

**TITLE:** BACTERIAL NITRIC OXIDE METABOLISM ORCHESTRATES POLYETHYLENE BIODEGRADATION

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**ABSTRACT**

The ubiquitous importance of plastics to the global economics panorama is unquestionable. They are among the most important products of petroleum, a central feedstock to the worldwide economy. Polyethylene (PE) is the most produced and utilized plastic with a global production that exceeds 140 million tons/year. Since these materials are extremely recalcitrant and inert, they accumulate on the environments taking over 100 years to decompose under natural conditions. Triggered by this crescent issue, we previously isolated from the Brazilian Cerrado soil nine novel bacterial strains from the genera *Comamonas*, *Delftia* and *Stenotrophomonas* capable of degrading unpretreated PE of very high molecular weight (191.000). Remarkably, the biodegraded PE chemical fingerprint revealed the unprecedented involvement of nitrogen metabolism in the chemical modification of PE, as reflected by the extensive nitro group formation (C–O–N=O) induced by these bacteria. We hypothesize that nitric oxide (NO) is synthesized by the microbes and diffuses through their outer membrane to the interface between bacteria and PE, where it is oxidized by O<sub>2</sub> to the reactive nitrogen dioxide ( $\bullet$ NO<sub>2</sub>) ultimately attacking PE. Whole-genome analyses of the PE-degrading bacteria revealed the presence of not only different genes related to nitrification, denitrification and nitric oxide synthesis, as well as several oxidases, monooxygenases, dioxygenases, laccases and peroxidases, which are reported to be participants of PE biodegradation process. Thus, we investigated this novel biodegradation strategy by confirming the bacterial capability of producing nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) from ammonia as the sole nitrogen source, which indicates

these microbes are capable of heterotrophic nitrification – aerobic denitrification that leads to NO release. NO synthesis was also estimated under the same culture conditions. Furthermore, chemical analysis of PE films exposed to abiotic treatments with minimal synthetic media (MSM) supplemented with nitrite, iron/nitrite and nitrite solutions suggests that chemodenitrification is also involved in nitro formation. In addition, MSM/nitrite solution induced a substantial formation of alkoxy groups on PE, showing that its oxidation through chemodenitrification is not restricted to nitro formation. We are also investigating the effects of the overexpression of NO-related genes to the PE molecule. Our findings indicate that these microbes have original biochemical mechanisms to break and oxidize the long hydrophobic PE's chain in order to utilize the oxidized fragments as carbon sources. Moreover, since the genes and enzymes that mediate heterotrophic nitrification – aerobic denitrification are not described yet, our perspective is to understand this mechanism at a molecular level and investigate its impact on the overall PE biodegradation process.

**KEYWORDS:** biodegradation; polyethylene; nitric oxide; nitrogen metabolism.

**DEVELOPMENT AGENCIES:** CNPq