활성슬러지에 의한 이온성 액체 1-butyl-3-methylpyridinium bromide의 생분해: 분해과정과 중간체 증명

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Biodegradation of the Ionic Liquid 1-butyl-3-methylpyridinium bromide by Activated Sludge: Identification of Pathways and Metabolites

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1. Introduction

Ionic liquids (ILs) are low melting organic salts that have emerged as promising "green" replacements for volatile organic solvents [1]. Despite of their non-measurable vapor pressure, some quantities of ILs will soon be present in effluent discharges since they do have significant solubility in water. Recently, the toxic effects of ILs towards aquatic communities have been intensively investigated, but little information is available concerning the biodegradability of these compounds. In the present study, we aim to understand the fate of ILs through their life cycle in the aquatic milieu using microorganisms from activated sludge. Degradation products were analyzed and tentatively identified by means of liquid chromatography/mass spectrometry (LC/MS). The pyridinium moiety, i.e. 1-butyl-3-methylpyridinium bromide, was selected in this research since pyridinium compounds are an important class of chemicals used widely as biocides, cationic surfactants, drugs, and herbicides.

2. Materials and Methods

2.1. Chemical

1-butyl-3-methylpyridinium bromide [BMPy][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. Biodegradation study

The biodegradability of the tested compound was evaluated using the modified OECD screening test (OECD 301E) [2]. 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incubator maintained at 70 rpm under dark condition at 25 ± 2°C. At intervals of 28-day incubation period, samples were withdrawn for HPLC and LC/MS analysis.

2.4. Analytical methods

HPLC: [BMPy] [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 150 *m/z* was used as target mass.

3. Results and Discussion

Initial attempts to ascertain the biodegradation of pyridinium salts were performed using HPLC with 0.1% formic acid as the ion pair agent. It was found that after 18 days of incubation, microorganisms from activated sludge was able to break down [BMPy] [Br]. Over a 28-day period, the biotransformation resulted in complete use of [BMPy] (Fig. 1).

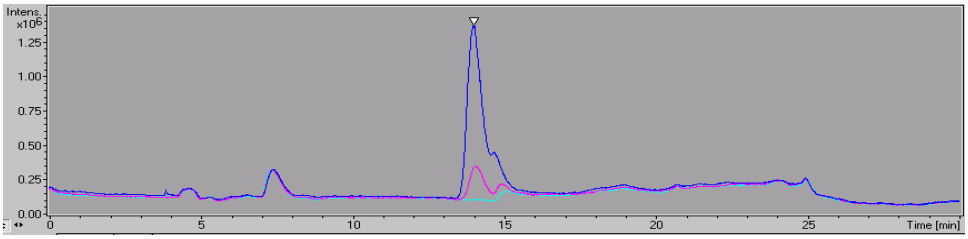

Fig. 1. HPLC chromatograms showing the biodegradation of 1-butyl-3-methylpyridinium cation after 18, 21 and 28 days of incubation with activated sludge

To determine the mass and to deduce the structures of the intermediate compounds, samples containing mixtures of [BMPy] [Br] and its degradation products were directly injected into the MS. Samples taken after 28 days of [BMPy] [Br] degradation yielded data (Fig. 2) that showed a

progressive diminution of the substrate ([BMPy], *m/z* 150) concomitant with the synthesis and consumption of the metabolites. The mass spectrum (*m/z* 165) was consistent with a fragment of 1-hydroxybutyl-3-methylpyridinium containing a hydroxyl group which is located in an unknown position between C_1 and C_4 of the butyl chain. A second metabolite was tentatively identified as 1-(2-hydroxybutal)-3-methylpyridinium with the *m/z* value of 180. The ion at *m/z* 138 represents a fragment 1-(2-hydroxyethyl)-3-methylpyridinium. The ultimate product with *m/z* 93 corresponds to methylpyridine. On the basis of the data obtained, the biodegradation pathways were deduced as illustrated in Fig. 3.

