Biotreatability of selected choline-based deep eutectic solvents

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Abstract

Deep eutectic solvents (DES) are used in laboratory and industrial scale in different processes. One of the most widespread components used for the formation of DES is choline chloride with good solubility in water resulting in their presence in wastewaters. The aim of our study was to compare biotreatability of two choline-based eutectic solvents, made of choline chloride with oxalic and malonic acid. Toxicity to microorganisms was assessed by determination of inhibition of bioluminescence by *Vibrio fisheri* and by determination of inhibition of oxygen consumption by activated sludge. Both DESs were very toxic to heterogenic and nitrifying microorganisms. Their toxicity to *Vibrio fisheri* was lower and they were well biodegradable (90-95%) at non-toxic concentrations. Respirometric measurements in open respirometer, simulating conditions in actual aerobic biological wastewater treatment plant, confirmed biotreatability of both investigated DES at low concentrations.

Keywords: Biotreatability, biological wastewater treatment plant, deep eutectic solvents, toxicity

1. Introduction

Many of widely used as well as newly designed chemicals are belonging to a group of organic pollutants expressing high environmental risk. Prevailing are usually industrial chemicals, used for different purposes (solvents, additives, stabilizations, accelerators, etc.), which are toxic, slowly (bio)degradable, bioactive and (bio)accumulative in terrestrial and aquatic ecosystems (Fent, 2003). Some of them express also endocrine disruptive effects. They could bio-concentrate at the end of the food chain, resulting in high concentrations in fish and seafood, consumed by humans. In the recent years, there has been intensive development of non-hazardous solvents and reaction media able to replace common organic compounds. As a result, ionic liquids (IL) and deep eutectic solvents (DES) were introduced and they are used in lab and industrial scale in different processes (bio-transformations, separation) (Dai, 2013) They are obtained by simply mixing of two low-cost, safe and biodegradable components capable to form eutectic mixture. One of the most widespread components used for the formation of DES is choline chloride, low-cost, biodegradable and non-toxic quaternary ammonium salt. This new phase is generally characterized by a lower freezing point than that of individual constituents (Durand, 2013; Zhang , 2012). Although DES can lower the risk of air pollution due to their non-volatility, they do have significant solubility in water and consequently, they could spread widely by wastewaters and affect large area in terms of chronic toxicity, bioaccumulation or even bio-magnification if they are persistent or poorly biodegradable in environmetal conditions (Fent, 2003; Dai, 2013). The most effective management of these solvents is thus their entrapment in industrial process as recycling or reuse or at least in wastewater collection system proceeded by effective end-of pipe treatment. Biological treatment of municipal and industrial wastewater is often used due to its reliability, simplicity and high cost-effectiveness and provides many advantages in term of biodegradable matter and nitrogen as well as phosphorous compounds removal. However, the efficiency of biological processes is strongly limited in presence of refractory or inhibitory compounds in wastewaters (Pitter, 1990). To assess the impact of newly applied substances on biological treatment plant, especially for chemicals with considerable technical and commercial potential, data on toxicity to activated sludge, and biodegradability must be acquired. The aim of our study was to determine treatability of two chlorine-based eutectic solvents in aerobic biological wastewater treatment plant using approach based on their toxicity and biodegradability.

2. Methods

Two choline-based eutectic solvents, made with mixing of choline chloride with oxalic acid (1:1 molar ratio) and choline chloride with malonic acid (1:1 molar ratio) were used. First, stability of both DES was checked by preparing solution with 0.04 v/v of each solvent and put in dark and light place. Stability was followed by dissolved organic carbon (DOC) measurements of non-filtered samples for 28 days (ISO 8245, 1999). Freeze-dried luminescent bacteria *Vibrio fischeri* (DR. LANGE LUMIStox, 2001) were used for bioluminescence inhibition test and toxicity was evaluated as 30minEC_{50} value (ISO 11348-2, 1998). Activated sludge (1500$mg_{MLVSS}.L^{-1}$) from regional municipal wastewater treatment plant was used for activated sludge oxygen consumption inhibition test (ISO 8192, 2007) and toxicity was evaluated as 30/180minEC_{50} value for different types of present microorganisms. The toxic impact was evaluated for all microorganisms of activated sludge and heterotrophic and nitrifying separately as well. Test was performed twice and average values were calculated. The impact of pH to toxicity was also investigated. To assess final impact to aerobic biological wastewater treatment plant, experiments in open respirometer were accomplished. 1 L of activated sludge

