



“Gheorghe Asachi” Technical University of Iasi, Romania



---

## WHAT IS HIDDEN BEHIND ACTIVATED SLUDGE SUPERNATANT? FLUORESCENT STAINING AND LASER GRANULOMETRY INVESTIGATION SUPPORTED BY MACHINE LEARNING

Magdalena Domańska\*, Magdalena Kuśnierz, Justyna Stańczyk

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

---

### Abstract

Studies on the biological composition of activated sludge flocs in the operating conditions of wastewater treatment plants are generally limited to the estimation of respiratory activity or to the analysis of images made with optical microscopy. The results of these studies indirectly provide information on the microbiological composition. To date, molecular methods, although very promising, have not found a wider application in operational monitoring of wastewater treatment plants. In this paper, the supernatant was under discussion as a potential source of sampling for analyzing microbial quality changes. The results of 270 activated sludge and treated wastewater samples showed that the smallest flocs leaching to the outflow constitute a group of microorganisms that is most numerous. The studies carried out using the fluorescence *in situ* hybridization method have shown that the microorganisms responsible for the nitrification processes occur both in activated sludge and supernatant. Image analysis of microorganisms from activated sludge and supernatant stained with Live/DEAD reagent indicate that microflocs and bacteria in the outer flocs, which are relatively loosely attached to the flocculent matrix are more exposed to external factors. The results suggest that it is advisable to find information about the condition of the whole community especially for group of particles in the supernatant. In addition, the authors recommend using machine learning methods to evaluate predicting anomalies in biological composition of activated sludge flocs.

**Keywords:** activated sludge, fluorescence in situ hybridization method, machine learning, particle size distribution, wastewater treatment

Received: July, 2020; Revised final: November, 2020; Accepted: December, 2020; Published in final edited form: July, 2021

---

---

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