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# A NEW BIOTECHNOLOGICAL MEDIUM FOR BIOTRANSFORMATION OF SUBSTRATES WITH DIFFERENT WATER-SOLUBILITY

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***Abstract***

*Reverse micellar system (RMS) provide an excellent medium for nonaqueous biocatalytic studies, being used for enzymatic conversion of aqua-low-solubility reactants. RMS is characterized by hydration degree, $w_0$, defined as the molar ratio of water and surfactant. This parameter induce most of the structural and physico-chemical properties of RMS, being more important even then the absolute quantity of water or surfactant in the system. The reaction of alcohol oxidase from Hansenula polymorpha solubilized in AOT-isooctane reverse micelles was used as a small scale model, both for experimental study and theoretical discussions. The potential of the new reaction medium for biotransformation-catalyzed alcohol oxidase was evaluated using substrates with different hydrophilic / hydrophobic balance. The efficiency of the bioconversion of aliphatic alcohols in RMS has been analyzed.*

***Key words*:** *reverse micelles, biotransformation, alcohol oxidase, AOT*

## INTRODUCTION

One of the nanostructured medium with scientific and biotechnological applicative potential is the reverse micellar system (RMS), a representing of the colloidal chemistry [1], based on the self-assembling capacity of amphiphilic molecules of surfactants in organic solvent (min 95%). The resulting spherical or ellipsoidal shape surfactant aggregates are thus closely packed globules where the polar head group of the surfactant molecules occupies the interior of the aggregates whereas the hydrophobic tails extend into the bulk apolar solvent, with water encapsulated in compartments [2].
They are characterized by the hydration degree, $w_0$, defined as the molar ratio of water and surfactant [3]. This parameter induce most of the structural and physico-chemical properties of RM, being more important even then the absolute quantity of water or surfactant in the system.

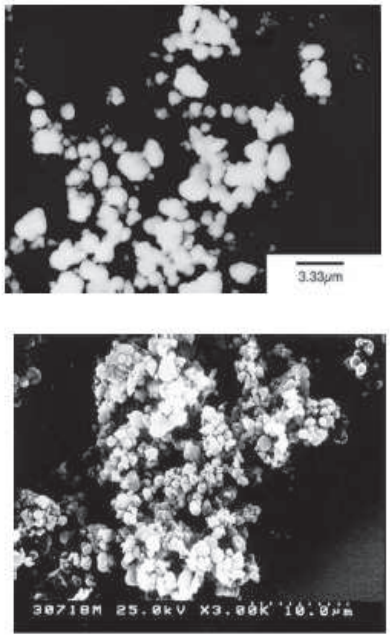


Fig.1. Electron microscope images of reverse micelles

RM media are able to solubilize proper solution, the main driving forces responsible for the solute distribution between the organized assembly and the organic medium being considered to be mainly hydrophobic effects and hydrogen bond interactions. However,

other effects such as chemical and electrostatic interactions must be considered when charges or zwitterionic molecules are involved. In these supramolecular systems, a solute can be located in a variety of microenvironments namely the surrounding organic solvent, the water pool or at the micellar interface [4]. To know the location of molecular probes in the aggregates, can give information about their residing place in biological systems.

RMS provide an excellent medium for nonaqueous biocatalytic studies, being used for enzymatic conversion of aqua-low-solubility reactants or to improve the unfavorable thermodynamic yield by shifting the reaction equilibrium due to the decrease of water content. They have two important characteristics: first, are a cell membrane-mimetic medium [5] and, second, are a macroscopically pseudo-homogeneous and optical transparence system [6], so all spectroscopic techniques can be applied.

In the present studies, we are focusing on the oxidation activity of alcohol oxidase **(**EC 1.1.3.13, alcohol:oxygen oxidoreductase) in a reverse micellar system of sodium bis(2-ethylhexyl) sulfosuccinate (AOT), in isooctane. Alcohol oxidase, a peroxisomal enzyme, plays a major role in the metabolism of methanol resulting in the formation of formaldehyde and has a significant practical role in analytical determination of alcohols and yielding aldehydes, hydrogen peroxide, and various heterologous proteins [7]. The main aim of the study is to evaluate the potential of the enzymatic oxidation of aliphatic alcohols in a AOT-isooctane reverse micelles.

## MATERIAL AND METHOD

***Reverse Micelles Preparation***

Reverse micelles were prepared by injection of different reactants into 50 mM AOT-isooctane stock solution, followed by gentle shaking, until a completely transparent solution was formed.

