https://doi.org/10.30970/apmicro.2026.08

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IDENTIFICATION OF NOVEL SUBSTRATE FOR THE YEAST ALCOHOL OXIDASE AND APPLICATION OF THIS ABILITY FOR ASSAY OF NITRATE ESTER EXPLOSIVE – PETN

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Abstract. For the first time, it was shown that pentaerythritol is a substrate for the yeast alcohol oxidase (AOx). This phenomenon was exploited to develop the novel optical methods for detection of an explosive material, pentaerythritol tetranitrate (PETN), using pentaerythritol tetranitrate reductase–alcohol oxidase (PETNR-AOx) cascade. The sensing concept relies on PETNR-catalyzed reductive denitration of PETN to generate alcohol-containing intermediates, which are subsequently oxidized by AOx with concomitant formation of hydrogen peroxide, monitored in peroxidase reaction.
Keywords: yeast *Komagataella phaffii* (*Pichia pastoris*), alcohol oxidase, pentaerythritol, pentaerythritol tetranitrate (PETN), enzymo-fluorometric assay.

Alcohol oxidase (AOx, EC 1.1.3.13) is a flavin-dependent enzyme that catalyzes the oxidation of primary alcohols to the corresponding aldehydes, accompanied by the reduction of molecular oxygen to hydrogen peroxide. Owing to its high catalytic activity and direct generation of hydrogen peroxide, AOx has been widely employed in biosensing, biocatalysis, and analytical applications. Although methanol is considered the natural substrate of AOx, the enzyme exhibits a relatively broad substrate specificity and is capable of oxidizing a variety of primary alcohols and polyhydroxylated compounds. The hydrogen peroxide generated during AOx-catalyzed oxidation has been extensively exploited in coupled enzymatic systems employing horseradish peroxidase and chromogenic or fluorogenic substrates for sensitive optical detection of target analytes, first of all, methanol and ethanol.

Despite the broad substrate scope reported for AOx, no information is currently available regarding its ability to oxidize pentaerythritol (2,2-bis(hydroxymethyl)propane-1,3-diol), a tetrahydroxylalcohol that represents the complete denitration product of pentaerythritol tetranitrate (PETN). Establishing whether pentaerythritol and PETN-derived hydroxyl-containing intermediates can serve as AOx substrates is important not only from a biocatalytic perspective but also for the development of novel enzymatic sensing strategies based on peroxide generation.

PETN is one of the most powerful nitrate ester explosives widely used in military formulations, detonating cords, blasting caps, and plastic explosive mixtures because of its high detonation velocity, chemical stability, and explosive performance.

For the first time, we demonstrate that pentaerythritol serves as a substrate for alcohol oxidase (AOx) and exploit this newly identified enzymatic activity to develop the novel optical methods for PETN determination based on a coupled pentaerythritol tetranitrate reductase–alcohol oxidase (PETNR-AOx) cascade. The sensing concept relies on PETNR-catalyzed reductive denitration of PETN to generate alcohol-containing intermediates, which are subsequently oxidized by AOx with concomitant formation of hydrogen peroxide. The generated H_2O_2 was quantified using either a horseradish peroxidase (HRP)/chromogen system or an HRP/fluorogen one, providing spectrophotometric and fluorometric detection formats, respectively.