Nanobioconjugates of *Candida antarctica* lipase B and single-walled carbon nanotubes in biodiesel production

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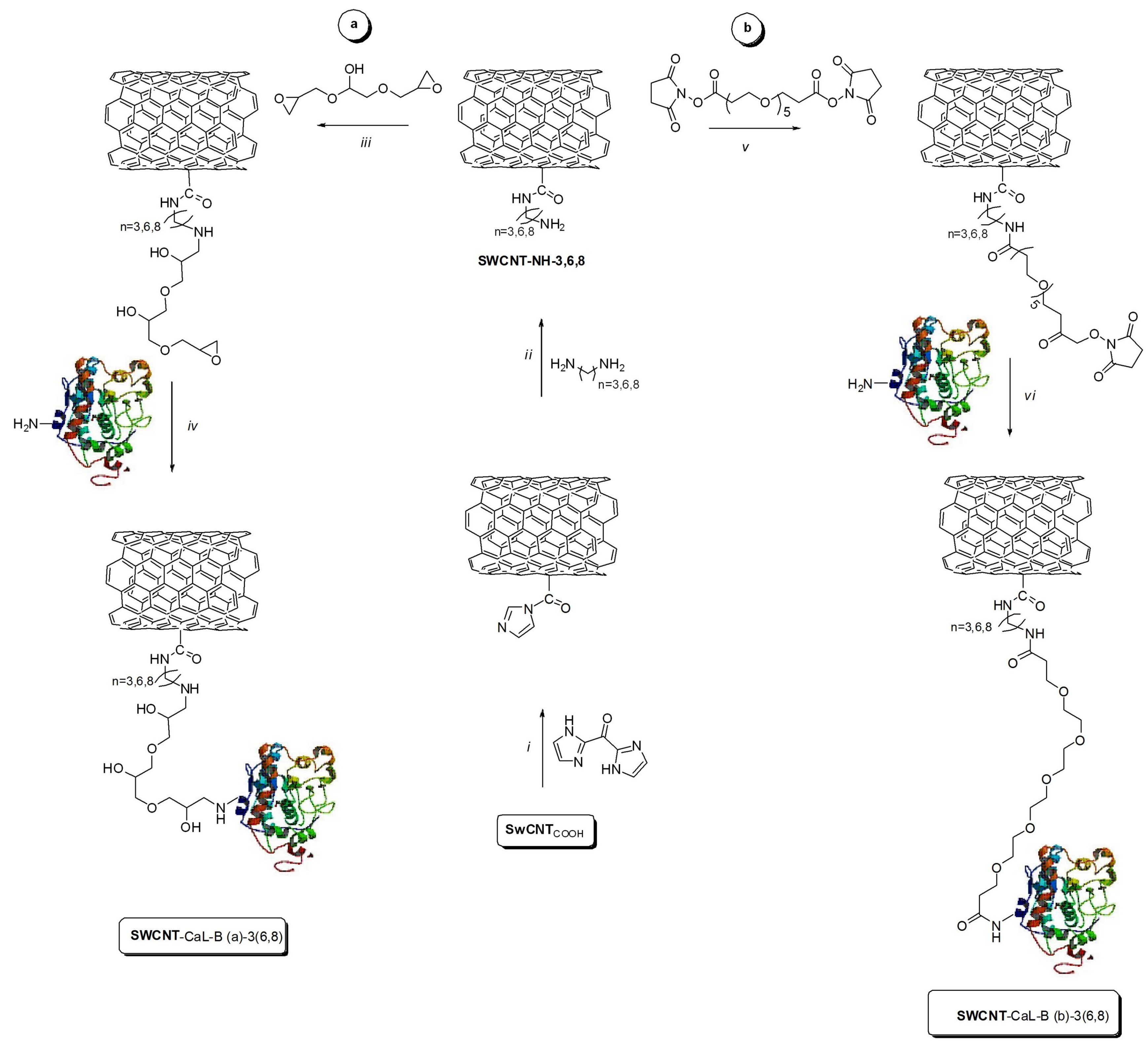
Introduction

