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Aerobic Denitrification of *Pseudomonas aeruginosa* Monitored by Online NAD(P)H Fluorescence

Fan Chen, Qing Xia and Lu-Kwang Ju*

Department of Chemical Engineering, The University of Akron, Akron, Ohio 44325

Abstract:

Continuous cultures of *Pseudomonas aeruginosa* (ATCC 9027) maintained at different dissolved oxygen concentrations (DO) were studied for the effects of DO on various culture properties, especially aerobic respiration and denitrification. The DO was varied from 0 mg/liter (completely anoxic conditions) to 1.3 mg/liter and measured with optical sensors that could accurately determine very low DO based on oxygen-quenched luminescence. The strain was found to perform aerobic denitrification; while the specific rate decreased with increasing DO, denitrification persisted at approximately 1/8 of the maximum rate (1.7 mmol/g of cells/h) even at relatively high DO (1 to 1.3 mg/liter). In the presence of nitrate, the culture's Monod half-rate saturation constant for O₂ was very small, <0.1 mg/liter. Aerobic denitrification appeared to function as an electron-accepting mechanism supplementary to or competitive with aerobic respiration. The shift of the culture's respiratory mechanism was also clearly detected with a fluorometer targeting intracellular NAD(P)H, i.e., the reduced forms of the NAD(P) coenzymes. Comparatively, the NAD(P)H fluorescence under the anoxic, denitrifying conditions (NFU_{DN}) was highest, that under fully aerobic conditions (NFU_{OX}) was lowest, and that under conditions in which both denitrification and aerobic respiration occurred (NFU) was intermediate.

Representing a quantitative measure of the culture's "fractional approach" to the fully denitrifying state, the normalized fraction $(NFU - NFU_{OX}) / (NFU_{DN} - NFU_{OX})$ was correlated with DO and the calculated fraction of electrons accepted by denitrification. The NFU fraction decreased with increasing DO, following an empirical exponential relationship. The fraction of denitrification-accepted electrons increased with the NFU fraction: the increase was gradual and approximately linear at DO of >0.1 mg/liter but much sharper at lower DO. Online NAD(P)H fluorescence was demonstrated as a feasible technique for effective monitoring and quantitative description of the microaerobic state of microorganisms.