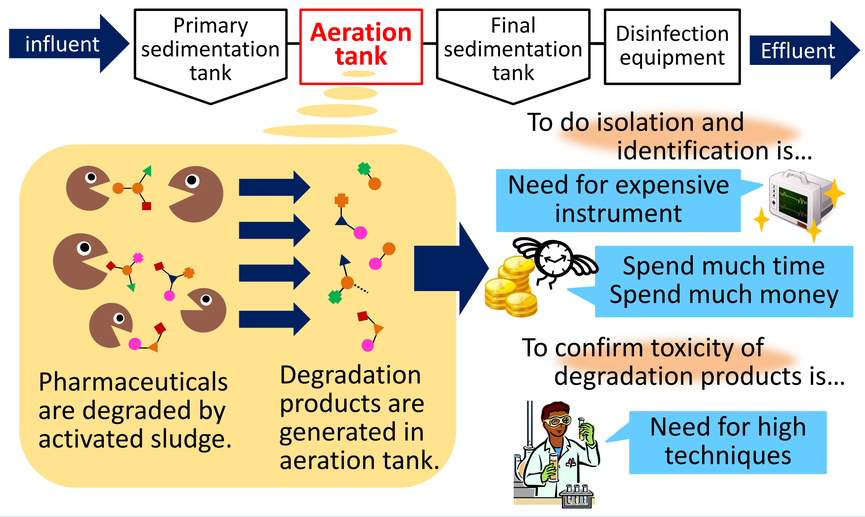
Use Of Simple Bioassay To Detect The Formation Of Toxic By-products During Activated Sludge Process

Makoto SEKINE*, Masahiro TOKUMURA**, Susumu KONUMA***, Shigeki MASUNAGA**

* Graduate School of Environment and Information Science, Yokohama National University
79-1 Tokiwadai, Hodogaya-ku, Yokohama 240-8501 Japan
** Faculty Environment and Information Science, Yokohama National University
79-1 Tokiwadai, Hodogaya-ku, Yokohama 240-8501 Japan
*** Department of Civil Engineering, College of Science and Technology, Nihon University
1-8-14 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8308 Japan

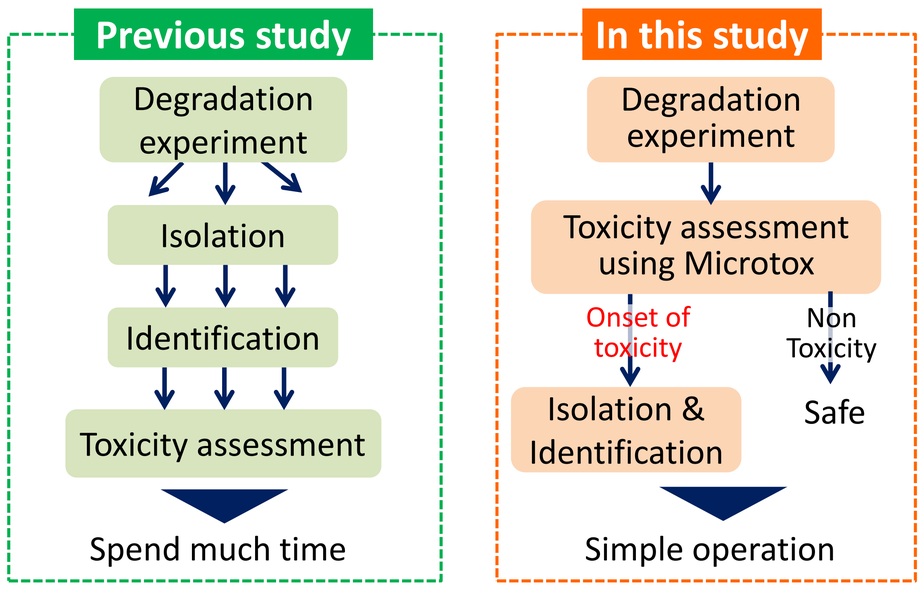
INTRODUCTION

It is expected that biological treatment decomposes toxic organic pollutants and toxicity of the wastewater decreases. However, there are occasions that **some biotransformation intermediates are formed during the treatment process and their toxicity is higher than their parent compounds.** In the previous study, ecotoxicological assessment was carried out and photo transformation products of naproxen were found to be more toxic than their parent compound in both acute and chronic toxicity tests (Isidori *et al*., 2005). In the EU, the REACH legislation requires that transformation products should be included in assessment of chemical substances produced or imported in amounts exceeding 100 ton/year. However, **assessments of degradation products are difficult because isolation, identification and toxicity test of degradation products requires high technology, a lot of work and much costs.** In this study, we found the occurrence of toxic degradation products form Ibuprofen (IBP), an anti-inflammatory drug using the Microtox test without identification and isolation of degradation products. **Direct application of biodegradation reaction mixture to Microtox test was found to be a sample and feasible screening tool to find the formation of toxic degradation products.**



