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<b>Author(s)</b>	Fukahori, S.; Fujiwara, T.; Ito, R.; Funamizu, N.
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# Sulfonamide antibiotic removal and nitrogen recovery from synthetic urine by the combination of rotating advanced oxidation contactor and methylene urea synthesis process

S. Fukahori\*\*\*\*\*, T. Fujiwara\*\*\*\*\*, R. Ito\*\*\*\*\*, N. Funamizu\*\*\*\*\*

\*Paper Industry Innovation Center of Ehime University, 127 Mendoricho Otsu, Shikokuchuo, Ehime 799-0113, Japan

\*\*Research and Education Faculty, Natural Sciences Cluster, Agriculture Unit, Kochi University, 200 Monobe Otsu, Nankoku, Kochi 783-8502, Japan

\*\*\*Graduate School of Engineering, Department of Environmental Engineering, Hokkaido University, Kita-13, Nishi-8, Kita-ku, Sapporo 060-0808, Japan

\*\*\*\*Japan Science and Technology Agency, CREST, Japan

## Abstract

The combination of nitrogen recovery and pharmaceutical removal processes for livestock urine treatment were investigated to suppress the discharge of pollutants and recover nitrogen as resources. We combined methylene urea synthesis from urea and adsorption and photocatalytic decomposition of sulfonamide antibiotic using rotating advanced oxidation contactor (RAOC) contained for obtaining both safe fertilizer and reclaimed water. The methylene urea synthesis could recover urea in synthetic urine, however, almost all sulfonamide antibiotic was also incorporated, which is unfavorable from a safety aspect if the methylene urea is to be used as fertilizer. On the other hand, RAOC could remove sulfonamide antibiotic without consuming urea. It was also confirmed methylene urea could be synthesized from synthetic urine treated by RAOC thus we concluded that RAOC should be inserted prior to nitrogen recovery process for effective treatment of urine and safe use of methylene urea as fertilizer.

**Keywords:** Rotating advanced oxidation contactor; Nitrogen recovery; Sulfonamide antibiotics

## Introduction

Nonpoint source pollution owing to excessive fertilization of agricultural land or spreading of livestock manure causes widespread contamination of local systems, including organic pollution, eutrophication, and groundwater pollution. In livestock excreta, many investigators have reported methods of treating livestock excreta including composting (Maeda *et al.*, 2010) and anaerobic co-digestion (Zarkadas and Pilidis, 2011). Because almost all nitrogen is contained in urine, appropriate treatment of urine is necessary to prevent nitrogen pollution. Funamizu *et al.* suggested a differentiable method of household wastewater treatment in which urine, feces, and gray water were separated and treated individually to suppress the discharge of pollutants and recover nitrogen and phosphorous as resources (Hotta and Funamizu, 2008).

Pharmaceuticals contained in human or livestock urine and feces also pose a risk to the environment because they can lead to the development of antibiotic-resistant bacteria. Sulfonamide antibiotics are popular antimicrobial agents commonly used in animal food production. These materials are excreted in urine and not completely removed from biological wastewater treatment, resulting in their often being detected in secondary effluent of wastewater treatment plants or in local aquatic systems (Holm *et al.*, 1995; Ternes, 1998; Hirsch *et al.*, 1999; Lapworth *et al.*, 2012; Kim *et*

*al.*, 2013; Li *et al.*, 2013); accordingly, safe and cost-effective methods for their removal are required. Advanced oxidation including ultraviolet irradiation, ozonation, Fenton reaction and photocatalytic decomposition, and adsorption are commonly applied to remove pharmaceuticals and personal care products from wastewater (Fukahori *et al.*, 2012; Prieto-Rodriguez *et al.*, 2012; Cao *et al.*, 2013; Gao *et al.*, 2014; Ji *et al.*, 2014; Lian *et al.*, 2015). Additionally, our group found that high-silica zeolite was effective for adsorption of sulfonamide antibiotics from porcine urine even though high amounts of co-existing materials such as urea, ions, and organic materials were also present (Fukahori *et al.*, 2013). Furthermore, a composite of high-silica zeolite and TiO<sub>2</sub> photocatalyst more effectively removed sulfonamide antibiotics from secondary effluent relative to either zeolite or TiO<sub>2</sub> alone (Ito *et al.*, 2014).

