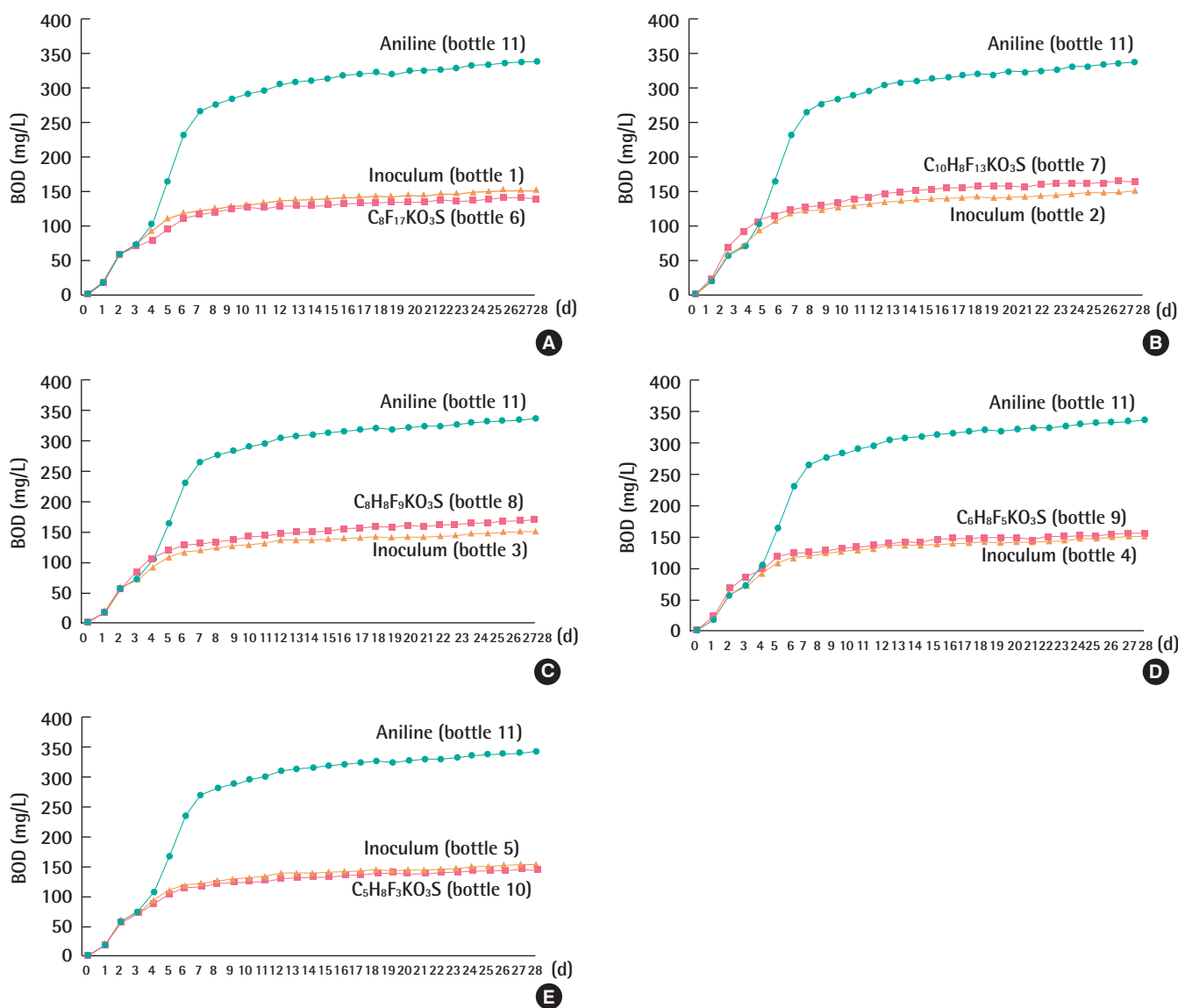


Figure 1. Biochemical oxygen demand (BOD) results for the perfluorooctanesulfonic acid (PFOS) alternatives and the PFOS salt. (A) BOD of $C_8F_{17}KO_3S$ (PFOS salt), (B) BOD of $C_{10}H_8F_{13}KO_3S$, (C) BOD of $C_8H_2F_9KO_3S$, (D) BOD of $C_6H_8F_5KO_3S$, and (E) BOD of $C_5H_8F_3KO_3S$.