was aerated to obtain constant oxygen concentration value and then oxygen uptake rate was determined after addition of different amounts of both DES. Deep eutectic solvents were added into the system in concentration of 0.029 and 0.125 v/v.% (DES A - choline chloride and oxalic acid) and 0.031 and 0.125 v/v.% (DES B - choline chloride and malonic acid). Lower concentrations were selected on the basis of toxicity test with activated sludge to avoid severe toxic impact, while higher ones were in the range of toxicity aimed to see the impact to activated sludge. Specific heterotrophic endogenous (Ri) and exogenous respiration (Re) were determined (Pitter, 1990; Derco, 2013). For biodegradability assessment method by analysis of oxygen consumption was applied (ISO 9408, 1999). Deep eutectic solvents were added into the test mixtures in concentration of 0.0147 v/v.% (choline chloride and oxalic acid) and 0.0156 v/v.% (choline chloride and malonic acid). Concentrations were chosen to avoid any toxic impact to activated sludge (as high as $30minEC_{20}$ value determined on the basis of previously mentioned activated sludge oxygen consumption inhibition test). Abiotic degradation was evaluated in the system without microorganisms. (Bio)degradation (D_t, %) was plotted versus time to obtain (bio)degradation curves. They were used to determine maximal extent of biodegradation (%) or degradation (%) in the case of abiotic system.

3. Results

Results of stability test with both investigated DES are presented in Table 1. Results indicated, that DOC did not decrease more than 5% in 28 days of the stabilisation study in light and dark conditions. It has been confirmed, that concentration of the DES did not change due to the external parameters in further toxicity testing. Components of tested mixtures are not volatile and thus the initial concentrations should be maintained constant during tests. Toxicity of investigated DES to luminescent bacteria and mixed culture of activated sludge is presented in Table 2. Both DES had low pH of 2.0 and 2.7, respectively, due to the presence of acidic components. In the test with *Vibrio fischeri* initial pH was corrected to 7.0 ± 0.2 to assure optimal conditions for test organisms, but in the case of DES A (choline chloride and oxalic acid) precipitate was formed and we were unable to perform toxicity test. DES B was less toxic to marine bacteria *Vibrio fischeri* than to microorganisms of activated sludge. However, both solvents expressed high but comparable toxicity to microorganisms of activated sludge, being more toxic to nitrifying then heterotrophic microorganisms. This could cause a problem in nitrification process during biological treatment, but toxic impact decreased with time of incubation (30/180 min). With second DES B, toxicity test was performed also after pH regulation to 7.0 and much lower toxicity was observed; $30minEC_{50}$ for total microorganisms was 1.4 v/v.%, $30minEC_{50}$ for heterotrophs was 2.6 v/v.%, and $30minEC_{50}$ for nitrifyers increased up to 0.2 v/v.%. As a result, pH regulation was recommended as one of the pretreatment options for wastewater, containing investigated eutectic solvent. Results of treatability testing in open respirometer are presented in Table 3. Oxygen uptake rate was used as an alternative means to confirm biodegradability and toxicity measurements (Zhang, 2012).

Table 3. Oxygen uptake rates in the tests with eutectic solvents (DES A = choline chloride and oxalic acid; DES B = choline chloride and malonic acid) in open respirometer.

