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Ionic liquids with a theophyllinate anion

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Experimental

Stability of the synthesized salts

The stability of the synthesized ILs in contact with air as well as in aqueous and alcohol solutions was tested over the period of 7 days at room temperature. Each day the surfactant content was determined by a direct two-phase titration technique EN ISO 2871-2010. The surfactant content did not change in any the studied samples.

Solubility of the synthesized salts

The solubility of the synthesized salts at room temperature was determined according to the technique employed by Vogel (A. I. Vogel, *Preparatyka organiczna,* WNT, Warszawa, 1984). The solubility was established as good when 1 g of the studied salt was dissolved in 1 cm^3 of the solvent. When the salt was dissolved in a higher volume of the solvent (1-3 cm^3), the solubility was established as poor.

Microorganisms

A bacterial consortium with a high biodegradation potential towards studied salts was selected from the group of 218 consortia isolated from petroleum-contaminated soil[22]. The genetic characterization of the bacterial consortium based on the analysis of 16s rRNA sequences revealed the following taxa: *Alcaligenes* (AlcP); *Sphingobacterium* (SphiP); *Citrobacter* (CKK); *Achromobacter* (AchrP); *Comamonadaceae* (ComP); *Pseudomonas* (PseuP); *Variovorax* (VariP).

Preparation of the preculture

The microbial consortium has been stored at -80°C in a 30% (v/v) glycerol stocks. In order to prepare inoculums for biodegradation tests, a stock suspension (1 cm^3) was transferred to a 250- cm^3 Erlenmeyer flask containing 50 cm^3 mineral medium[23] and diesel fuel (0.5%, v/v). Afterwards, the microorganisms were cultivated at 25°C. After 24 h, an aliquot (1 cm^3) of the cell suspension was transferred to a new enrichment flask and the culture was grown for 3 days in the same conditions. This step was repeated three times and cells from the last enrichment were centrifuged at 10,000×g, washed twice with 40 cm^3 of mineral medium and used as a to inoculate the samples for biodegradation tests. Aerobic conditions were provided during all steps. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) revealed that the community in the glycerol stock was the same as the communities in the enrichment cultures.

Preparation of biodegradation tests

The biodegradation tests were carried out in loosely closed 100- cm^3 Erlenmeyer flasks, which contained 50 cm^3 of the mineral medium and approx. 0.025 g of the studied salt as a sole source of carbon and energy. The initial inoculum was adjusted to reach an OD_{600} value of 0.1±0.01 by adding approx. 1 cm^3 of dense cell suspension from the aerobically grown preculture. Afterwards, the cultivation was carried out at 25 °C and 120 rpm for 30 days. The samples were prepared in triplicates. Samples lacking biomass served as control to account for potential abiotic losses. The biodegradation efficiency was calculated as follows: biodegradation efficiency = (IA – RA) / IA x 100, where IA – initial amount of the studied compound; RA – residual amount of the studied compound determined by HPLC-MS.

HPLC-MS analysis of IL residues after biodegradation tests

After finishing the biodegradation tests, the biomass was separated from the supernatant by centrifugation (10.000 g for 10 min.). The separated biomass was rinsed three times with the mineral medium (5 cm^3) and the aliquotes were combined with the supernatant. About 10 cm^3 of the supernatant were subjected to ultrasound-assisted extraction with methanol (3 x 1 cm^3). The extracts were combined, filtered through a 0.2 μm PTFE syringe filter and diluted with methanol:water solution (80:20 v/v).

The chromatographic system UltiMate 3000 RSLC from Dionex (Sunnyvale, CA, USA) was used. Five μL samples were injected into a Hypersil GOLD column (100 mm x 2.1 mm I.D.; 1.9 μm) with a 2.1 mm I.D. filter cartridge (0.2 μm) from Thermo Scientific (Waltham, MA, USA). The mobile phase consisted of 5 mmol dm^{-3} ammonium acetate in water and methanol at a flow rate of 0.2 cm^3/min. Gradient elution was performed by linearly increasing the percentage of organic modifier from 85 to 100% in 4 min and maintained at 100% for 3 min. The LC column effluent was directed to the API 4000 QTRAP triple quadrupole mass spectrometer from AB Sciex (Foster City, CA, USA) through the electrospray ionization source (Turbo Ion Spray) operated in positive ion mode for analyses of cations and in negative ion mode for analyses of anion. The dwell time for each mass transition detected in the MS/MS multiple reaction monitoring mode was set to 200 ms. All the ions were detected using the following settings for the ion source and mass spectrometer: curtain gas 10 psi, nebulizer gas 40 psi, auxiliary gas 45 psi, temperature 400 °C and collision gas medium. The ion spray voltage was 4500 V for cations and –4500 V for anion. The declustering potential was 50 V for cations and –50 V for anions.