



“Gheorghe Asachi” Technical University of Iasi, Romania



QUANTIFICATION OF PROTEOBACTERIA WITH FLUORESCENCE *IN SITU* HYBRIDIZATION AND NEXT-GENERATION SEQUENCING

Magdalena Domańska*, Joanna Kamińska

Wroclaw University of Environmental and Life Sciences, Institute of Environmental Engineering,
Department of Mathematics, pl. Grunwaldzki 24, 50-375 Wroclaw, Poland

Abstract

Determining the microbial community structure as well as the quantitative and qualitative changes in the composition of activated sludge provide valuable information on the operation of biological reactors. Since microorganisms that are of key importance for wastewater treatment processes often do not grow on the medium, the use of molecular methods becomes necessary. Despite the dynamic development of these methods, they are still not commonly used in the operation of wastewater treatment plants. Therefore, the research concerned the identification and quantification of the main classes of *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria*) in five activated sludge samples using fluorescence *in situ* hybridization (FISH) and next-generation sequencing (NGS). To compare the methods, statistical analysis was used. The results of the conducted research confirm both the usefulness of the applied methods and the difficulties in interpreting the results. The number of photographs taken affected the result of the analysis. The Wilcoxon test for 30 photographs taken during the study showed that the results of both methods were in agreement in 12 out of 20 cases under consideration. In 13 out of 20 cases, it was enough to take 15 photographs to achieve agreement with a 95% confidence interval.

Key words: fluorescence *in situ* hybridization, image analysis, next-generation sequencing, Proteobacteria, Wilcoxon signed rank test

Received: August, 2021; *Revised final:* March, 2022; *Accepted:* April, 2022; *Published in final edited form:* June, 2022

* Author to whom all correspondence should be addressed: e-mail: magdalena.domanska@upwr.edu.pl; Phone: +48 713205553; Fax: +48 71320 5579