Sample	DES A		DES B	
Concentration (v/v.%)	0.029	0.125	0.031	0.125
R_i (g_{O2} g_{sludge}^{-1} day^{-1})	0.093	0.103	0.106	0.104
R_e (g_{O2} g_{sludge}^{-1} day^{-1})	0.177	0.062	0.308	0.018
R_e/R_i (/)	1.89	0.60	2.91	0.17

During the endogenous respiration phase, microorganisms utilize oxygen at a constant endogenous specific respiration rate R_i (g_{O2} g_{sludge}^{-1} day^{-1}) over a relatively long period of time. The addition of substrate to the respirometric cell causes a temporary increase in the respiration rate and thus a specific rate of substrate oxidation Re (g_{O2} g_{sludge}^{-1} day^{-1}) can be computed (Pitter, 1990). Data in Table 3 showed that increased amount of DES added increase toxicity resulting in reduced oxygen consumption. This could be seen from reduced R_e/R_i ratio. Toxicity of both DES, determined in toxicity test (Table 2) was therefore confirmed by measurements in open respirometer. However, due to relative high R_e, indicating good biodegradability, further biodegradation test was accomplished to confirm biodegradability of tested mixtures. Both eutectic solvents degraded well. Biodegradation of DES A and B reached 91 ± 2% and 94 ± 1% after 1.4 days of lag phase, respectively. Both eutectic solvents were assessed as ready biodegradable without any abiotic degradation (3 ± 2%) as indicated also in stability test (Table 1). It should be emphasized, that biodegradability tests were accomplished with non-toxic concentrations of DES.

4. Conclusion

The aim of our study was to determine treatability of two almost identical choline-based eutectic solvents in terms of their toxicity to organisms of activated sludge at different conditions as well as their biodegradability. Investigated mixtures were choline chloride/oxalic acid and choline chloride/malonic acid, both in 1:1 molar ratio. They were readily biodegradable but quite toxic to microorganisms of activated sludge as also confirmed by measurements in open respirometer. Results pointed out, that pH regulation in case of these acidic DES is a good and low-cost treatment option to reduce toxicity to activated sludge, but remained toxicity is still high enough. Consequently, recycling seems to be the most viable option.

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Table 1. Stability of investigated eutectic solvents in light and dark conditions (DES A = choline chloride and oxalic acid; DES B = choline chloride and malonic acid)

	Conditions	Parameter	Time [Days]							
			0	1	2	3	4	14	21	28
DES A	Light	TC (mg L^{-1})	365750	371750	359250	353500	364250	356000	365250	358921
		IC (mg L^{-1})	<1	<1	<1	<1	<1	<1	<1	<1
		TOC (mg L^{-1})	365750	371750	359250	353500	364250	356000	365250	358921
	Dark	TC (mg L^{-1})	361564	364258	357985	348921	355772	357941	358661	357213
		IC (mg L^{-1})	<1	<1	<1	<1	<1	<1	<1	<1
		TOC (mg L^{-1})	361564	364258	357985	348921	355772	357941	358661	357213
DES B	Light	TC (mg L^{-1})	515250	513750	510250	510250	510500	503000	514750	512672
		IC (mg L^{-1})	<1	<1	<1	<1	<1	<1	<1	<1
		TOC (mg L^{-1})	515250	513750	510250	510250	510500	503000	514750	512672
	Dark	TC (mg L^{-1})	511452	513462	512500	511369	513200	514200	512500	513214
		IC (mg L^{-1})	<1	<1	<1	<1	<1	<1	<1	<1
		TOC (mg L^{-1})	511452	513462	512500	511369	513200	514200	512500	513214

TOC = Total Organic Carbon; IC = Inorganic Carbon; TC = Total Carbon

Table 2. Toxicity of investigated eutectic solvents (DES A = choline chloride and oxalic acid; DES B = choline chloride and malonic acid) to microorganisms of activated sludge.

Test organism	EC_{50} (v/v.%)	DES A	DES B
Vibrio fischeri	30minEC_{50}	/	3.2 ± 0.5
Activated sludge:			
• Total microorganisms	30minEC_{50}	0.024 ± 0.002	0.031 ± 0.002
	180minEC_{50}	0.027 ± 0.001	0.041 ± 0.005
• Heterotrophic microorganisms	30minEC_{50}	0.028 ± 0.003	0.039 ± 0.003
	180minEC_{50}	0.030 ± 0.003	0.047 ± 0.002
• Nitrifying microorganisms	30minEC_{50}	0.017 ± 0.001	0.023 ± 0.001
	180minEC_{50}	0.024 ± 0.002	0.033 ± 0.002

/...Not determined.

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