

It is assumed that the eutectic mixtures (a mixture of two or more substances of specified composition, which is separated from the solution liquid at a certain temperature) based on non-toxic salt of choline and glycerol or urea, can be used as an alternative to the water environment for enzymatic reactions and microbial cultures. The objective of this project is to determine the activity of selected enzymes in non-aqueous media, both in *ex vivo* (outside the body) or *in vivo* (in the living body). Cultures of microorganisms producing these enzymes, will be carried out in suitably designed eutectic mixtures with a limited water content, which will give the answer to the question: Is it possible to live without water?

It is planned to design at least 10 mixtures of ionic liquids (i.e., organic or inorganic salts with low melting points) with suitable properties for this project. These will include in their composition the choline salt and urea or glycerol. The activity of three enzymes of microbial origin: subtilisin (an enzyme that breaks down proteins), chitinase (chitin-degrading enzyme) and  $\beta$ -galactosidase (lactose-degrading enzyme) has been verified by performing the reaction in each of the prepared ionic liquid and the reference reaction in an aqueous medium carried out under the same conditions. We will perform similar tests on the microorganisms producing these enzymes. Each will be grown in the test ionic liquids with additional growth control carried out in aqueous medium. Exposed samples will be analyzed by chromatographic methods (high performance liquid chromatography or gas chromatography) and colorimetric methods (method consists in determining the concentration of a substance by comparing the color of the test solution and reference - colorimeter) appropriate for reaction products of selected enzymes. Suitable colorimetric methods are also going to be used to determine the metabolic activity of microorganisms.

This project aims to check the biocompatibility of a new type of ionic liquids, not studied so far in this regard. It will also show what sort of relationship involves the activity of enzymes in such extreme conditions and the survival of living organisms responsible for their production. Given the interest in the subject of ionic liquids by industry, it can be expected that usefulness of those results as a theoretical basis for further projects, strongly focused on the commercial development of technology. It is estimated that the use of ionic liquids as solvents may facilitate isolation of the product and lead to continuous production processes. It will also limit waste production. It should be expected that the implementation of this type of technology will increase the availability of a wide variety of products manufactured using biocatalysis (chemical or biochemical reactions occurring in a living organism), such as medicines, food and household chemistry. It is also likely to reduce the environmental impact of their production and emerging waste in the process. The project can also contribute to the discovery of previously unknown reactions and enzymatic activities, which in the long term may enable discovery or creation of new metabolic pathways. Finally, the results may have purely scientific significance for the biology of extremophiles (organisms that could live and thrive in extreme conditions), such as astrobiology, *inter alia*, by helping to explain whether and how carbon-based life can exist without water.