Continuous removal and degradation of β-lactam antibiotics using organoclay-packed column

Saıtoh T.[1,*], Oiwa M.[1], Saıto A.[1], Fujıoka H.[2], And Shıbayama T.[3]

[1]Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology, Koen-cho 165, Kitami 090-8507, Japan

[2]Department of Physical Science and Engineering, Faculty of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

[3]Department of Materials, Physics and Energy Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

*Corresponding author:

e-mail: saito@chem.kitami-it.ac.jp, saitoh@mail.kitami-it.ac.jp

Abstract

β-Lactam antibiotics in water were rapidly removed and degraded by using didodecyldimethylammonium bromide (DDAB)-bentonite (BT) organoclay. The removal increased with increasing the amount of DDAB in the organoclay and could be correlated to the aqueous-octanol distribution coefficient of antibiotic. The degradation of antibiotics significantly increased by the organoclay sorption. Even under the mild conditions (pH 7 and 25°C), penicillin G (m/z = 335) and oxacillin (m/z = 402) completely degraded into their penicilloic acids (m/z = 353 and 420, respectively) missing β-lactam ring within 24 h. An organoclay-packed column was successfully used for continuous removal and degradation of oxacillin in water and synthesized hospital wastewater. Adjusting flow rate allowed the occurrence of the effluent containing no oxacillin but only its degraded products. Application of this method may reduce environmental risk of antibiotics that can interfere microbial ecosystem.

Keywords: β-Lactam antibiotics. Organoclay. Removal. Degradation. Continuous treatment

1. Introduction

Occurrence of antibiotics has become significant environmental concern because of their potential risk in microbial ecosystem. According to recent literatures, more than 60% of commercially available antibiotics using in the world are β-lactam compounds (Homem and Santos; 2011). They are not chemically stable in water and therefore almost degrade during conventional activated sludge processes (Watkinson, *et al*.; 2007, Le-Minh, *et al*.; 2011). However, the degree of degradation is largely dependent on the kinds of antibiotics and often insufficient (Michael, *et. al.;* 2013). These antibiotics can give damages to microbial community in sewage systems and aquatic environment (Kümmerer; 2009, Ding and He; 2013) as well as induce the bleeding of antibiotic-resistant bacteria (Li, *et al*.; 2009, Bouki, *et al*.; 2013). However, the application of advanced methods tend to boost the cost of wastewater treatment. Recently, we designed a simple and efficient non-microbial methods for eliminating β-lactam antibiotics from water (Saitoh and Shibayama; 2016). Didodecyldimethyl-ammonium bromide (DDAB)-montmorillonite organoclay was useful sorbent, because of its stability in water and high capacity in the sorption of β-lactam antibiotics. Moreover, an antibiotic, penicillin G, sorbed on the organoclay rapidly transfered to its degraded product, penicilloic acid. Therefore, the use of organoclay sorption method may reduce the environmental risk of these antibiotics. In the present study, experimental conditions for the organoclay sorption and degradation of different β-lactam antibiotics were studied. LC-MS studies were performed to identify the degraded products of penicillin G and oxacillin. Moreover, continueous treatment was examined by using a laboratogy-scale organoclay-packed column. The requirement for the practical application was discussed.

2. Experiment

2.1. Materials

Didodecyldimethylammonium bromide (DDAB) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Montmorillonite K-30 (MT, surface area: 330 m^2/g) and β-lactam antibiotics including penicillin G potassium sal, oxacillin sodium salt monohydrate, and Bis-Tris were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bentonite (BT, Wako Pure Chemical, Osaka, Japan) was also used for column experiment. A standard material of penicilloic acid, (4S)-2-[carboxy[(phenylacetyl)amino]-methyl]-5,5-dimethyl-thiazolidine-4-carboxylic acid, was from LGC Standards (Teddington, UK). Other chemicals used were analytical or HPLC grade agents. Water was purified with a Milli-Q Integral Water Purification System (Merck Millipore, Billerica, MA, USA) having a UV irradiation unit.