Fujiwara applied the above techniques to develop an innovative water management system that employed decentralized water reclamation and a cascading material cycle for treatment of agricultural waste streams with consideration of climate change (Fujiwara, 2012). In this system, a nitrogen and phosphorous recovery process were combined with a pharmaceutical removal technique for the safe recovery and reuse of nutrients in livestock urine. To accomplish this, we developed a novel rotating advanced oxidation contactor (RAOC) equipped with a composite TiO<sub>2</sub>-zeolite sheet for selective removal of sulfonamide antibiotics from livestock urine. We have already reported that functional powders such as TiO<sub>2</sub> or zeolite can be immobilized into sheet-like materials via a papermaking technique (Fukahori *et al.*, 2003). In this study, P-25 and high-silica zeolite HSZ-385 were immobilized using glass and pulp fibers as a supporting matrix. The retention was above 95% and the prepared sheet was strong enough to be handled and attached to the stainless steel disk. In addition, our group developed a nitrogen recovery process from urine in which methylene urea was directly synthesized using formaldehyde and then applied as nitrogen fertilizer (Ito *et al.*, 2013).

In the present study, we combined methylene urea synthesis and wastewater treatment using RAOC for obtaining both safe fertilizer and reclaimed water. Application of methylene urea synthesis as the first step enables effective recovery of nitrogen; however, the final product may contain antibiotics because some antibiotics may be incorporated into methylene urea, indicating that its safety cannot be guaranteed. Conversely, application of RAOC treatment first removes pharmaceuticals from urine, but a portion of the urea is consumed through photocatalytic decomposition. Thus, the objective of this study is to propose the appropriate order of the system. We compared two systems, one is methylene urea synthesis then RAOC treatment and another is RAOC treatment then methylene urea synthesis. The appropriate order was determined from the viewpoints of both nitrogen recovery and antibiotic removal.

## **Materials and Methods**

### **Materials**

High-silica Y-type zeolite (HSZ-385; surface area, 600 m<sup>2</sup>/g; SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> = 100) and TiO<sub>2</sub> (P-25; surface area, 50 m<sup>2</sup>/g; anatase) were purchased and kindly provided by Tosoh Ltd. and Nippon Aerosil Ltd., respectively. Sulfamethazine (SMT, Aldrich), urea, formaldehyde and inorganic salts (Wako) were reagent grade and used without

further purification. A mixture of 3% potassium peroxodisulfate and 4% sodium hydroxide solution prepared in house was used for the measurement of total nitrogen.

### Quantitative analysis

The urea concentration in supernatant was measured by ultra performance liquid chromatography-tandem mass spectrometry (LC/MS/MS, ACQUITY UPLC-Xevo TQ; Waters). The weight of methylene urea was measured with an electric balance.

Total nitrogen in the supernatant was quantified using a nitrogen auto analyzer (BRAN+LUEBBE AACS-II) equipped with a Cu-Cd reduction column. Briefly, an aliquot was diluted using ultrapure water to keep the final nitrogen concentration below the maximum determination limit (1.2 mg-N/L). Next, 25 mL of diluted sample was mixed with 5 mL of 3% potassium peroxodisulfate-4% sodium hydroxide solution and autoclaved at 120°C for 30 min to oxidize all of the nitrogen to nitrate. The solution was then neutralized using 2 M hydrochloric acid and the total volume was set to 50 mL.

### Preparation of synthetic urine

Synthetic urine was prepared as described in a previous study (Ito *et al.*, 2013). The concentrations of SMT, urea, and ions in synthetic urine are given in Table 1. The pH of synthetic urine was adjusted to 2 for methylene urea synthesis.

### Synthesis and characterization of methylene urea

A total of 200 mL of urea solution (25 g urea/L) or synthetic urine containing SMT was stirred and 10 mL of 30% formaldehyde was then added. During the reaction, a portion of the suspension was filtered using filter paper and precipitate (solid methylene urea) was separated. The molecular structure of the precipitate was analyzed using infrared spectroscopy (Nicolet 6700, Thermo Scientific Inc.).

