# EFFECT OF INCREASED INFLOW AND DILUTION ON THE ACTIVATED SLUDGE PROPERTIES AND VIABILITY OF MICROBIAL COMMUNITY

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*The purpose of the paper was to study the dilution-induced effect caused by high hydraulic load on the activated sludge structure and properties. A high hydraulic load rate was simulated in the laboratory. The analysis included area and perimeter automated measurements of activated sludge flocs under different dilution rates. The area of activated sludge flocs has decreased as a result of the initial dilution due to the mechanical disaggregation of large flocs. At the end of the experiment, the level of flocs disintegration was higher, but the cause was the massive cellular lysis as a result of nutrient deprivation.*

**Key words:** wastewater, activated sludge, flocs

## 1. Introduction

The hydraulic parameters are critical in operating Wastewater Treatment Plants (WWTPs). Variations in hydraulic loading rate (HLR) [1], hydraulic retention time (HRT) [2][3], sludge retention time (SRT) [4] [5] could affect the activated sludge quality, its structure and activity. WWTPs efficiency might be affected by sudden variation of influent wastewater loadings, occurring, for example, during rainfalls. Increased inflow could dramatically change a series of parameters such as the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) and could promote lowering of total suspended solids (TSS) contents due to the loss and dilution of activated sludge [6]. The purpose of this paper is to analyse by using laboratory simulation conditions the effect of an increased water inflow, especially the effect of dilution, on the properties of activated sludge and viability of the microbial community. Biodegradation of the organic matter existing in wastewaters is largely influenced by the characteristics of activated sludge flocs (size, shape and microbial composition). Examining the morphology of the flocs is a relatively simple way to evaluate the ability of activated sludge to support an optimal pollutants removal. Useful criteria to assess the operating efficiency of WWTPs are size, structure and the number of flocs.

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Recently, there is an increased interest in using the automated image analysis [7] [8] to estimate in real time the properties of activated sludge based on the flocs' morphology [9] [10] [11]. Based on these ideas, the paper presents preliminary laboratory data on the quantitative characteristics of flocs (area, perimeter) due to increased water inflow and dilution. Supplementary, the changes of viability of heterotrophic bacteria in consequence of dilution and increased water inflow have been estimated.

## 2. Materials and methods

In order to evaluate the effect of increased inflow and dilution, a simplified experimental laboratory model has been developed. Freshly collected activated sludge samples were diluted in different proportions - ½ (variant B), ¼ (variant C), and 1/8 (variant D) with sterile saline solution (0.85% NaCl). Variant A was undiluted and used as a control sample. All variants were kept in laboratory conditions ($t^oC=18\text{-}22^oC$) with continuous aeration and mechanical stirring. Subsamples were taken initially ($T_0$) and after two weeks (2 W). For microscopic examination, 10 uL of subsamples were pipetted onto clean sterile microscope slides and covered carefully with cover slip to avoid air bubbles trapping. Image capture was made on an Axio Imager microscope (Carl Zeiss) equipped with AxioVision LE camera (AxioVision LE 4.6 software) and examination was made with a calibrated 10x objective. For each sample, particles and flocs existing in each microscopic field were automatically enumerated as well as the automatic calculation of their perimeters and area. Measurements were further processed with Microsoft Excel, v.8. In parallel with microscopic examination, samples were inoculated on solid nutrient media to evaluate the total count of heterotrophic microorganisms. 1 mL was taken from each variant at the initial moment and after 2 weeks, diluted and inoculated onto Nutrient Agar (peptone, 0.5 g; beef extract, 0.3 g; NaCl, 0.5 g; distilled water, 100 mL, pH=7.4-7.6). Agar plates were incubated 24-48 h at $28^oC$. Subsequently, the developed colonies were counted, multiplied with the dilution factor and expressed as Colony Forming Units (CFU)/mL.

