

Abstract

Activated sludge, in its various forms, is one of the most widely used wastewater treatment processes. The main feature to successfully perform the wastewater purification is the complex community of microorganisms involved in the activated sludge process. Despite the numerous studies conducted to know the community of bacteria in the activated sludge, the structure and population dynamics are not completely understood.

Knowledge of the microbial population and their possible relationships with the operational parameters of the wastewater treatment plant (WWTP) can greatly contribute to the optimization of the processes involved and therefore have an important impact on operating costs.

In this study, detection of bacteria and archaea populations present in a continuous aeration activated sludge reactor has been assessed using a molecular technique, fluorescence in situ hybridization with 16S rDNA probes labeled with fluorophores (FISH).

Bacteria and archaea were studied at phylum and class level. Other bacteria such as nitrifying bacteria, denitrifying bacteria, phosphorus accumulating organisms, glycogen accumulating organisms, methanotrophic bacteria, sulfate-reducing and methanogenic organisms were studied at functional groups level. An average of 80% of the total population present in the samples at the level of phylum and classes was detected. *β-Proteobacteria* class of the phylum *Proteobacteria* was the dominant during the sampling, followed by *α-Proteobacteria* and *γ-Proteobacteria* classes. At the level of functional groups, it has been detected less than 50% of this population, being the dominant group the sulfate-reducing bacteria, followed by denitrifying bacteria. Presence of methanogenic archaea and ammonia oxidizing archaea was also detected.

When working with FISH technique the complexity of simultaneous detection of Gram negative and Gram positive cells is an important issue. The fixation method depends on the structural differences at membrane level and the type of sample being tested.

Moreover ellipsoidal shapes, possibly ascospores of the phylum *Ascomycota*, with glucuronidase and phosphatase enzyme activity, have been also detected by FISH. *Ascomycetes* are saprophytic decomposers and can contribute to the treatment process, but its capacity to degrade organic matter in the activated sludge process has not been yet established.

The diversity of the community of organisms in the activated sludge process depends on the characteristics of the water to be treated, the operational parameters (such as dissolved oxygen, sludge age and others) and physicochemical parameters. In this study, the dynamic community of bacteria and archaea was studied during one year. Samples were taken from the biological reactor of the Carraixet WWTP with a

bi-weekly sampling frequency. Different multivariate analysis were carried out such as CCorA (canonical correlation analysis); CA (correspondence analysis) and CCA (canonical correspondence analysis), to study the relations of each population present in the samples with the physico-chemical and operational parameters.

Bacteria and archaea abundance by taxonomic group (phylum, class) was higher related to operational variables, while functional groups abundance were higher related to the physico-chemical variables.

Relationships between protozoa population, bacteria population and archaea were studied by multivariate analysis. Results showed a relationship between some protozoa communities and some bacteria or archaea, indicating that they can share habitat characteristics.

In general in activated sludge systems with continuous aeration, operational and physical-chemical variables of mixed liquor play an important role in the population dynamics of bacteria and archaea.