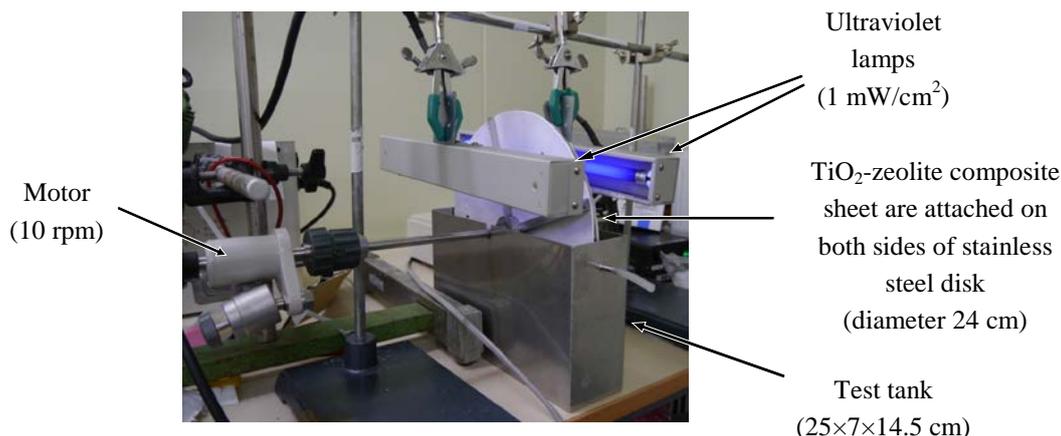
The amount of nitrogen derived from low-molecular-weight methylene urea (soluble methylene urea) was calculated by subtracting that derived from urea, creatinine, and ammonium chloride from the total nitrogen.

### Preparation of TiO<sub>2</sub>-zeolite composite sheet

The TiO<sub>2</sub>-zeolite composite sheet consisted of a catalytic layer and a supporting layer. To prepare the catalyst layer, P-25, HSZ-385 powder (7 g each), and glass fiber (2 g) were dispersed into 4 L water and vigorously stirred. The suspension was then mixed with poly(diallyl) dimethyl ammonium chloride solution (PDADMAC, 0.05% total solids), followed by the sequential addition of anionic polyacrylamide solution (A-

Table 1 Components of synthetic urine

Chemicals	Concentration g/L
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.65
NaCl	4.6
Na <sub>2</sub> SO <sub>4</sub>	2.3
Na <sub>3</sub> (C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> )·2H <sub>2</sub> O	0.76
KCl	1.6
C <sub>4</sub> H <sub>7</sub> N <sub>3</sub> O	1.1
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.65
KH <sub>2</sub> PO <sub>4</sub>	4.2
NH <sub>4</sub> Cl	1.0
(NH <sub>2</sub> ) <sub>2</sub> CO	25
Na <sub>2</sub> (COO) <sub>2</sub>	0.02
C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S (SMT)	0.01



**Fig. 1** Rotating advanced oxidation contactor

PAM, 0.05% total solids) and pulp fiber (8 g) suspension in that order at 3-min intervals. Hand sheets with a grammage of 384 g/m<sup>2</sup> were prepared according to the TAPPI Test Methods T205. The supporting layer was prepared using only glass fiber (6 g) and pulp fiber (3 g) and was laminated with a catalyst layer. Following pressing at 350 kPa for 5 min, the wet sheets were dried in an oven at 105°C for 30 min. After being soaked in an alumina sol, the composite sheets were pressed at 350 kPa for 5 min, then ignited at 700°C for 1 h to remove organic pulp and improve physical strength by calcination of the alumina binder (Fukahori *et al.*, 2003).

