



## Influence of pH on 2,4,6-trinitrotoluene degradation by *Yarrowia lipolytica*

Ayrat M. Ziganshin<sup>a,b</sup>, Rimma P. Naumova<sup>b</sup>, Andy J. Pannier<sup>a</sup>, Robin Gerlach<sup>a,c,\*</sup>

<sup>a</sup> Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA

<sup>b</sup> Department of Microbiology, Kazan State University, Kazan 420008, Russia

<sup>c</sup> Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT 59717, USA

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### ABSTRACT

The microbial reduction of the aromatic ring of 2,4,6-trinitrotoluene (TNT) can lead to its complete destruction. The acid-tolerant yeast *Yarrowia lipolytica* AN-L15 transformed TNT through hydride ion-mediated reduction of the aromatic ring (as the main pathway), resulting in the accumulation of nitrite and nitrate ions, as well as through nitro group reduction (as minor pathway), resulting in hydroxylamino- and aminoaromatics. TNT transformation depended on the yeasts' ability to acidify the culture medium through the production of organic acids. Aeration and a low medium buffer capacity favored yeast growth and resulted in rapid acidification of the medium, which influenced the rate and extent of TNT transformation. This is the first time that nitrate has been detected as a major product of microbial TNT degradation, and this work demonstrates the importance of pH on TNT biotransformation. The ability of *Y. lipolytica* AN-L15 to reduce the TNT aromatic ring to form TNT-hydride complexes, followed by their denitration, makes this strain a potential candidate for bioremediation of sites contaminated with explosives.

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### 1. Introduction

Remediation of soils, surface water, and groundwater contaminated with TNT and related compounds can be achieved using physical, chemical, and biological methods (Rodgers and Bunce, 2001; Boparai et al., 2008; Fuller et al., 2009). Each of these strategies provides advantages and disadvantages regarding the fate and detoxification of TNT and its metabolites. Incineration, for example, is probably the most effective treatment method for TNT-polluted soils, but this technology can become very expensive due to its energy demand, the need for excavation, transport of the contaminated soil off site, and basically complete destruction of the treated soil (Esteve-Núñez et al., 2001; Rodgers and Bunce, 2001). In recent studies weathering and rainfall-driven dissolution were also shown to be effective for remediation of soils polluted with explosives (Furey et al., 2008; Taylor et al., 2009a,b), however treatment durations are significantly longer than for excavation and incineration. Irreversible covalent binding of explosives and their transformation products to organic materials in soil or groundwater is believed to be a major pathway for their elimination from contaminated environments (Achtlich et al., 1999, 2000; Yang et al., 2008).

The potential advantages of bioremediation are public acceptance, low cost, and ease of operation (Esteve-Núñez et al., 2001; Rodgers and Bunce, 2001). However, most TNT bioremediation processes result, at least temporarily, in the accumulation of nitro group reduction intermediates (Funk et al., 1993; Stahl and Aust, 1995; Nepovim et al., 2005), which may cause aplastic anemia, cataracts, liver damage, and tumors in urinary systems (Hathaway, 1985; Yinon, 1990; Leung et al., 1995). Hence, the development of improved TNT biotransformation methods that reduce the accumulation of such nitro group reduction products and increase the rate of the aromatic ring destruction could help to decrease the risk to humans and the environment.

TNT nitro group reduction by microbial enzymes can result in the production of nitroso- and hydroxylamino-dinitrotoluenes, which can covalently bind to proteins and nucleic acids (Fu, 1990; Leung et al., 1995). In contrast, the direct reduction of the TNT aromatic ring (also often referred to as TNT-hydride or Meisenheimer complex formation) can result in the denitration of TNT and destruction of the aromatic system (Pak et al., 2000; Jain et al., 2004; Ziganshin et al., 2007; Wittich et al., 2008). The principal possibility of the elimination of a nitro group from the TNT-mono-hydride complex (3-H<sup>-</sup>-TNT) with simultaneous 2,4-dinitrotoluene (2,4-DNT) production has been described previously (Kim et al., 2002; Jain et al., 2004). 2,4-DNT production from TNT can be desirable, since 2,4-DNT mineralization has been demonstrated. This reaction can be initiated by a dioxygenase attack at the 4-nitro group position resulting in 4-methyl-5-nitrocatechol

\* Corresponding author. Address: Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA. Tel.: +1 406 994 1840; fax: +1 406 994 6098.  
E-mail address: [robin\\_g@biofilm.montana.edu](mailto:robin_g@biofilm.montana.edu) (R. Gerlach).