For three PFOS alternatives (bottle 7 with $C_{10}H_8F_{13}KO_3S$, bottle 8 with $C_8H_8F_9KO_3S$, and bottle 9 with $C_6H_8F_5KO_3S$) that were confirmed to undergo biodegration, the degrading microorganisms were separated and subjected to sequencing analyses. Samples from the $C_{10}H_8F_{13}KO_3S$ treatment showed greater than 99% of high homology with *Serratia marcescens, Acinetobacter* spp., and *Pseudomonas geniculata*. Samples from the $C_8H_8F_9KO_3S$ treatment had more than 99% of high homology with *Bacillus* spp., *Serratia marcescens*, and *Chryseobacterium* spp. Samples from the $C_6H_8F_5KO_3S$ treatment had more than 99% of high homology with *Bacillus cereus* and *Serratia marcescens* (Table 2).

**Table 1.** Results for the biodegradation of the PFOS alternatives and the PFOS salt

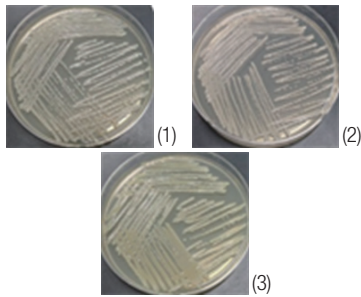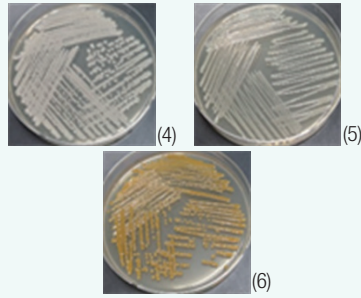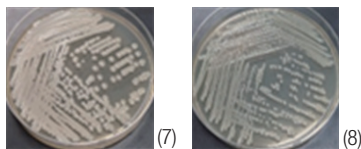| Chemicals | Biodegradation (%) | | |
| --- | --- | --- | --- |
| | 7 d | 14 d | 28 d |
| Bottle 6 ($C_8F_{17}KO_3S$, PFOS salt ) | 0.0 | 0.0 | 0.0 |
| Bottle 7 ($C_{10}H_8F_{13}KO_3S$) | 6.8 | 17.1 | 17.7 |
| Bottle 8 ($C_8H_8F_9KO_3S$) | 9.3 | 15.1 | 24.3 |
| Bottle 9 ($C_6H_8F_5KO_3S$) | 2.0 | 3.7 | 4.1 |
| Bottle 10 ($C_5H_8F_3KO_3S$) | 0.0 | 0.0 | 0.0 |

PFOS, perfluorooctanesulfonic acid.

## Discussion

$C_8F_{17}KO_3S$, which is the PFOS in a form of potassium salt, as well as potential PFOS alternative $C_5H_8F_3KO_3S$ with a decreased number of carbon and fluorine atoms did not undergo biodegradation at all. While $C_8H_8F_9KO_3S$, which has the same number of carbons as the parent compound but a reduced number of fluorines, showed the highest biodegradation rate followed by $C_{10}H_8F_{13}KO_3S$ and $C_6H_8F_5KO_3S$ with 6 carbon atoms and 5 fluorine atoms also exhibited susceptibility to biodegradation, but at a markedly low rate. Together, these results suggest that it may be it advantageous to develop PFOS alternatives with 8 carbons, the same as PFOS, but a reduced number of fluorines, as such chemicals are more susceptible to biodegradation than the parent compound.

Three microorganisms were confirmed to degrade $C_8H_8F_9KO_3S$ and $C_{10}H_8F_{13}KO_3S$, which showed a relatively high biodegradation rate. Two microorganisms were identified during the biodegradation of $C_6H_8F_5KO_3S$, which exhibited a relatively low biodegradation rate. Such results follow the general principle that there are more species of microorganisms that can act as degradators for chemicals with a higher biodegradation rate.

Commercialization of PFOS alternatives should proceed with

**Table 2.** Separated bacteria strains from the perfluorooctanesulfonic acid alternative experiments

| Chemicals | Strains | Colony |
| --- | --- | --- |
| $C_{10}H_8F_{13}KO_3S$ | (1) *Serratia marcescens* <br> (2) *Acinetobacter* spp, <br> (3) *Pseudomonas geniculata* |  |
| $C_8H_8F_9KO_3S$ | (4) *Bacillus* spp. <br> (5) *Serratia marcescens* <br> (6) *Chryseobacterium* spp. |  |
| $C_6H_8F_5KO_3S$ | (7) *Bacillus cereus* <br> (8) *Serratia marcescens* |  |

an overall consideration of ecotoxicity, human toxicity, functionalities, and economic feasibility. It is therefore necessary to perform additional studies regarding this matter.

## Acknowledgements

## Conflict of Interest

The authors have no conflicts of interest with material presented in this paper.

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