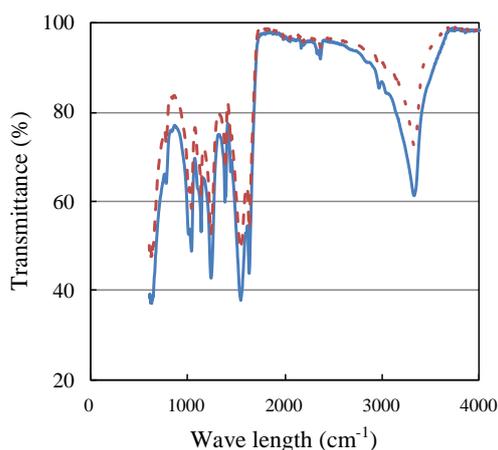
#### Removal of SMT using RAOC

The appearance of RAOC is shown in Fig. 1. The TiO<sub>2</sub>-zeolite sheets were fixed on both sides of a stainless steel disk with a diameter of 24 cm and the disk was mounted on a laboratory-scale test tank, while the bottom side was submerged in water. Next, 2 L of synthetic urine containing SMT was poured into the test tank and the disk was rotated slowly. For photocatalytic treatment, the top side of the disk exposed to air was irradiated with ultraviolet irradiation (FL287-BL365; Raytronics Corp.: maximum wavelength 365 nm) to overcome attenuation of light intensity.

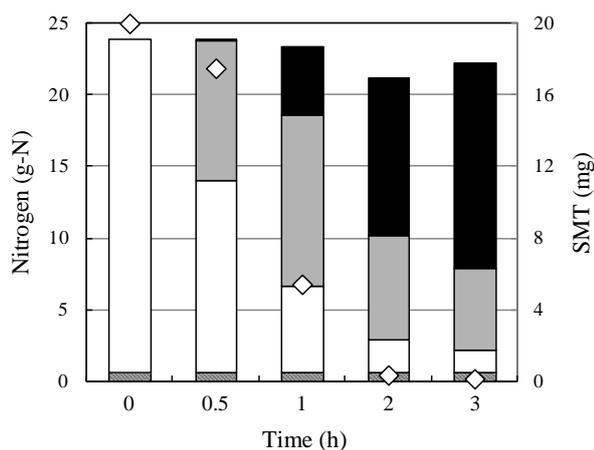
After treatment, synthetic urine was passed through a membrane filter (Dismic; pore size, 0.20 μm; Advantec, Ltd.) prior to quantitative measurement. Solid-phase extraction (SPE) was then conducted to determine the concentration of SMT in synthetic urine. The SMT concentration was measured with a standard addition method using liquid chromatography tandem mass spectrometry (LC/MS/MS; Acquity UPLC-Xevo TQ; Waters) following SPE. The detail of SPE is described in our previous study (Fukahori *et al.*, 2013). The urea concentration was also measured using LC/MS/MS after 10<sup>3</sup> fold dilution of a sample aliquot.

To measure the amount of SMT in the composite sheet, a piece of the composite sheet was cut and placed into 0.1 M sodium hydroxide solution. The solution was then sonicated for 1 min to elute SMT in the sheet, after which the supernatant was passed through a membrane filter. The SMT concentration in the supernatant was measured using LC/MS/MS and the amount of SMT contained in the sheet was calculated.

## Results and Discussion



**Fig. 2** IR spectra of precipitate obtained from synthetic urine (solid line) and urea solution (dash line).



**Fig. 3** Time courses of the amounts of nitrogen derived from urea (white), low molecular methylene urea (gray), methylene urea (black) and creatinine+NH<sub>4</sub>Cl (stripe) during methylene urea synthesis from synthetic urine. Diamond shows the amount of SMT contained in synthetic urine.