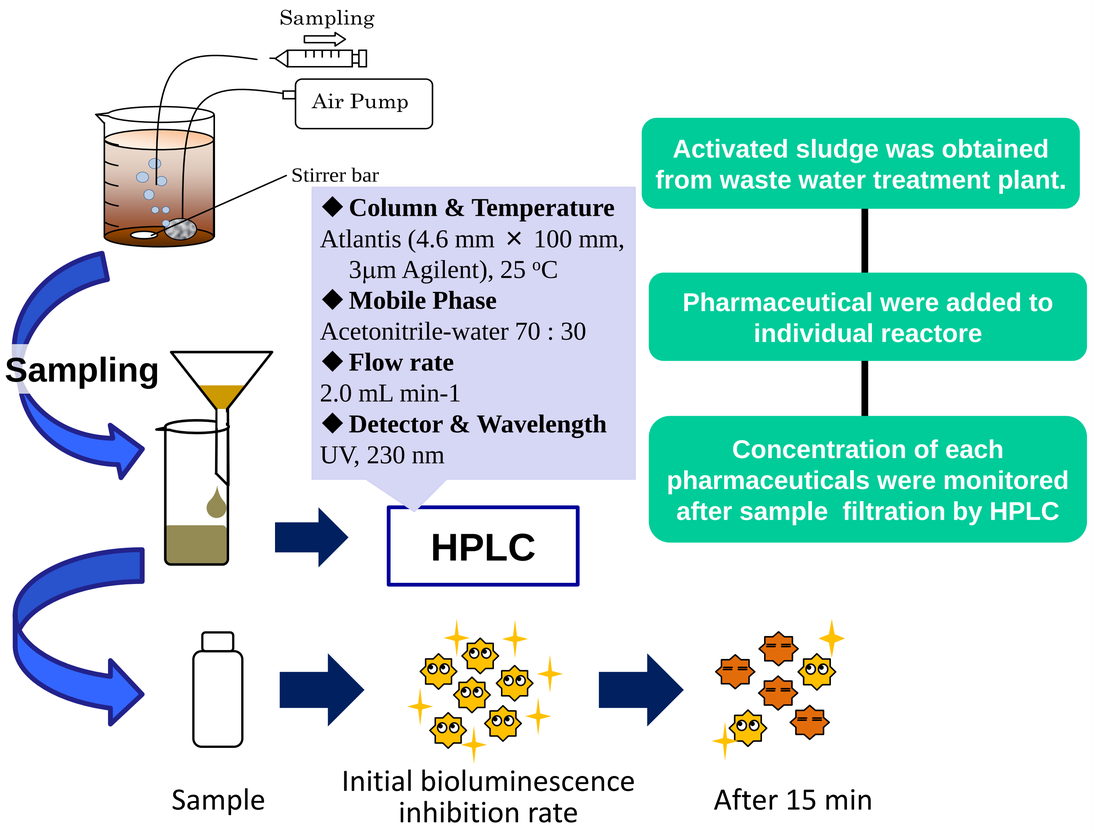
AIM OF THIS STUDY

To confirm the toxicity of degradation products in activate sludge without isolation and identification using Microtox test.



METHODS

Laboratory-scale biodegradation batch test of IBP was performed using activated sludge mixed liquor taken from a municipal wastewater treatment plant. Batch experiments consisted of a one-litre glass bottle with aeration at 2 L min^{-1} and continuous mixing by a magnetic stirrer. The experiment was performed at room temperature. The mixed liquid suspended solid was adjusted at proximately 2500 mg L^{-1}. IBP was added to the bottle at an initial concentration of 100 mg L^{-1}. Ten ml of mixed liquor was sampled from the bottle using a syringe. The sample was filtered using a glass filter paper to separate solids from liquid phase. The filtrate was analysed by High performance liquid chromatography (HPLC) to determine IBP concentration and also determined its toxicity by Microtox test.



Microtox test is one of the simply bioassay test

- ◆Possible to assess of organic toxicity
- ◆Operation is easy
- ◆Measurement time is very short
- ◆Possible to assess include degradation products

RESULTS & DISCUSSION

Results of IBP degradation experiment showed that the concentration of IBP decreased gradually (Fig. 1 left). Some peaks were found in the HPLC chromatograms of reaction mixture (Fig. 1 right). In addition to the parent IBP peak (retention time (RT) = 2.9 min), **some unknown peaks were found. This implied the occurrence of degradation products. The peaks of degradation products gradually became higher.** In spite of the considerable decrease of IBP concentration, **the luminescence inhibition rate increased and peaked at 96 hours and then decreased.** This phenomenon indicated that **a degradation product that was more toxic than IBP was formed during the treatment of IBP.** The spectrums of unknown peaks were different from that of IBP (data not shown). The previous study reported that the toxicity of the treatment solution of Ibuprofen increased upon irradiation, indicating a higher toxicity of the first degradation products (Illés *et al*., 2013). **The increase of toxicity at 96 hours observed in this study might have been due to a degradation product produced during the treatment.** We showed that direct application of reaction solution to Microtox test could be a useful tool to screen the formation of toxic degradation products without isolation and identification.