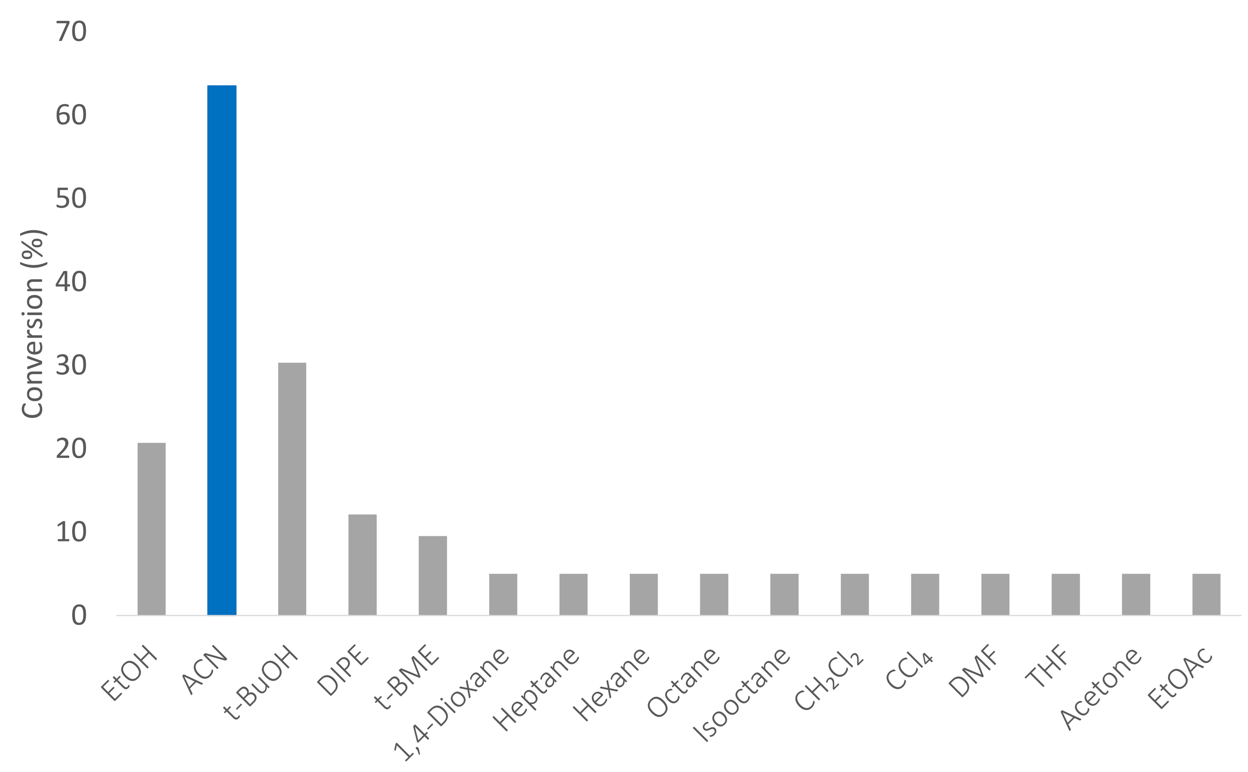
Biodiesel, a mixture of fatty acid alkyl esters, can be an ecofriendly alternative to the fossil fuels, since it can be obtained from vegetable oils or animal fat.[1] The attention towards the lipase-catalyzed biodiesel synthesis increased due to the mild reaction conditions, cleaner technology and small number of operation phases in comparison with the chemically catalyzed processes.[2] The use of various immobilized forms of lipases has the advantages of enhanced stability and easy removal from the reaction mixture.[3] Carbon nanotubes are widely used as support for the immobilization of biomacromolecules, exploiting their remarkable mechanical, thermal, electrical properties and general biocompatibility.[4]

Experimental

The commercially available $SWCNT_{COOH}$ activated with carbonyldiimidazol were coupled with three different alkyldiamines (propane-, hexane-, octane-), yielding the amino-functionalized $SWCNT_{COOH}$. Furthermore, using two different types of crosslinkers, glycerol diglycidyl ether (GDE) (Scheme 1a) or PEGylated bis (sulfosuccinimidyl) suberate ($BS(PEG)_5$) (Scheme 1b), amine-functionalized SWCNT-NHs were activated for the linkage of the amine groups from the enzyme surface to the support. The activity of SWCNT-CaL-B immobilized enzyme preparations was tested in the ethanolysis of sunflower oil. The reaction conditions were optimized, the optimization reactions included the effect of organic solvents, the water content of the reaction mixture, the effect of substrate–solvent ratio, the substrate–nucleophile ratio, and the effect of the temperature upon the enzyme activity.