2.2. Preparation of organoclays

MT or BT was rinsed with 50 mM Bis-Tris solution (pH 7) for 2 h and washed with water before use. Then, it was mixed with 5 mM Bis-Tris (pH 7) solution containing prescribed amount of DDAB for 2 h for the preparation of DDAB-MT or DDAB-BT organoclay. A Jasco FT/IR-4200 infrared spectrometer (Hachioji, Japan) was employed for measuring IR spectra (ν_{C-H} = 2980 cm^{-1}) of freeze-dried organoclays. The amount of DDAB sorption was estimated from the difference in its initial and residual concentrations determined by an Orange II extraction method (Scott; 1968). The amounts of DDAB sorption were typically 400 mg/(g dry) for MT and 50 mg/(g dry) for BT and used for batch experiment and column experiment, respectively

2.3. Procedures for organoclay sorption

For batch experiment, 10 mL of 1 mM Bis-Tris (pH 7) containing 1.0 mg/L of antibiotic was added into a 15 mL polypropylene tube in which organoclay composing of 40 mg of MT and 16 mg of DDAB was placed. The solution was gently (60 rpm) mixed for 1 min. After the solution was filtrated by passing through an Advantec Dismic$^{®}$ syringe filter (hydrophilic PTFE, filter size: 13 mm, pore size: 0.45 μm, Tokyo, Japan), a 5 μL-portion was introduced into a Waters LC/MS system composing of an Alliance e2695 separation module and a 3100 mass spectrometric detector (Milford, MA, USA) with an InertSustain$^{®}$ C18 column (length: 150 mm, inner diameter: 3.0 mm, particle size: 5 μm, GL Sciences,
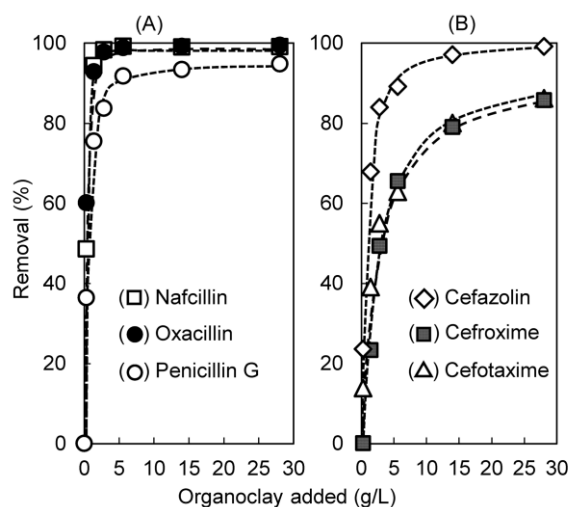

Fig. 1 Effect of the amount of DDAB-MT organoclay onthe removal of penicilin-type (A) and cepharosporin-type (B)β-lactam antibiotics (pH 7, 25°C).

Tokyo, Japan) for the separation and determination of antibiotics. The flow rate and composition of mobile phase were 0.5 mL/min and 20%(v/v) acetonitrile containing 0.27 mM formic acid and 4.73 mM ammonia (pH 5). Ionization potential and souse temperature were 30 V and 350°C, respectively. For column experiment, organoclay composing of 1 g BT and 50 mg DDAB was packed in a glass column (inner diameter: 15 mm, length: 300 mm). The height of organoclay layer was ca. 7 mm. Aqueous solution (1 mM Bis-Tris, pH 7) of 10 mg/L oxacillin or

synthesized wastewater containing the same amount of oxacillin was supplied by using a Tokyo Rika MP-3000 peristaltic pump (Tokyo, Japan). Eluting solutions were collected by using a Tokyo Rika DC-1500C fraction collector. The method for monitoring oxacillin and its degraded products the same as above described. Temperature was adjusted to 25 ± 1°C for all experiments.

3. Results and discussion

3.1. Organoclay sorption

As clearly shown in Fig. 1, the removal of different β-lactam (A: penicillin-type, B: cepharosporin-type) antibiotics increased with increasing the amount of DDAB-MT organoclay added. The removal ratios became constant and leached to the maximum values within 1 min, indicating the applicability of the organoclay sorption method for rapid removal of antibiotics from water. The binding constant, K_b, of antiniotic to DDAB in the organoclay can be represented by the following equation.

