Development of simple toxicity assay for degradation products of pharmaceuticals

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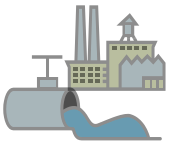
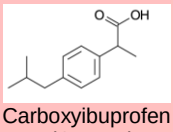
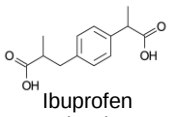
Introduction

Sewage treatment plant

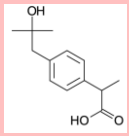




Carboxyibuprofen (CA-IBP)



Ibuprofen (IBP)



Hydroxyibuprofen (OH-IBP)

Unknown intermediates

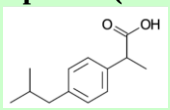
- ☆ When IBP is degraded by activated sludge, CA-IBP, OH-IBP and other unknown intermediates are formed.
- ☆ Their toxicities are not known and their risk has not been considered.

Aim of this study is to apply a simple toxicity assay to find the occurrence of toxic intermediates during degradation of pharmaceuticals without isolating or identifying the intermediates.

Material & Method

Activated sludge was obtained from Tsuzuki sewage treatment plant.

Ibuprofen (IBP)

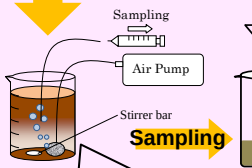


Log K_{ow}: 3.97
MW: 206.3 g mol^{-1}

HPLC Conditions

- **Column & Temperature** Atlantis (4.6 mm × 100 mm, 3 μm Agilent), 25°C
- **Mobile phase** Acetonitrile-water 70:30
- **Flow rate** 2.0 mL min^{-1}
- **Detector & Wavelength** UV, 230 nm

IBP Biodegradation Experiment

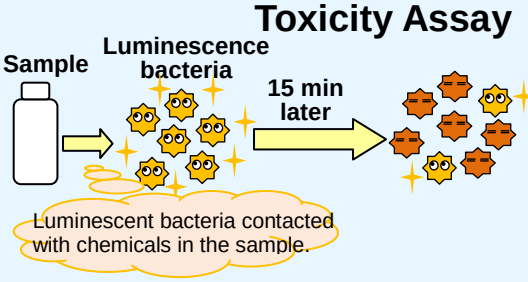


Solid phase Solid phase sample was extracted by methanol and water using ultra-sonication.

Liquid phase Liquid phase sample was measured by HPLC.

MLSS: 2000 mg L^{-1}, Temperature: 25 °C
Aeration rate: 2 L min^{-1}

Toxicity Assay



Toxicity of liquid phase sample was estimated by Microtox assay.

- ➢ Luminescence bacteria are inhibited by chemicals in the sample.
- ➢ Toxicity is evaluated by luminescence inhibition rate.

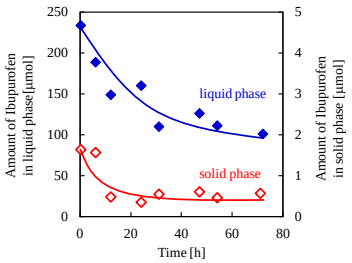
Conclusions

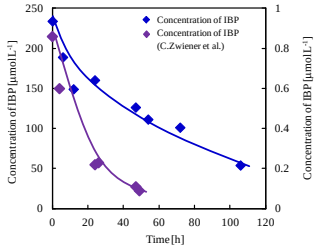
- ➢ 50% of IBP in liquid phase was degraded in 75 h.
- ➢ With the IBP biodegradation, the inhibition rate decreased at 40 h and increased at 75 h.
- ➢ Biodegradation products may have higher toxicity than IBP.
- ➢ Toxicity of biodegradation products should be considered.

Future Work

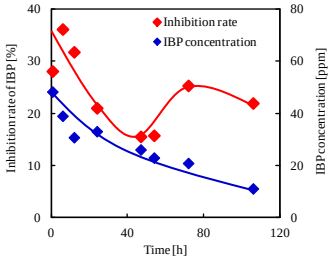
- ✧ To identify biodegradation products having higher toxicity.
- ✧ To apply the simple toxicity assay to other pharmaceuticals.

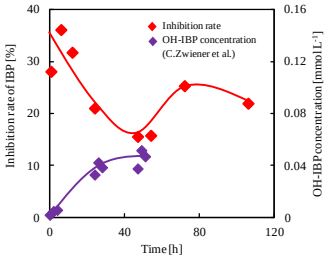
Results & Discussion





- ➢ IBP in liquid phase was degraded 51% by activated sludge in 75 h.
- ➢ Constant solid phase concentration was achieved within 15 hours.
- ➢ As compared with the literature, IBP biodegradation rate in this study was slower.
- ➢ It may be attributed that activated sludge used this study was not acclimated to IBP biodegradation.





- ➢ The inhibition rate at 30 min was 30%.
- ➢ With the IBP biodegradation, the inhibition rate decreased.
- ➢ In 40 hours, the inhibition rate decreased to 15%.
- ➢ Then, the inhibition rate increased to 30% at 75 h.
- ➢ According to the literature, OH-IBP concentration increased during IBP biodegradation.
- ➢ Some of biodegradation products may have higher toxicities than IBP.

Reference

Zwiener, C., Seeger, S., Glauner, T. and Frimmel, F.H. (2002) Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments. Anal Bioanal Chem 372(4), 569-575.