***Enzyme assay***

The standard procedure for alcohol oxidase assay was performed according to Janssen and Ruelius [8]. The initial rate of reaction was recorded at 415 nm.

***Experimental conditions***

The variation of the hydration degree was obtained by changing the water volume and maintaining constant the AOT concentration. The overall enzyme concentration was kept constant through the whole range of $w_0$ – values.

## RESULTS AND DISCUSSIONS

Certain enzymes, especially oxidoreductases, have the potential for the biotransformation of hydrophobic compounds as substrates. However, the poor solubility of such compounds in water is a critical problem for industrial applications. To overcome this problem, enzymatic reactions of alcohol oxidase in a non-conventional medium, such as reverse micelles, have been tested.

Substrates susceptible to the conversion catalyzed by alcohol oxidase are differentiated by their degree of polarity which is materialized in the case of AOT-isooctane reverse micelles through different miscibility of the substrates between the two major phases of the reaction environment: polar aqueous microenvironment and apolar organic phase.

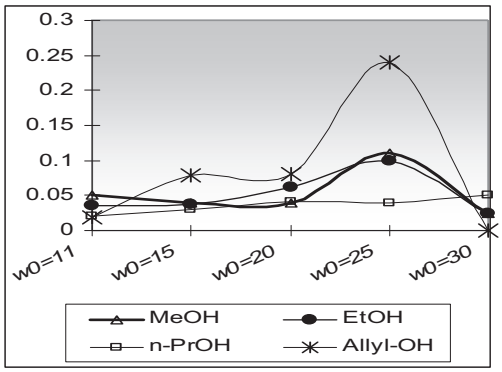
$$S_{solvent} \rightleftharpoons S_{interface} \rightleftharpoons S_{water\text{-}pool}$$

The catalytic activity of the studied enzyme, in the presence of various substrates, was performed in the range of w0 = 11-30, taking into the account that hydration degree is considering the defining parameter for the caracteristics of the reverse micellar environment [9], [10].

Methanol is characterized by immiscibility in the organic phase of reverse micellar system, i.e. isooctane, and therefore it can be considered that this substrate is accumulated only in the intramicellar aqueous cavity (the solubility of methanol in isooctane being negligible).

Experimental data not presented show that the dependence of alcohol oxidase activity vs the concentration of methanol was a Michaelis-Menten type and the catalytic activity, in the range of $w_0$ = 11 to 30, has a maximum value at $w_0$ =25 (Fig.2 A).

**A.**




**B.**

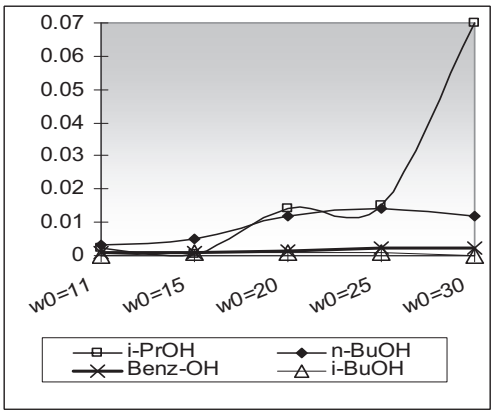



Fig. 2. Catalytic activity of alcohol oxidase in the presence of different substrates (in the range of $w_0$ = 11-30)

Generally, the dependence of enzyme activity vs.hydration degree was determined to be a Gaussian curve, which implies an optimum w0, where the enzyme presents maximum activity in reverse micelles environment [11].

The upturn in the curves is described either by changes in the aqueous microenvironment [12], or conformational distortion of the enzyme molecule at low values of the degree of hydration [13]. The downward of the curve is determined by variation of intramicellar pH or local dilution of reactants [14].

Unlike methanol, miscible only in the aqueous phase, other substrates, such as aliphatic alcohols with 2 or 3 atoms of carbon (ethanol, n-propanol, i-propanol) or unsaturated alcohols, such as allyl alcohol (3 propene-1-ol), are characterized by miscibility both in aqueous environment and isooctane phase. In this case, the substrates will be distributed between the two phases of RMS, according to the individual partition coefficient of each substrate.

Despite this difference, for ethanol and allyl alcohol, biotransformation profile vs $w_0$ ($w_0$ =11–30) is Gaussian, similar with those for methanol (Fig. 2A).