$$K_b = X\,V/q\,(100 - X)$$

Here, X denotes the difference in the removal (%) of antibiotic with unmodified MT and that using DDAB-MT organoclay, V is solution volume (L), and q is the amount of DDAB (kg) sorbed on MT. Figure 2 shows the
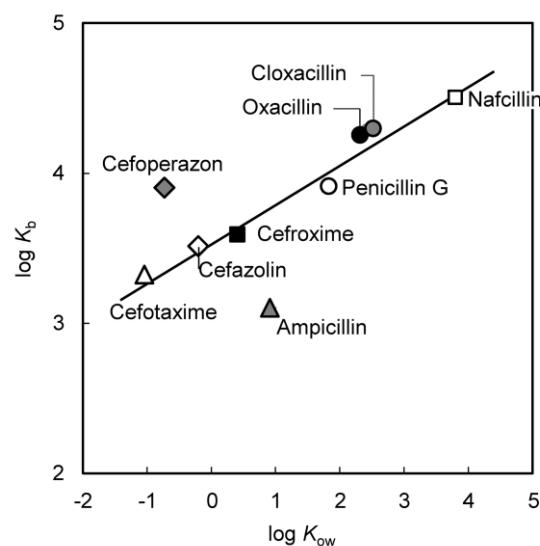

Fig. 2 Relationship between log K_b and log K_{ow}.

correlation between logarithmic binding constant (log K_b) and logarithmic aqueous-octanol distribution coefficient (log K_{ow}) being a measure of hydrophobicity of a compound. Fairly good correlation was obtained for most antibiotics, indicating that hydrophobic interaction plays an important role for organoclay sorption. However, the K_b value for cephoperazon is higher than the value that can be predicted from the corelation. On the other hand, the value for ampicilline is lower than the precicted value. Electrostatic interaction or repulsive force as well as structual factors may also influence the sorption on the organoclay composing of negatively charged clay mineral and positively charged surfactant molecules.

3.2. Organoclay-catalyzed degradation

Time-dependent degradation profiles of different classes of β-lactam (A: penicillin-type, B: cepharosporin-type) antibiotics are summarized in Fig. 3, in which the amount of remaining antibiotic is represented by the ratio of remaining concentration to initial one in the eluting solution. All antibiotics examined apparently degraded by their organoclay sorptin, although the degrees of degradation in bulk aqueous solution (pH 7) for 2 h were very small (<2%). Organoclay was found to act as the catalysis for the degradation of these β-lactams. Based on the mass spectra of the respective peaks in the chromatograms, penicillin G (m/z = 335) and oxacillin (m/z = 402) hydrolyzed into their penicilloic acids (m/z = 353 and 420, respectively) missing β-lactam ring. Chemical and biological stabilies are largely dependent on the structures of β-lactams (Page; 1992, Deshpande; 2004). Generaly, cephorosporin-type antibiotics having a 6-memberd heterocyclic ring are stable against acid-catalyzed hydrolysis rather than penicillin-type ones having 5-membered hetrocyclic ring. However, Fig. 3 indicates the catalytic function of organoclay for all
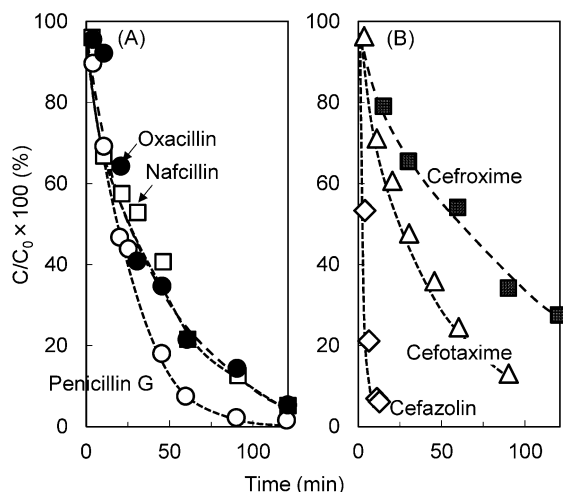
Fig. 3: Degradation of penicilin-type (A) and cepharosporin-type (B) β-lactam antibiotics during DDAB-MT organoclay sorption (pH 7, 25°C).