***Scheme 1**. Immobilization of CaL-B on $SWCNT_{COOH}$, a) immobilization route with GDE: i) CDI in CH_2Cl_2; ii) $H_2N(CH_2)_3NH_2/H_2N(CH_2)_6NH_2/H_2N(CH_2)_8NH_2$ in water; iii) glycerol diglycidyl ether in CH_2Cl_2; iv) CaL-B in PBS buffer (20 mM Na_2HPO_4, 150 mM NaCl, pH 7); b) Immobilization via $BS(PEG)_5$: i) CDI in CH_2Cl_2; ii) $H_2N(CH_2)_3NH_2/H_2N(CH_2)_6NH_2/H_2N(CH_2)_8NH_2$ in water; v) $BS(PEG)_5$ in DMSO; vi) CaL-B in PBS buffer (20 mM Na_2HPO_4, 150 mM NaCl, pH 8).*



Figre 1. *The effect of the nature of the solvent on the ethanolysis of sunflower oil (4 h)*

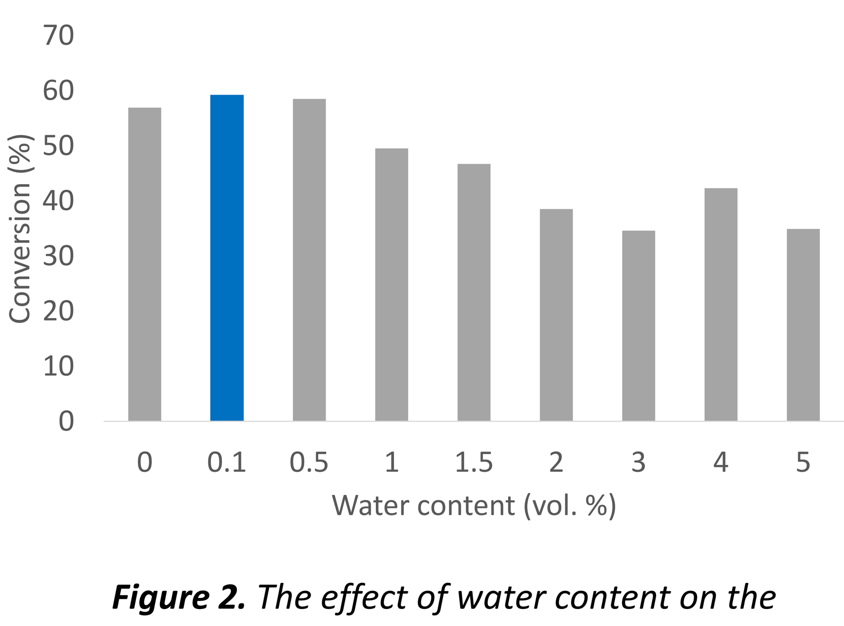


***Figure 2.** The effect of water content on the biodiesel production (3 h)*

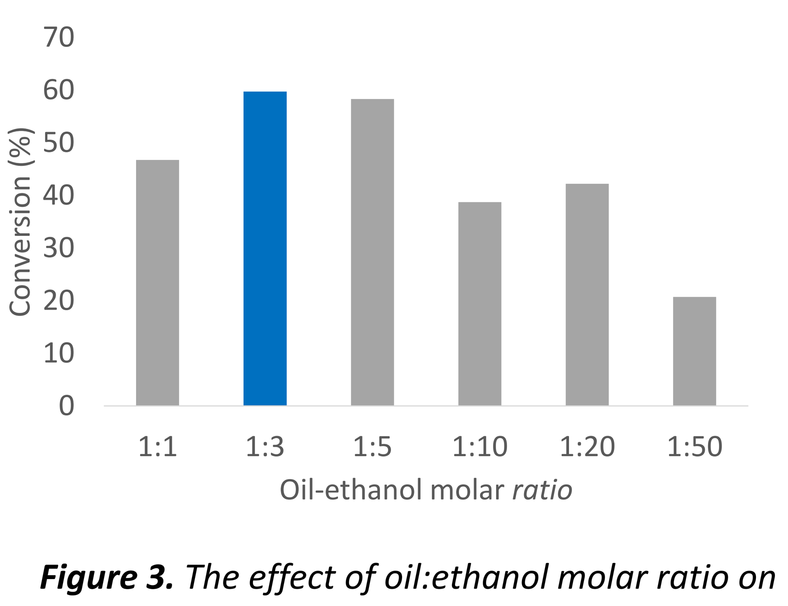


***Figure 3.** The effect of oil:ethanol molar ratio on the ethanolysis process (3 h)*

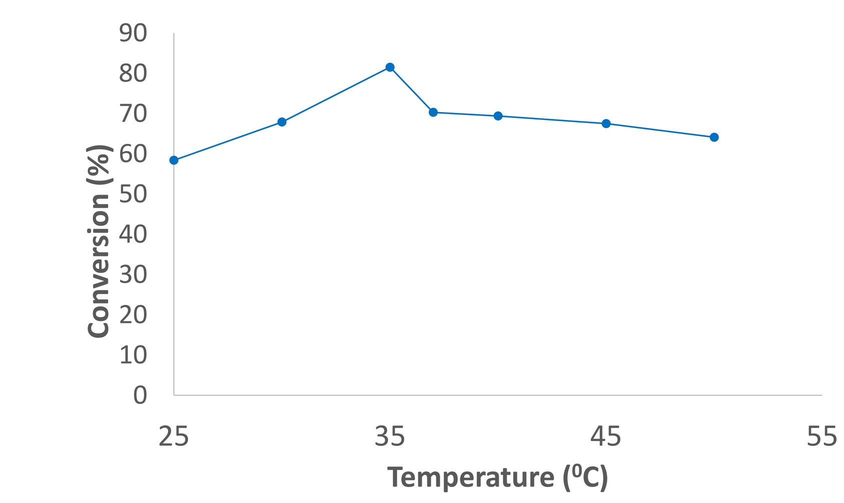


***Figure 4.** The temperature effect on the enzymatic transesterification reaction (3 h)*

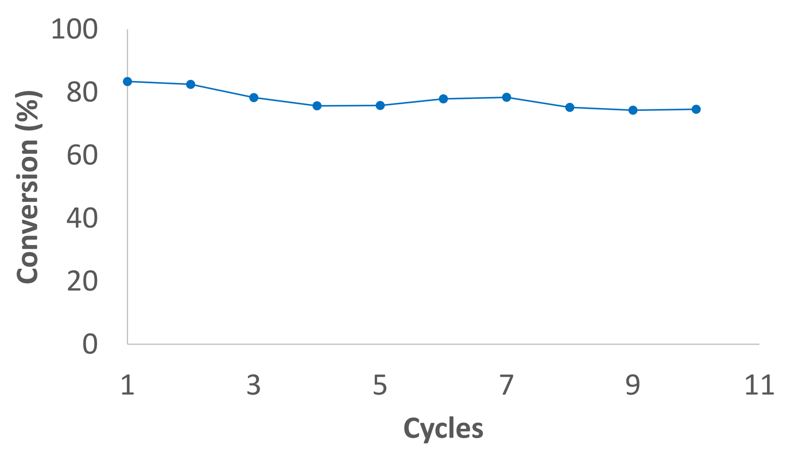


