활성슬러지를 활용한 1-butyl-3-methylimidazilium bromide의 독성과 생분해성 평가

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Using activated sludge to explore the inhibitory effects and biodegradability of 1-butyl-3-methylimidazolium bromide

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1. Introduction

Green sustainable chemistry involves the designing of chemical processes, with a view to reduce or even eliminate the use and production of hazardous materials. Recent endeavors have focused on limiting the use of organic solvents, with their replacement by new, environmentally benign media. Of the commonly used alternative solvents, ionic liquids (ILs) have gained increasing attention due to their attractive properties [1]. Although ILs do not contribute to atmospheric pollution, due to their negligible vapor pressure, some have significant solubility in water; therefore, this is the most likely medium through which they will enter the environment. In the present study, we aim to understand the environmental behavior of an imidazolium-based IL by assessing the toxic effects as well as performing the biodegradation study with microorganisms from activated sludge. Degradation products were analyzed and tentatively identified by means of liquid chromatography/mass spectrometry (LC/MS).

2. Materials and Methods

2.1. Chemical

1-butyl-3-methylimidazolium bromide [BMIM][Br] was obtained at 98% of purity from C-tri Co., Korea and was used as supplied without any pre-treatment.

2.2. Activated sludge

As inoculum, activated sludge collected from the aeration tank of a municipal wastewater treatment plant (Jeonju, Korea) was used. After pre-treatment, the sludge was resuspended in mineral medium to yield a concentration of 5 g SS/l and aerated thereafter.

2.3. Toxicity study

Toxicity of ILs was assessed by determining the inhibition of respiration rate [2]. Activated sludge after pre-treatment was mixed with distilled water, synthetic sewage and the test substance at various concentrations. The control was prepared in same manner but contained no IL. After 30 min exposure time, a part of the mixture was transferred to a respiration vessel and respiration rate was determined by an oxygen electrode. The specific respiration rate and respiration rate inhibition by the test substance against the control were calculated.

2.4. Biodegradation study

The biodegradability of the tested compound was evaluated using the modified OECD screening test (OECD 301E) [3]. The IL concentration applied was 7 ppm. The previously prepared sludge and the test compound as sole source of organic carbon were inoculated into 100 ml Erlenmeyer flasks containing 40 ml of mineral medium. Test chambers were placed in the incubator maintained at 70 rpm under dark condition at 25 ± 2°C. At intervals of incubation period, samples were withdrawn for HPLC and LC/MS analysis.

2.5. Analytical methods

HPLC: [BMIM][Br] degradation fragments were separated using an acetonitrile-water gradient method. A reverse-phase column (Xterra C_{18}, 5 μm, 4.6 by 150 mm; Waters) was connected to a Agilent 1100 binary HPLC pump system equipped with a Agilent 1100 series autosampler and a PDA detector. Detector wavelength was set at 264 nm. The elution gradient used consisted of a combined flow rate of 1 ml min^{-1} of 97% A (water containing 0.1% formic acid) and 3% B (acetonitrile containing 0.1% formic acid) for 3 min, which then decreased to 40% A and 60%B for the following 27 min.

LC/MS: The LC/MS experiments were performed using an Agilent 1100 Series LC/MSD Trap SL ion trap mass spectrometer coupled to an Agilent 1100 Series capillary LC system. The column, gradient program and solvents were exactly the same as described above for the HPLC analysis. The ion trap mass spectrometer was operated with the electrospray (ESI) source in positive ion mode with a standard mass range of 50-250 *m/z* and 139 *m/z* was used as target mass.

3. Results and Discussion

3.1. Toxic effects of ILs

As can be seen in Fig. 1, when IL concentration increases, the inhibitory effect on respiration rate of activated sludge becomes strong. However, it was observed that only the highest concentration yielded a significant decrease in respiration rate compared to the control. At concentrations ranging between

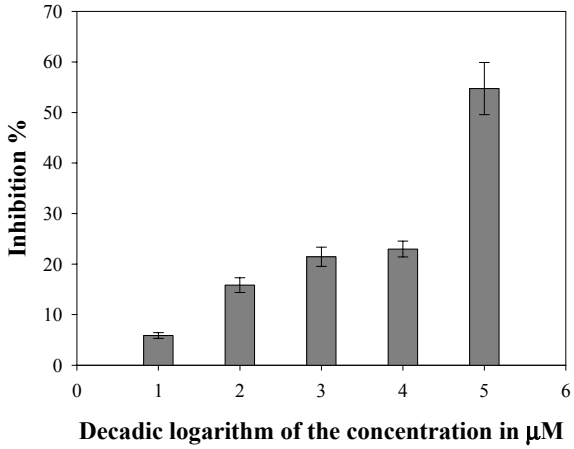

Fig. 1. Respiration rate inhibition of activated sludge in different concentrations of [BMIM][Br].