## 3. Results and discussions

*Effect of dilution on the activated sludge structure*

In order to identify the effect of dilution on the activated sludge quality, microscope analyses and measurements of flocs size have been carried out under different magnitude of dilution at the initial time ($T_0$) and after two weeks (2 W) of incubation in laboratory conditions. The perimeter and area of particles and flocs have been determined in all experimental variants. In variant A a reduction of number of particles and flocs and a decrease of perimeter and area after two weeks were observed. Since there was no other disturbing factor, this fact was attributable to the lack of nutrients and physiological stress caused by starvation. Under intense aeration, most part of the nutrients was consumed during the first week. The shift from normal nutrient environment to a low nutrient level had as a main effect the lysis of filamentous bacteria and disintegration of flocs with formation of smaller flocs. At the same time, there was also a reduction of total number of particles and flocs (Fig. 1A).

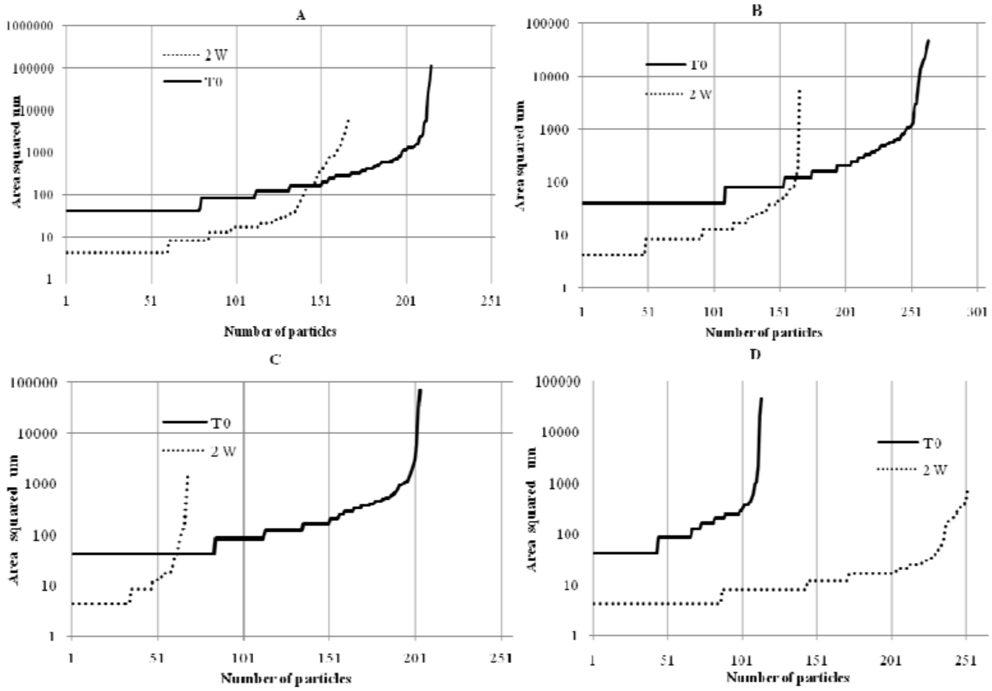


Fig. 1. Area of particles and flocs at the initial stage and after two weeks (2 W) of incubation of activated sludge without nutrients addition and diluted ½ (B), ¼ (C) and 1/8 (D).

Variant A is the undiluted control sample. In the sample B case there (Fig. 1 B) was an initial reduction of area as a result of the dilution in comparison with variant A. After two weeks a significant decrease in area of activated sludge flocs has been recorded, but the cause was different from that occurring at initial starting point, relying on the lysis of nutrient-deprived microbial cells.

A similar situation has been observed in the case of the other two samples (Fig. 1C and D).

However, in case of the C sample, a higher number of flocs with relatively large area were conserved. Instead, in the case of sample D, the flocs undergone an intense process of lysis followed by the release of a high number of particles with smaller area (Fig. 1D).