***Figure 5**. The reusability study of the immobilized enzyme preparation*

Discussion

The obtained enzyme preparations were characterized by reproducible and high immobilization yields (>99% of the CaL-B bound to $SWCNT_{COOH}$), with an enzyme loading of 0.33 mg protein per mg of functionalized carbon nanotubes. By the optimization procedures it was found that acetonitrile is the best solvent (Figure 1), low amount of water has a beneficial effect upon the transesterification reaction (Figure 2), the optimum substrate–solvent ratio is 1:5 (Figure 6), and the minimal stoichiometric oil–ethanol ratio (1:3) enables the highest reaction rates (Figure 3). In all cases the immobilization procedure with the shorter diglycidyl ether crosslinker provided better results, than those with the longer BS(PEG)5 crosslinker (Figure 7) . The effect of the temperature was also investigated, the enzyme activity showed a maximum at 35 C, further increase of temperature causing a decrease of the enzyme activity (Figure 4). The reusability experiments show that the biocatalysts retained more than 90% of its initial activity after 10 repeated batch reactions (Figure 5).

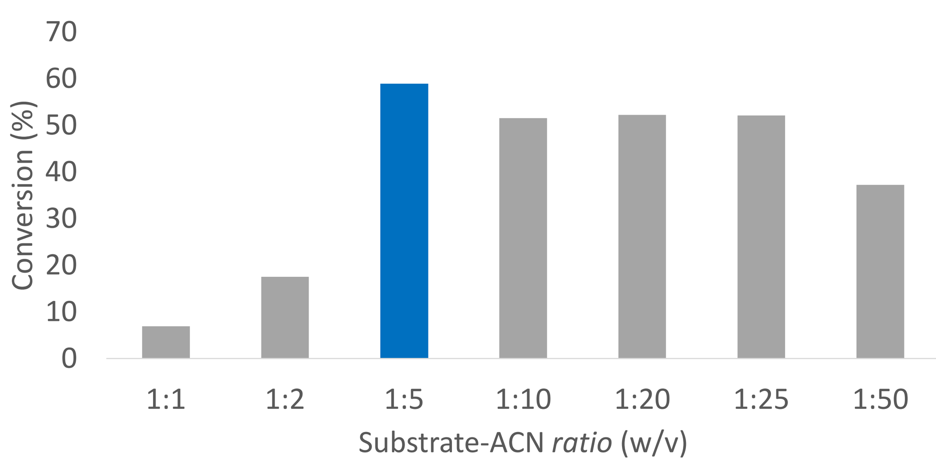


***Figure 6.** The effect of substrate concentration upon the biodiesel production (3 h)*

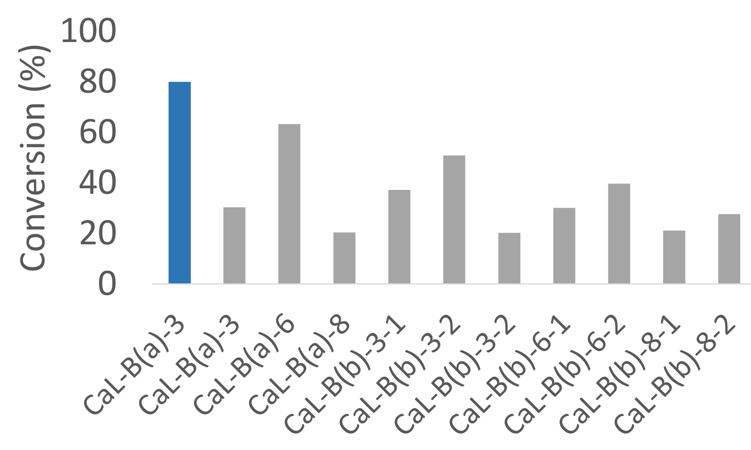


***Figure 7.** The effect of immobilization type on biodiesel production*

Conclusions

By covalent immobilization of lipase B from *Candida antarctica* in presence of TWEEN-80, a non-ionic surfactant, on carboxy-functionalized single-walled carbon nanotubes a useful biocatalyst for biodiesel production was developed. The most active enzyme preparation, CaL-B immobilized with glycerol diglycidyl ether on propane-1,3-diamine functionalized $SWCNT_{COOH}$ (SWCNT-CaL-B(a)-3), through several optimization rounds provided high conversion for the ethanolysis of sunflower oil in acetonitrile. Recycling studies demonstrates that the immobilized enzyme preparation has high operational stability, preserving more than 90% of its original activity after 10 reaction cycles.

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