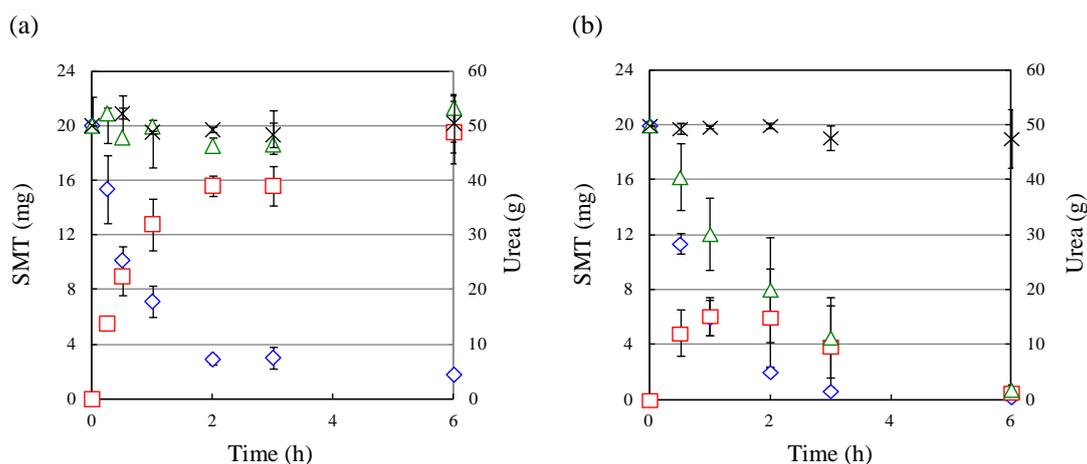
### Synthesis of methylene urea from synthetic urine and fate of SMT

After addition of formaldehyde to urea solution or synthetic urine, white precipitates were observed. Figure 2 shows the infrared spectra of the precipitates. The spectra were quite similar, with three major peaks observed at 3500 cm<sup>-1</sup>, 1800 cm<sup>-1</sup>, and 1500 cm<sup>-1</sup>, which were attributed to an N-H bond, C=O bond, and C-N. These findings are in accordance with the results of a previous study (Ito *et al.*, 2013), and together, these findings indicate that urea was synthesized during the process.

The time courses of the amounts of urea, methylene urea, and SMT during methylene urea synthesis are depicted in Fig. 3. The results indicated that the amount of urea contained in synthetic urine decreased and that of precipitates increased with time, respectively. The total nitrogen was maintained, and ca. 80% of urea was eventually converted to methylene urea as previously reported (Ito *et al.*, 2013). Conversely, the amount of SMT remaining in synthetic urine decreased drastically after the reaction had proceeded for 1 h. No interaction of SMT with urea, formaldehyde, ions, or organics contained in synthetic urine was observed (data not shown), therefore we deduced that SMT was incorporated into methylene urea, which is unfavorable from a safety aspect if the methylene urea is to be used as fertilizer.

### Adsorptive removal of SMT using RAOC

Figure 4 shows the time courses of the amounts of SMT and urea in water and SMT adsorbed inside the composite sheet when synthetic urine containing SMT was treated by RAOC under dark condition. After 3 h treatment, ca. 85% of SMT was removed from synthetic urine (Fig. 4-a). Measurement of the amount of SMT in the TiO<sub>2</sub>-zeolite composite sheet by alkaline extraction confirmed that SMT adsorbed inside the composite sheet could be quantified by alkaline extraction and the total amount of SMT was unchanged without UV irradiation, indicating SMT was removed from synthetic urine through adsorption. We have already revealed that HSZ-385 could selectively adsorb sulfonamide in synthetic urine (Fukahori *et al.*, 2013) and there is



**Fig. 4** Time courses of the amount of urea and SMT when treated by RAOC without (a) or with (b) UV irradiation: urea (crosses) and SMT (diamonds) in synthetic urine, SMT in TiO<sub>2</sub>-zeolite composite sheet (squares) and total system (triangles).

no affinity between SMT and P-25 (Fukahori *et al.*, 2012); thus, the decrease of SMT in synthetic urine must be attributed to adsorption on HSZ-385 immobilized inside the composite sheet,

A few hours were required for RAOC treatment to reach adsorption equilibrium, and the adsorption rate by RAOC treatment was slower than that by HSZ-385 powder (Fukahori *et al.*, 2013). This occurred because HSZ-385 particles are aggregated inside the sheet through the use of two types of flocculants during the sheet-making process, which might have reduced accessibility of the SMT molecules to the surfaces of HSZ-385 in the composite sheet, however, it was clarified that the composite sheet could adsorb SMT in synthetic urine and SMT could be separated from synthetic urine.

