Environmental Engineering and Management Journal

March 2012, Vol.11, No. 3, Supplement, S132 http://omicron.ch.tuiasi.ro/EEMJ/



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## MICROBIAL RESPONSES TO XENOBIOTIC SHOCK LOADS IN ACTIVATED SLUDGES

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## Abstract

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In wastewater treatment, ammonia undergoes nitritation, i.e. oxidation to nitrite by Ammonia Oxidizing Bacteria (AOB). Nitrite Oxidizing Bacteria (NOB) then further oxidize nitrite to nitrate, completing the nitrification process. AOB activity, essential for the ammonia removal and COD abatement, can be disrupted by organic pollutants present in the wastewater stream, such as 3-chloroaniline (3CA). Currently the assessment of wastewater treatment plant systems' efficiency is based on measuring basic functions (inhibited nitrification, decreased carbon removal, modification of the sludge physical properties). However the biological catalyst remains a black box. The definition of the structure and functionality of the microbial community in the sludge is a key-point to be addressed for the management of the system (Microbial Resource Management), since microbial community metabolism drives the process efficiency and stability.

This work aimed at indentifying a set of microbial based parameters useful to predict the system functionality. In addition, it was also evaluated if any adaptation, resistance or resilience to 3CA shock loads are possible. WWTP sludge containing aerobic Semi-Continuous-Activated-Sludge reactors were set up. After their initial stabilization, 1.5 mg 3CA (L sludge)<sup>-1</sup> was added either immediately or gradually (by daily adding small amounts of 3CA); non-amended reactors were incubated alongside as controls. Eight days later, each reactor was split to be either suddenly shock-loaded with 250 mg 3CA (L sludge)<sup>-1</sup> or left under normal operating conditions. Microbial community dynamics were monitored in all the reactors throughout incubation by PCR-DGGE directed to the 16S RNA and the 16S DNA of both total bacteria and AOBs.

In all the 3CA shock loaded reactors, nitritation was inhibited and accumulation of ammonia occurred, thus indicating that no adaptation was achieved. RNA-based analyses, which reflects the functionality of the targeted bacteria, showed a quick change in response to the shock load. Conversely, DNA-based analyses detected bacteria independently from their activity. The abundance of this resilient community could be used to infer the possibility of restoring the disrupted functionality. Finally, the combination of three DGGE deduced parameters ((i) the range-weighted richness (Rr) reflecting the carrying capacity of the system, (ii) the dynamics (Dy) reflecting the specific rate of species coming to significance, and (iii) Community Organization (Co) allowed to draw a user-friendly graph representing a breakthrough solution to predict possible functional failures and their management.