100 μM and 0.01 M, the toxicity of IL was similar. At the lowest tested concentration (10 μM), the inhibition percent was only 6%, indicated that at this concentration, [BMIM][Br] caused no remarkable effect toward microbial community in activated sludge.

3.2. Biodegradability of ILs

Initial attempts to ascertain the biodegradation of imidazolium salt were performed using HPLC with 0.1% formic acid as the ion pair agent. Fig. 2 demonstrated that microorganisms from activated sludge were able to break down [BMIM][Br] after 45 days of incubation.

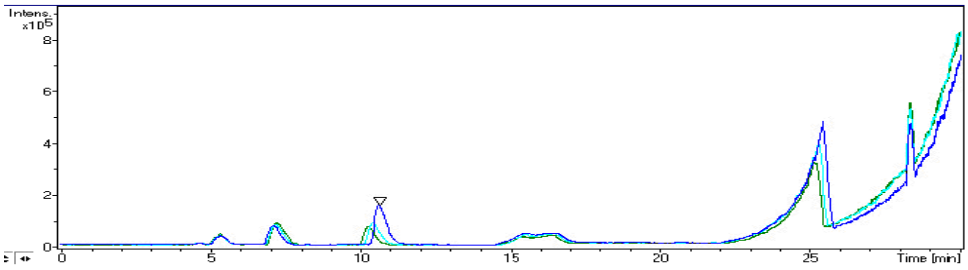

Fig. 2. HPLC chromatograms showing the biodegradation of 1-butyl-3-methylimidazolium cation after 35, 45 and 70 days of incubation with activated sludge compared to the standard sample.

To determine the mass and to deduce the structures of the intermediate compounds, samples containing mixtures of ILs and their degradation products were directly injected into the MS. Based on the molecular mass and prominent fragment of the metabolites (data not shown), the metabolites was surmised to be 1-(1-hydroxybutyl)-3-methylimidazolium, 1-(3-hydroxybutyl)-3-methylimidazolium, 1-butyl-3-hydroxymethylimidazolium, 1-methyl-3-(3-oxobutyl)imidazolium, methylimidazole and butylimidazole. Based on the data obtained, the biodegradation pathways were deduced as illustrated in Fig. 3.

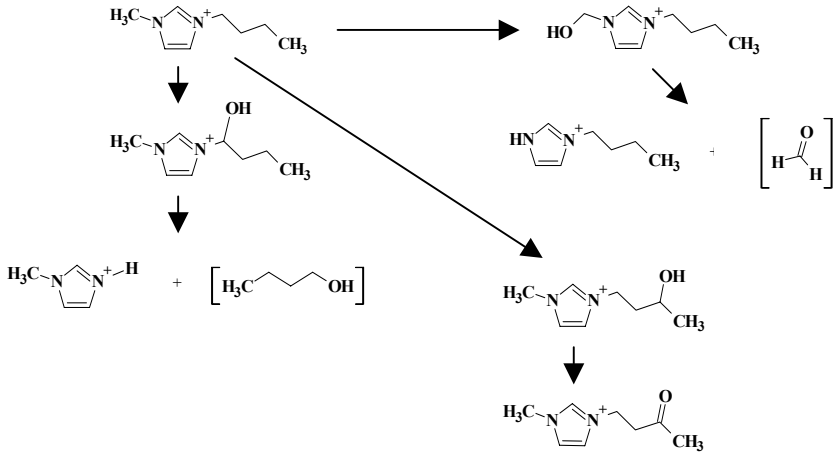

Fig. 3. Biodegradation pathways of 1-butyl-3-methylimidazolium entity by organisms in activated sludge. The intermediates shown in brackets were not detected

From the theoretical prediction of metabolisms [4] and metabolite analysis, it was observed that the metabolism of imidazolium- and pyridinium-based ILs by activated sludge appeared to undergo oxidation reactions catalyzed by cytochrome P_{450} located in the endoplasmatic reticulum of cells. In

the report of Jastorff and coworkers [4], the products of a cytochrome P_{450}-catalyzed hydroxylation of the 1-butyl-3-methylimidazolium cation were proposed according to a theoretical model involving a so-called "oxygen rebound" step. Regarding this mechanism, an "iron-oxo" species reacts by abstracting a hydrogen atom from the substrate to yield a radical intermediate. This radical then reacts with the iron hydroxide species via a hemolytic substitution reaction [5]. Thus, the IL cation can be oxidized at different positions in the alkyl side chains.

4. Conclusion

The present study is the first report to demonstrate the biodegradation of imidazolium IL together with an identification of resulting metabolites. Since the lack of readily accessible biodegradation data is a big gap in our knowledge when considering the employment of ILs on a pilot or manufacturing scale, the results of the metabolic fate of [BMIM][Br] may be useful in alleviating the environmental impacts related to the introduction of this commonly used IL into the environment. Further experiments about the transformation pathways and kinetics of ILs under different environmental conditions and within organisms are highly recommended.

5. References

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