Molecular assessment of microbial community structure and dynamics along mixed olive oil and winery wastewaters biotreatment

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The major parcel of biodegradation that occurs during all kind of wastewater biotreatments is performed by microorganisms either from the native microbiota or due to added microbial inocula. Although biotreatments are carefully monitoring along time in order to assure their efficiency, little attention was paid during decades to the microbial communities present and responsible for the treatments. Besides the need to identify and quantify these microorganisms, it is essential to determine the correlation between their specific metabolic functions and the effectiveness of the biotreatment. The classical approach to characterise a microbial community is based on culturing, identify and count the members of the community and then assign functions to them, according their physiologic characteristics. Nowadays, a large range of molecular methodologies are commonly applied to analyse and compare environmental samples, providing fast and reliable solutions to overcome the bias of culture-dependent methods and allowing a more complete assessment of the microbial community composition and dynamics.

The present work can be considered as a case-study, reporting the biotreatment of mixed olive oil and winery wastewaters. These effluents were chosen, due to their importance in the Portuguese agro-industrial sector, and also to their strong negative impact in the environment.

The experiment was performed in a jet-loop reactor, under aerobic conditions and at room temperature, using native microbiota from the crude wastewater as inoculum. Biotreatment was monitored along time, covering the initial start-up batch phase and the continuous regimen, testing two hydraulic retention times (HRT) of 6.0 d and 4.5 d. Microbial communities were characterized in samples routinely collected during the biotreatment.

The bacterial community structure was compared using two fingerprinting methods: Temperature Gradient Gel Electrophoresis (TGGE) and Length Heterogeneity-PCR (LH-PCR) analysis of 16S rRNA gene fragments. For TGGE analysis the variable domain V3 of bacterial 16S rDNA was amplified using primers 341F-GC and 534R. For LH-PCR analysis, genomic DNA was amplified with a PCR using a fluorescently labeled forward primer 27F (5'-6FAM) and unlabeled reverse primer 534R. TGGE bands were reamplified and sequenced to identify the community members. Phylogenetic analysis showed the presence of bacteria affiliated with five main phylogenetic groups: alpha-Proteobacteria (40%), beta-Proteobacteria (5%), gamma-Proteobacteria (15%), Firmicutes (20%) and Bacteroidetes (20%). Within these groups, eight genera were identified: *Gluconacetobacter*, *Novosphingobium*, *Sphingobium*, *Sphingomonas*, *Ralstonia*, *Klebsiella*, *Pseudomonas*, *Lactobacillus*, and *Prevotella*. Bacterial populations have shown predominance of Gram- groups during all the biotreatment. LH-PCR analysis distinguished nine predominant fragments (468, 471, 474, 496, 499, 521, 524, 555 and 559 bp) in the sample that presented the highest performance (COD removal rates of 67 up to 75%), probably representing the members of the corresponding microbial consortia. Numerical analysis of both TGGE and LH-PCR fingerprinting profiles established five main clusters, with similarity coefficients higher than 79% (TGGE) or 62% (LH-PCR), showing that the main shifts observed in the microbial community structure were related with changes in tested HRT conditions.

A bioreactor operation depends upon the microbial consortia ability to grow in this man-modified environment whose physical and chemical conditions are subject to numerous and unpredictable fluctuations, that influences the microbiota metabolic functions. In this context, TGGE bands corresponding to the samples collected along the biotreatments were correlated with all the environmental data available (TRH, temperature, pH, COD, pO_2, NH_4 and NO_3), using canonical correspondence analysis (CCA). Obtained data shows that changes observed on temperature and O_2 level were the main responsible for the shifts in microbial consortia composition, during the biotreatment.

Furthermore, several raw effluent samples were tested for their metabolic activities using the "Ecoplate" system (Biolog, Inc). Results revealed the presence of microbial communities with marked degradation of phenolic compounds that can be of potential interest for industries applications.

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