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TOXICITY OF CHOLINE AMINO ACIDS-BASED IONIC LIQUIDS TOWARD YARROWIA LIPOLYTICA CELLS

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PURPOSE OF THE ABSTRACT

Ionic liquids (IL) are widely regarded as "green" solvents, mainly due to their negligible vapour pressure and non-inflammability. Nevertheless some studies have shown that commonly used imidazolium and pyridinium-based ILs are not environmentally friendly and biodegradable as expected at first. Choline amino acids-based ionic liquids have been considered as non-toxic or practically harmless for various microorganisms and some aquatic organisms [1-3]. Aiming their use in microbial biocatalysis to produce high-value chemicals, biofuels, and bioproduct, in addition to biomass pretreatment, it is important to determine toxicity to microbial platforms such as *Yarrowia lipolytica*. The viability of *Yarrowia lipolytica* IMUFRJ50682, were evaluated in the presence of [Ch][Gly] choline glycinate, [Ch][His] choline histidinate, [Ch][Ala] choline alaninate, [Ch][Asn] choline asparaginate, [Ch][Asp] choline aspartate, [Ch][Pro] choline proline, [Ch][Gln] choline glutamate, [Ch][Glu] choline glutamate, [Ch][Ser] choline serinate. Maximum non toxic concentration (MNTC) was determined following standard methods of CLSI (Clinical and Laboratory Standards Institute) by turbidity. Table 1 shows the MNTC values obtained. For the majority of ionic liquids the MNTC values were similar, with the exception of 3 ionic liquids that showed the following ascending order of toxicity: [Ch][His] > [Ch][Gly] > [Ch][Gln]. Such similar results make it difficult to establish any standard response to the structure of these compounds. A slight increase in MNTC was observed for amino acid with a hydroxyl group on their side chain, [Ch][Gln], when compared to its analogue [Ch][Glu], which has an amide group instead of hydroxyl. However, this same behavior could be expected between [Ch][Asp] and its analogue with amide group [Ch][Asn], which did not present a difference in MNTC. [Ch][His] was the most toxic among those evaluated for *Y. lipolytica*. The presence of aromatic ring, an imidazole group in this case, may represent this increased toxicity for [Ch][His]. A previous study showed that ionic liquids with imidazolium were more toxic for this yeast than choline cation [4]. pH values measured in the MNTC indicated that, in higher concentration, these amino acid-based IL can significantly increase the pH of the medium, which can result in a higher toxic effect for *Y. lipolytica*. This phenomenon was also discussed for bacteria when in the presence of ionic liquids based on [Ch][amino acids] [5]. Although *Y. lipolytica* has shown a high tolerance to these ionic liquids when taking into account the concentrations values applied in the ecotoxicological tests (1000 mg / L), perhaps, the MNTC values presented here are not suitable to perform biocatalytic processes. This determination will depend on the process to be performed.

1. Foulet A, Ghanema O, El-Harbawi M, et al (2016) Understanding the physical properties, toxicities and anti-microbial activities of choline-amino acid-based salts: Low-toxic variants of ionic liquids. *J Mol Liq* 221:133-138.

2. Ghanema O Ben, Papaiconomou N, Mutalib MIA, et al (2015) Thermophysical properties and acute toxicity towards green algae and *Vibrio fischeri* of amino acid-based ionic liquids. *J Mol Liq* 212:352?359.
3. Yazdani A, Sivapragasam M, Leveque JM, Moniruzzaman M (2016) Microbial Biocompatibility and Biodegradability of Choline-Amino Acid Based Ionic Liquids. *J Microb Biochem Technol* 08:415?421.
4. Santos AG, Ribeiro BD, Alviano DS, Coelho MAZ (2014) Toxicity of ionic liquids toward microorganisms interesting to the food industry. *RSC Adv* 4:37157?37163 .
5. Hou X-D, Liu Q-P, Smith TJ, et al (2013) Evaluation of Toxicity and Biodegradability of Cholinium Amino Acids Ionic Liquids. *PLoS One*.

FIGURES

Table 1 Maximum Non Toxic Concentration (MNTC) values for choline amino acids-based ionic liquids toward *Yarrowia lipolytica* cells.

Ionic liquid	MNTC % (w/v)	MNTC mg/L	Upper limit (mg/L)	Lower limit (mg/L)	pH in MNTC
[Ch][His]	0,625	6250	300000	195	7,50
[Ch][Gly]	0,81	8100	400000	253	7,89
[Ch][Gln]	0,94	9400	150000	146	8,62
[Ch][Ala]	1,25	12500	200000	195	8,81
[Ch][Asn]	1,25	12500	400000	390	8,57
[Ch][Asp]	1,25	12500	400000	390	8,36
[Ch][Pro]	1,25	12500	400000	390	8,51
[Ch][Glu]	1,25	12500	200000	195	6,96
[Ch][Ser]	1,25	12500	200000	195	8,99

The upper and lower limits refer to the highest and lowest concentrations tested in the assay, respectively.

FIGURE 1

Table 1 Maximum Non Toxic Concentration (MNTC) values for choline amino acids-based ionic liquids toward *Yarrowia lipolytica* cells.

Table 1

FIGURE 2

KEYWORDS

Ionic liquids | *Yarrowia lipolytica* | Toxicity | Choline

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3. Yazdani A, Sivapragasam M, Leveque JM, Moniruzzaman M (2016) Microbial Biocompatibility and Biodegradability of Choline-Amino Acid Based Ionic Liquids. *J Microb Biochem Technol* 08:415–421.
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