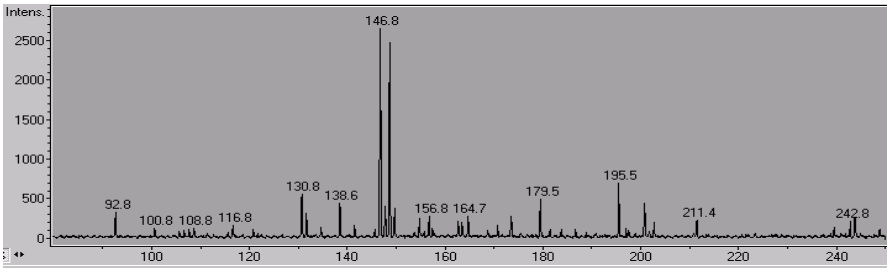

Fig. 2. Mass spectra of the putative metabolites generated from activated sludge cultures incubated with 1-butyl-3-methylpyridinium IL after 28 days

From the theoretical prediction of metabolisms [3] and metabolite analysis, it was observed that the metabolism of [BMPy] [Br] by activated sludge appeared to undergo oxidation reactions catalyzed by cytochrome P_{450} located in the endoplasmatic reticulum of cells. In the first stage, the HEME system of P_{450} enzymes is activated by the dioxygen molecule iron complex, which can react with the carbon-hydrogen bond via a radical mechanism. In the report of Jastorff and coworkers [3], 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 [4]. Thus, the IL cation can be oxidized at different positions in the alkyl side chains. This mechanism might be applied for elucidating the biotransformation of pyridinium salts since the reaction steps only relevant to the alkyl chain and the final degradation product still contained pyridinium core as being shown in the data obtained. In this respect, 1-butyl-3-methylpyridinium compound was converted to 1-hydroxybutyl-3-methylpyridinium with the addition of a hydroxyl functional group. It is likely that subsequent oxidation of the hydroxyl group at C_4 of the butyl chain into carbonyl group concomitant with the addition of a hydroxyl group into C_2 lead to the formation of 1-(2-hydroxybutal)-3-methylpyridinium, which then decomposes to 1-(2-hydroxyethyl)-3-methylpyridinium and acetaldehyde (Fig. 3, pathway I). On a simple approach, hydroxyl functional group might be added into [BMPy] salt at C_2 position of the butyl chain. In this way, the generated 1-hydroxybutyl-3-methylpyridinium was degraded to produce 1-(2-hydroxyethyl)-3-methylpyridinium

and ethane (Fig. 3, pathway II). The methylpyridine detected during [BMPy] [Br] degradation may be generated by via the elimination of ethanol from 1-(2-hydroxyethyl)-3-methylpyridinium. Taken as a whole, the data suggest that microorganisms in activated sludge degrades [BMPy] cation by two pathways, one of which is mediated by the formation of 1-(2-hydroxybutal)-3-methylpyridinium and the other deals with the generation of 1-(2-hydroxyethyl)-3-methylpyridinium directly from 1-hydroxybutyl-3-methylpyridinium.

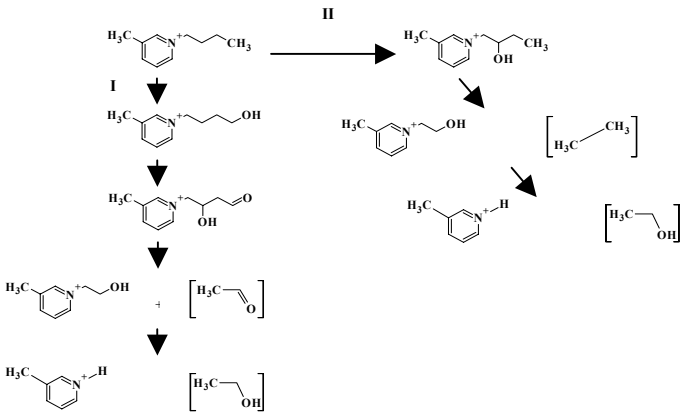

Fig. 3. Biodegradation pathways of 1-butyl-3-methylpyridinium entity by microorganisms in activated sludge. The intermediates shown in brackets were not detected

4. Conclusion

The present study is the first report to demonstrate the biodegradation of pyridinium 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 [BMPy] [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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