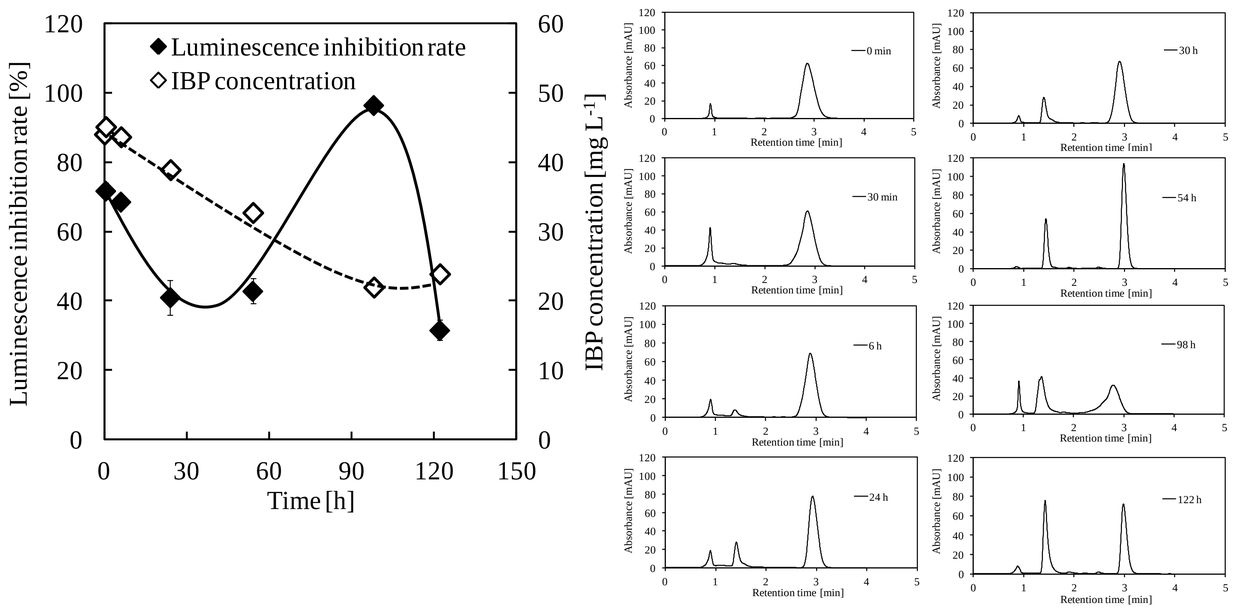
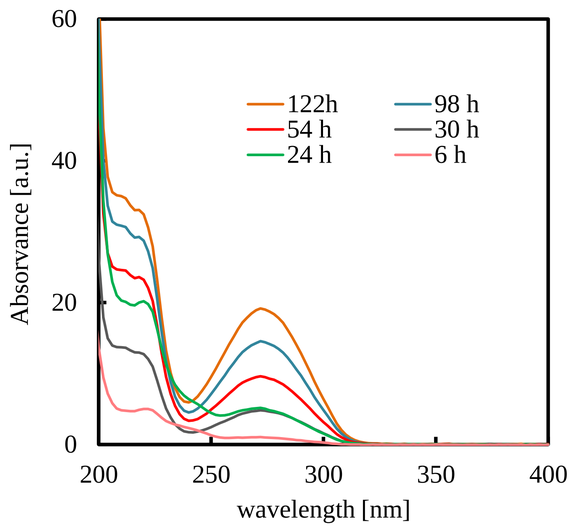


Fig. 1 Time changes in IBP concentration and luminescensce inhibition rate (left) and HPLC chromatograms of IBP and degradation products (right)



The UV absorption spectra of IBP degradation products which found peaks (RT = 1.4 min) in HPLC chromatograms was depicted on Fig 2. **The intensity of the band around 272 nm becomes higher by time because of the formation of changed aromatic molecules.** The previous study reported that this change in absorbance is attributed to the formation of products hydroxylated in the aromatic ring (Illés et al., 2013). Hence, This phenomenon indicated that **degradation products are generated by bacterium in activated sludge.**

Fig. 2 UV absorption spectrum of IBP degradation products

CONCLUSION

- ◆ In spite of the considerable decrease of IBP concentration, the luminescence inhibition rate increased and peaked at 96 hours and then decreased.
- ◆ The increase of toxicity at 98 hours observed in this study might have been due to a degradation product produced during the treatment.
- ◆ Degradation product that was more toxic to aquatic ecological than IBP was formed during the treatment of IBP.
- ◆ We showed that direct application of reaction solution to Microtox test could be a useful tool to screen the formation of toxic degradation products without isolation and identification.

Reference literature

- Erzsébet Illés, Erzsébet Takács, András Dombi, Krisztina Gajda-Schrantz, Gergely Rácz, Katalin Gonter, László Wojnárovits, 2013. Hydroxyl radical induced degradation of ibuprofen. Science of the Total Environment 447, 286 - 292
- Marina Isidori, Margherita Lavogna, Angela Nardelli, Alfredo Parrella, Lucio Previtera, Maria Rubino, 2005. Ecotoxicity of naproxen and its phototransformation products. Science of the Total Environment 348, 93 - 101

If you have any question, please send me an Email : sekine-makoto-cb@ynu.jp