Detection of D/N functional genes during a biotreatment of mixed olive oil and winery wastewaters

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Portuguese wineries and olive oil mills are often located in the vicinity of each other which makes possible to share wastewaters treatment plants (WWTP) to treat both effluents, before being discharged into municipal collectors and/or transport to one WWTP. This economy of resources is very important since Portuguese wine and olive oil is produced by small or medium companies.

Biotreatment monitoring is the unavoidable factor to characterise, control and improve the process, which will lead to new strategies for better treatments efficiency.

In order to optimize biotreatments for mixtures of the two referred effluents, a study was carried out in a jet-loop aerobic reactor, adding a winery effluent during the final phase of an olive oil wastewater biotreatment. On-line determination of the main physical/chemical parameters (pH, NO_3, temperature, and $\%pO_2$) was performed by sensors connected to new software developed to this aim associated with an acquisition data system plate (National Instruments). Off-line determination of COD, VSS and TSS was done following standard methods, and off-line NH_4^+ measurements were done with a probe (Crison) selective for NH_4 ion.

Bioreactor started-up under a batch phase, followed by a continuous regimen, testing two hydraulic retention times (HRT) of 6.0 d and 4.5 d, corresponding to feeding flow rates of 3.75 L/d and 5.0 L/d, respectively. Winery effluent was fed at a HRT of 6.0 d (3.75 L/d). COD olive oil wastewater removal rates reached more than 65%, at a loading charge of 16.1 g $COD.l^{-1}.d^{-1}$ with a HRT of 4.5 d.

Effluent biotreatments efficiency also depends upon biological nitrogen removal carried out by denitrifying/nitrifying (D/N) microorganisms. To detect the functional genes involved in the nitrogen cycle (N/D processes), samples were collected at selected phases of the treatment, and total DNA was extracted, and was amplified by PCR with specific primers for *nif*H, *nap*A, *nir*K, *cnor*B, *nos*Z, *amo*A and *nor*B genes.

The relevance of the molecular methods is nowadays recognized as the most important set of tools to identify unknown microbiota but this methodology does not provide cultures of a desired microorganism. To access and identify cultivable members of the consortia, some samples were also subjected to a cultivation, isolation and tipification experimental procedure using classical microbiology techniques. For sequencing analysis, 16S rDNA of isolates was amplified by PCR with universal primers for Eubacteria. To determine the phylogenetic affiliation, similarity search was performed using the BLAST program. The nucleotide sequences were aligned by the CLUSTAL program and the phylogenetic tree was constructed. Eleven sequences of isolates affiliated with phylum Firmicutes, one with Actinobacteria, and one with sub-class alpha-Proteobacteria.

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Keywords olive oil wastewater; winery effluent; microbial consortia; jet-loop reactor; D/N functional genes