#### Adsorptive and photocatalytic removal of SMT using RAOC

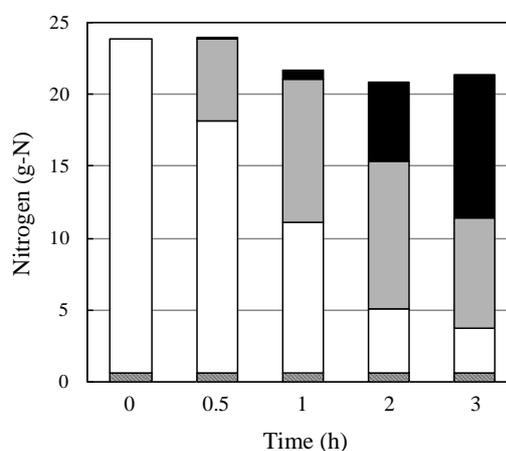
The time courses of SMT and urea when synthetic urine containing SMT was treated by RAOC with ultraviolet irradiation are shown in Fig. 4-b. SMT in synthetic urine decreased smoothly, and almost all SMT was removed after 6 h of treatment. The amount of SMT adsorbed onto the sheet increased for 2 h, then gradually decreased. Considering the behavior of SMT on RAOC treatment under dark conditions (Fig. 4-a), SMT adsorbed inside the composite sheet was decomposed through photocatalytic decomposition, indicating adsorption sites of zeolite will be regenerated and RAOC can remove SMT continuously. When powder photocatalyst is used, reaction efficiency decreases as the reactor size increases because light attenuation and scattering by water or TiO<sub>2</sub> powder itself occurs. In our RAOC reactor, the disk surface exposed to air was directly irradiated with an ultraviolet lamp, which enabled attenuation of light intensity to be overcome.

The amount of urea was almost unchanged during treatment, although SMT was apparently removed, indicating that RAOC treatment could remove SMT from synthetic urine without consuming urea. This is because the top side of the composite sheet exposed to air was irradiated with UV while most of urea was in water phase therefore not decomposed through photocatalysis which is one of the advantages of our apparatus. We previously investigated the photocatalytic behavior of SMT in pure

water and secondary effluent and found photocatalytic decomposition efficiency was drastically inhibited by ions and organic materials (Ito *et al.*, 2014). Although the NPOC content in synthetic urine was  $10^3$  times higher than that derived from SMT, RAOC could decompose SMT selectively because SMT was selectively adsorbed onto zeolite and photocatalytically decomposed inside the sheet.

### Synthesis of methylene urea from synthetic urine treated by RAOC

After RAOC treatment, a portion of the treated urine was subjected to methylene urea synthesis. The time courses of the amounts of urea and methylene urea (soluble fraction and precipitate) during methylene urea synthesis are shown in Fig. 5. Similar to the reaction using untreated synthetic urine, the amount of urea and precipitates decreased and increased with time, respectively. The total amount of elemental nitrogen was maintained and ca. 80% of urea was converted to methylene urea after 24 h of reaction, which was equal to that obtained using untreated synthetic urine.



**Fig. 5** Time courses of the amounts of nitrogen derived from urea (white), low molecular methylene urea (gray), methylene urea (black) and creatinine+NH<sub>4</sub>Cl (stripe) during methylene urea synthesis from treated synthetic urine.

To provide safe methylene urea as fertilizer, intermediates formed through photocatalytic decomposition of SMT in treated urine must also be considered. Therefore, we analyzed the treated urine for intermediates likely to be formed during the process; however, none were observed. The degradation mechanism of SMT using RAOC is currently under investigation and will be reported in the future.

## Conclusions

The combination of nitrogen recovery and sulfonamide removal from synthetic urine was investigated. Methylene urea synthesis was effective to recover urea in synthetic urine, however, SMT seemed to be incorporated in methylene urea. On the other hand, RAOC could remove SMT without consuming urea in the synthetic urine, thus we decide to insert RAOC prior to nitrogen recovery process.

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