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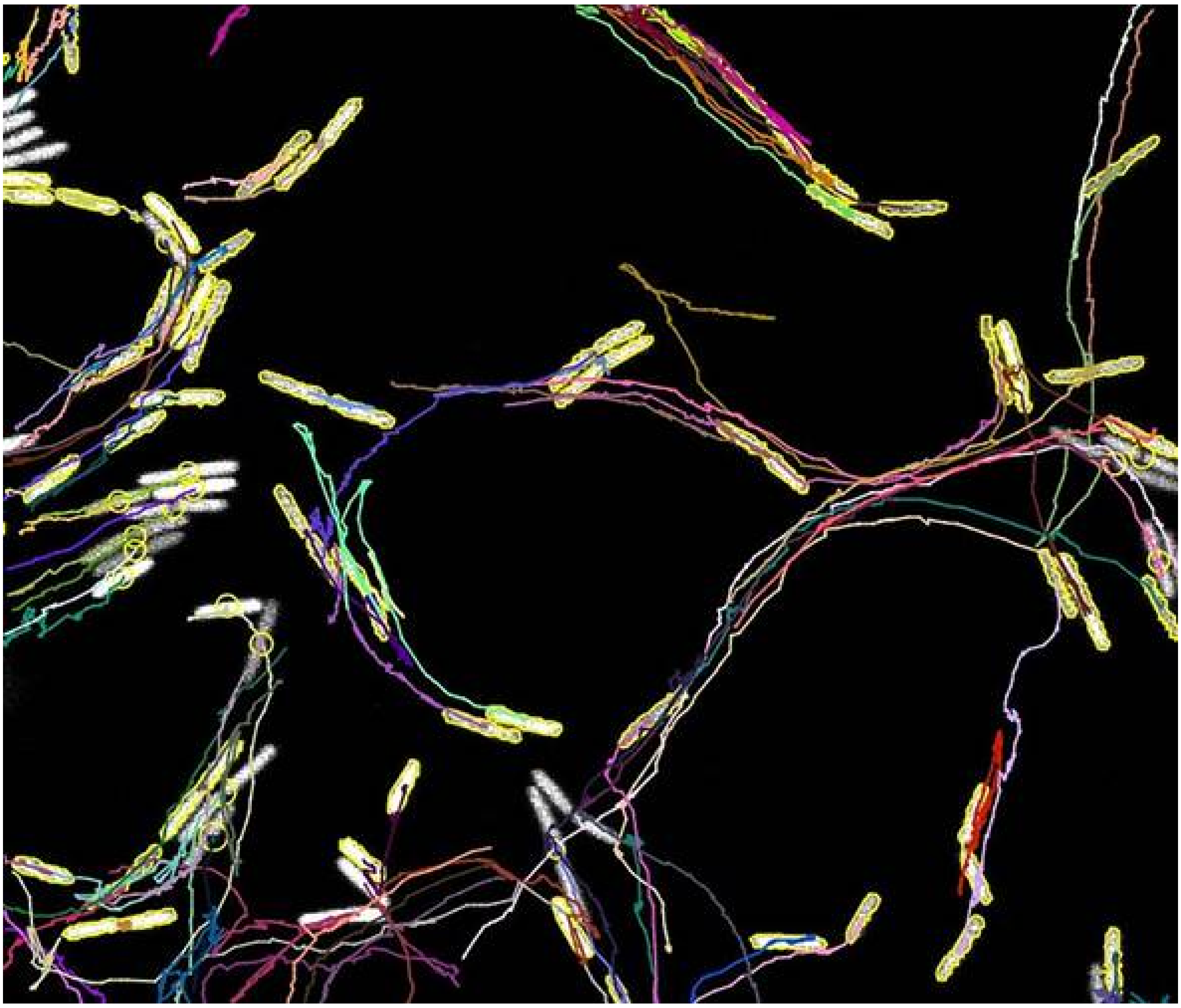

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

POTENTIAL DRIVEN REGULATION OF NITRATE INTERNALIZATION IN *Thiobacillus denitrificans.*

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The accumulation of nitrogen species, such as nitrate (NO32-) and nitrite (NO2-) ions in surface and groundwater, poses a direct threat to human health and ecosystems. The removal of nitrates from wastewater and contaminated water is typically achieved through biological denitrification. However, this process is often limited by the availability of suitable chemical species to serve as electron donors. To overcome this limitation, organic and inorganic electron donors are usually added to water and wastewater, increasing treatment costs and leading to secondary pollution due to unused donors. Alternatively, electroactive denitrifying microorganisms can use an electrode (cathode) as an inexhaustible source of electrons in a process called bioelectrochemical denitrification. A widely used model microorganism for bioelectrochemical denitrification is *Thiobacillus denitrificans*, a Gram negative betaproteobacterium, reported to bioelectrochemically reduce nitrate using an electrode as electron donor. Previous studies show that during the initial growth phase nitrate removal rate is much higher than that estimated from the electric current produced by the microorganisms. Herein, we explain this phenomenon, by revealing the occurrence of profuse nitrate internalization by the bacteria. For probing this, we grew a *Thiobacillus denitrificans* (DSM 12475) using a modified DSMZ 113 medium in three-electrode reactors operated in potentiostatic mode under anaerobic conditions, with graphite as working electrode. Nitrate was quantified by spectrophotometric measurements and internalized nitrate was obtained after cell lysis with tip sonicator. During the first few days after inoculation, we observed rates of nitrate removal which significantly exceeded the level supported by the respiratory reduction rate estimated from the produced electric current. For exploring inside the cells, cells were disrupted and 30% of added nitrate was recovered. This revealed that nitrate was not reduced but internalized by the cells. Cell internal/external nitrate distribution showed a statistically significant difference between polarized and non-polarized conditions, revealing that a negative potential applied to the electrode somehow drives nitrate internalization. During internalization significant differences between polarized and non-polarized conditions were observed in cell volume and number, despite cells didn’t use the electrode as energy source. This raises questions about the metabolic process that allows cell growth and how electrode potential may promote it. Internalization was observed both at the initial stages and after nitrate addition on a mature culture, indicating that this process persists even after the acclimation phase. Understanding how this process operates in denitrifying cells and how it could be stimulated and regulated could play a crucial role for improving nitrate removal at wastewater treatment plants.

Palabras clave: Keywords: Bioelectrochemical denitrification - Nitrate transport - Coulombic efficiency - Wastewater treatment