antibiotics examined. The results obtained in this study strongly suggest wide applicability of organoclay sorption method not only for the removal of antiniotics from water but for their degradation under the mild conditions (pH 7, 25°C). Before column experiment, batch experiment was also performed for the sorption and degradation of oxacillin. Since DDAB-MT composing of very fine particles was considerably inpermeable to water, DDAB-BT organoclay was used for column experiment. Moreover, the amount of DDAB sorbed was regulated to 50 mg/(g dry BT) as descriped in experimental section. Figure 4 shows time-dependent transision of chromatograms of oxacilin and its degraded products and mass spectra of the respective peaks. Because of lower net organoclay content, the degradation was slower than that obtained by using the same amount of DDAB-MT organoclay. However, the feature of the degradation in
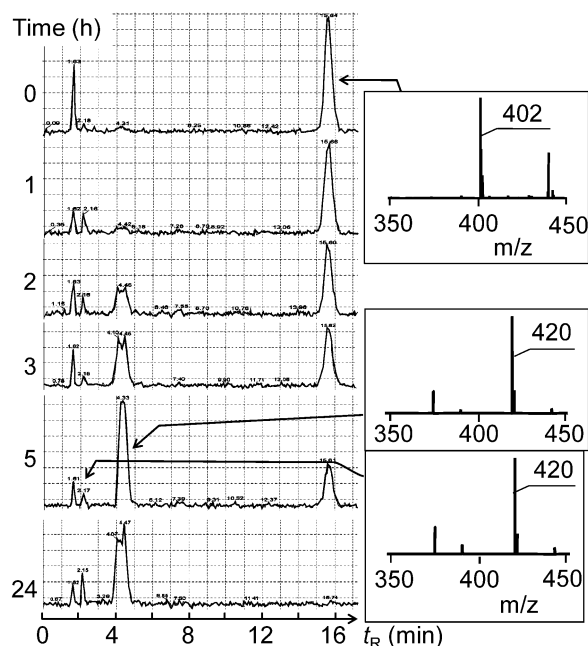
DDAB-BT was almost the same as the case in DDAB-MT.

Fig. 4 Chromatograms of oxacillin and its degaded products and mass sapectra for the respective peaks after different periods of DDAB-BT organoclay sorption.

With increasing the time of organoclay sorption, the peak hight of oxacillin (t_R = 15.6 min) decreased, while other peaks (t_R = 2.2 and 4.2 min) appeared. As clearly indicated in the mass spectra in Fig. 4, oxacillin (m/z = 402) hydrolyzed to its penicilloic acid (m/z = 420). Multiple peaks of penicilloic acid giving different retention times may be ascribable to the existance of the isomers owing to plural chiral carbons in penicilloic acid. Approximately 50% of oxacillin degraded for 3 h and and negligible oxacillin was detected within 24 h.
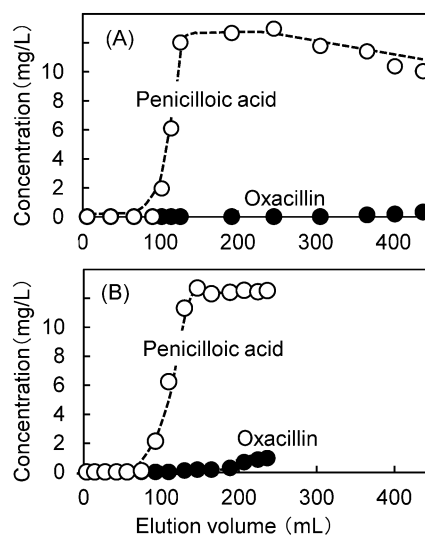
Fig. 5 Breakthrough curves of oxacillin and penicilloic acid obtained by passing 10 mg/L of oxacillin (A) or synthesized wastewater containing 10 mg/L of oxacillin (B) through DDAB-BT organoclay column.

Table 1. Qualities and ingradients of a syntesized wastewater.

pH	7.0
COD (mg/L)	570
Component	Concentration (mg/L)
NaCl	3000
KH_2PO_4	33
$NaNO_2$	220
CH_3COONH_4	110
Propionic acid	100
Butylic acid	50
Urea	1500
Creatinine	50

3.3. Continueous treatment

Feasilibity of the application to continuous treatment was studied by using a small scale DDAB-BT organoclay-packed column. Although the concentrations of most antibiotics in hospital effluents are normally far below µg/L-levels (Homem and Santos; 2011, Kümmerer; 2009, Frédéric and Yves; 2014), aqueous buffer solution containing 10 mg/L of oxacillin were used to clarify the applicability to highly contaminated effluents. Figure 5A shows the break through curve obtained by passing water containing 10 mg L^{-1} of oxacillin through the organoclay-packed column. Oxacillin was not detected up to 400 mL of elution. This fact seems to be ascribable to very strong interaction and high capacity in the organoclay sorption of oxacillin. On the other hand, apparent breakthrough of the degraded product, penicilloic acid, was observed more than ca. 80 mL of elution. As readily expected from the comparison of chemical structures as well as the retention behaviors in chromatogram (Fig. 4), hydrophobicity of penicilloic acid is lower than that of oxacillin. The elution of penicilloic acid from organoclay can be explained by its lower hydrophobicity. Finally, a synthesized wastewater containing 10 mg/L of oxacillin was also used for studying the applicability to wastewaters or contaimined waters. The water qualities measured and the components are listed in Table 1 and selected based on a synthesized hospital wastewater in a literature (Verlicchi, *et al*.; 2010). The result is shown in Fig. 5B. Similary to the feature in Fig. 5A, the elution of oxacillin is regulated, while breakthrough of degraded product, penicilloic acid, is observed. However, small leakage is obserbed more than ca. 200 mL elution, probably because of the irreversible sorption of dissolved organic components. Recently, organoclays have also been studied as barrier materials for polluted porcolated waters (Rodríguez-Cruz, *et al*., 2007, Zhao, *et al*., 2016). Modification of clay minerals with surfactants or polyelectrolytes improve the retention of different organic pollutants or heavy metals in soil. The results obtained in this study may useful not only for eliminating these pollutants but for reducing their toxicities.