The hydrofobicity of enzymatic substrates is increasing for aliphatic alcohols with 4 atoms of carbon (n-butanol and i-butanol) or with aromatic ring (benzyl alcohol). Thus, their miscibility in the apolar phase versus the miscibility in the polar phase of reverse micelles is also increasing.

Even if n-propanol, i-propanol, n-butanol, i-butanol and benzyl alcohol have a good miscibility in the organic phase of RM medium, the biotransformation yield is very poor, respecting the well-known substrate specificity of alcohol oxidase in aqueous medium.

In the case of alcohols with 3 or more atoms of carbon, branched or unbranched, the biotransformation profile is no longer Gaussian (Fig. 2B).

## CONCLUSIONS

From biotechnological point of view, it has demonstrated that AOT-isooctane reverse micelles with hydration degree in the range of w0=10-30 is a suitable media for enzymatic conversion of alcohols with alcohol oxidase, in a large range of their water solubility (from 100% to moderate).

The most important avantage of this new medium is the increasing of the range of substrates which are compatible with the enzymatic reaction and the performance of the reaction in a pseudo-homogeneous water-apolar solvent medium.

## ACKNOWLEDGEMENTS

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## REFERENCES

[1] Shrestha , L. K., Shrestha , R.G., Abe, M., Ariga, K., 2011*, Reverse micelle microstructural transformations induced by oil and water*, Soft Matter, 7, 10017-10024

[2] Tonova, K., Lazarova, Z., 2008, *Reversed micelle solvents as tools of enzyme purification and enzyme-catalyzed conversion*, Biotechnol. Adv., 26, 516–532
[3] Fenn, E. E.,Wong, D.B., Fayer, M.D., 2011, *Water dynamics in small reverse micelles in two solvents:Two-dimensional infrared vibrational echoes with two-dimensional background subtraction*, J.Chem. Phys., 134, 054512
[4] Shah, C., Sellappan, S., Madamwa, D., 2000, *Entrapment of enzyme in water-restricted microenvironment-amyloglucosidase in reverse micelles*, Process Biochemistry , 35 , 971–975
[5] Bru, R., Sanchez-Ferrer, A., Garcia-Carmona, F., 1995, *Kinetic models in reverse micelles*, Biochem. J., 310, 721-739
[6] Levashov, A.V., Klyachko, N/L., 2001, *Micellar enzymology: methodology and technique,* Russ.Chem.Bull., Int.Ed., 50 (10), 1718-1732
[7] Jassen, F.W., Ruelius, H.W.,1968, *Alcohol oxidase, a flavoprotein from several basidiomycetes species: Crystallization by fractional precipitation with polyethylene glycol*, Biochemica et Biophysica Acta , 151, 330-342
[8] Gvozdev, A. R., Tukhvatullin, I. A., Gvozde, R. I, 2010, *Purification and Properties of Alcohol Oxidase from Pichia putida*, Biochemistry (Moscow), 75 (2), 242-248.
[9] Martinek, K., Berezin, I.V., Khmelnitsky, Y.L., Klyachko, N.L., Levashov, A.V., 1987, *Kinetic theory of enzymatic reactions in reversed micellar systems*, Coll. Czech. Chem. Commun., 52 , 2589 – 2602
[10] Kabanov, A., Levashov, A.V., Klyachko, N.L., Namyotkin, S.N., Pshezhetsky, A.V., Martinek, K., 1988. *Enzymes entrapped in reversed micelles of surfactants in organic solvents: a theoretical treatment of the catalytic activity regulation*. J. Theor. Biol., 133, 327–343
[11] Luisi, P.L.,Giomini, M.,. Pileni, M.P., Robinson, B.H., 1988, *Reverse micelles as hosts for proteins and small molecules, Biochim. Biophys.* Acta , 947, 209-246,
[12] Klyachko, N.L., Levashov, A.V., Martinek, K., 1984, *Catalysis by enzymes entrapped into hydrated surfactant aggregates*, Mol. Biol., 18 , 1019 - 1031
[13] Luisi,P.L., Meier, P., Imre, V.E., Pande, A., 1984, *Reverse Micelles*, Ed. P.L. Luisi & B.E.Straub, Plenum Publ.Co., p. 323 - 337
[14] Fujii, H.., Kawai, T.., Nishikawa, H.., Ebert, G., 1982, *Determination of pH in reversed micelles .2. Application of the method proposed previously to other reversed micellar systems,* Colloid Polymer Sci., 260 , 697–699