4. Conclutions

Organoclay sorption method was useful for rapid removal of β-lactam antibiotics from water. The extent of removal was correlated to logarithmic aqueous-octanol distribution coefficient ot hydrophobiocity of the antibiotics. Moreover, β-lactam antibiotics sorbed on the organoclay gradually degraded into their hydrolyzed products under the mild conditions (pH 7, 25°C). Organoclay-packed column may be useful for continiues treatment of hospital wastewaters containing antibiotics.

References

Bouki C., Venieri D., Diamadopoulos E. (2013) Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review, Ecotoxicology and Environmental Safety, **91**, 1-9.

Deshpande, A.D., Baheti K.G., Chatterjee N.R. (2004) Degradation of β-lactam antibiotics, *Current Science*, **87**, 1684-1695.

Ding C., He J., (2010) Effect of antibiotics in the environment on microbial populations, *Applied Microbiology and Biotechnology*, 87, 925-941.

Frédéric O., Yves P. (2014) Pharmaceuticals in hospital wastewater: their ecotoxicity and contribution to the environmental hazard of the effluent, *Chemosphere*, **115**, 31-39.

Homem V., Santos L. (2011) Degradation and removal methods of antibiotics from aqueous matrices-a review, *Journal of Environment Management*, **92**, 2304-2347.

Kümmerer K. (2009) Antibiotics in the aquatic environment-a review part I, *Chemosphere*, 75, 417-434.

Le-Minh N., Khan S.J., Drewes J.E., Stuetz R.M. (2010) Fate of antibiotics during municipal water recycling treatment processes, *Water Research*, **44**, 4295-4323.

Li D., Yang M., Hu J., Zhan J., Liu R., Gu X., Zhang Y., Wang Z. (2009) Antibiotic-resistance profile in environmental bacteria isolated from penicillin production wastewater treatment plant and the receiving river, *Environmental Microbiology*, **11**, 1506-1517.

Michael I., Rizzo L., McArdell C.S., Manaia C.M., Merlin C., Schwartz T., Dagot C., Fatta-Kassinos D. (2013) Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review, *Water Research*, 47, 957-995.

Page M.I. (1992) *The mechanisms of reactions of β-Lactams,* in *The Chemistry of β-Lactams*, Page M.I. ed. Springer Science & Business Media, Dordrecht, pp.129-147.

Rodríguez-Cruz M.S., Sánchez-Martín M.J., Andrades M.S., Sánchez-Camazano M. (2007) Modification of clay barriers with a cationic surfactant to improve the retention of pesticides in soils, *Journal of Hazardous Materials*, **B139**, 363-372.

Saitoh T., Shibayamaba T. (2016) Removal and degradation of β-lactam antibiotics in water using didodecyldimethylammonium bromide-modified montmorillonite organoclay, *Journal of Hazardous Materials*, **317**, 677-685.

Scott G.V. Spectrophotometric determination of cationic surfactants with orange II, (1968) *Analytical Chemistry*, **40**, 768-773.

Verlicchi P., Galletti A., Petrovic M., Barceló D. (2010) Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options, *Journal of Hydrology*, **389**, 416-428, and references therein.

Watkinson A.J., Murby E.J., Costanzo S.D. (2007) Removal of antibiotics inconventional and advanced wastewater treatment: implications forenvironmental discharge and wastewater recycling, *Water Research*, **241**, 4164-4176.

Zhao Q., Choo H., Bhatt A., Burns S.E., Bate B. (2016) Review of the fundamental geochemical and physical behaviors of organoclays in barrier applications, *Applied Clay Science*, in press.