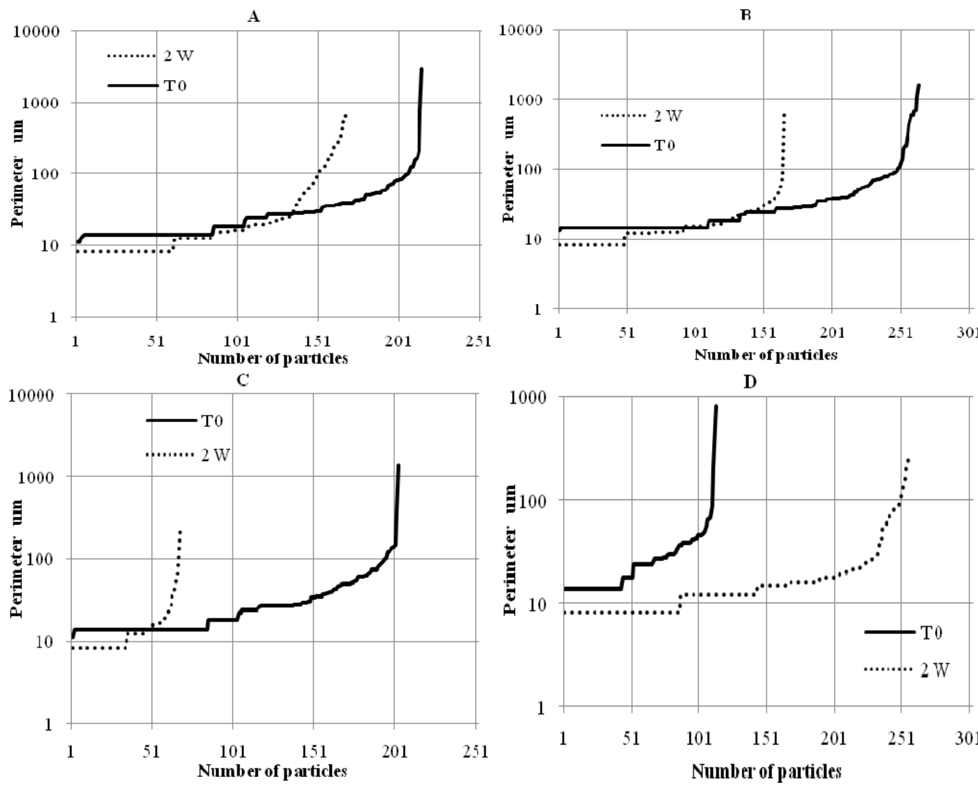


Fig. 2. Diameter of particles and flocs at the initial stage and after two weeks (2 W) of incubation of activated sludge without addition of nutrients and diluted ½ (B), ¼ (C) and 1/8 (D). A is undiluted control sample

A very similar pattern of flocs' size evolution was observed in case of the perimeter evaluation (Fig. 2A, B, C, D). It is worth mentioning the fact that at higher dilution rates the number of small flocs increased due to the lysis of bacterial cells and fragmentation of larger flocs.

Overall, microscopic measurements indicated a biomass decrease due to simulated high hydraulic loads and dilution. Observed facts are in accordance with Grijspeerdt and Verstraete [12] who found that sludge concentration can be estimated by microscope image analysis followed by calculation of projected area of the flocs. A very similar correlation has been reported by Amaral and Ferreira [13], which showed a close connection between total suspended solids (TSS) and total aggregate area. Sludge concentration prediction based on the total area can be described as a non-linear correlation due to the variable density of particles [14].

These changes in the quality of the activated sludge due to the water influx and dilution might obviously affect the activated sludge activity, as well as the dynamics of the biodegradation process.

Reduction in chemical oxygen demand (COD) and biochemical oxygen demand (BOD) due to high hydraulic load have been also reported by other authors [15] [16], supporting the idea that dilution interferes with optimal processing of wastes in WTTPs. In our case, the reduction of microbial metabolic intensity correlated with the decrease in dehydrogenase activity (DHA), the results being the subject of a future paper.

*Dynamics of total count of heterotrophic bacteria*

Bacteria form an essential component of the microbial consortium representing the activated sludge [17] [18]. Their numeric fluctuations can reflect the efficiency of activated sludge to process the organics found in wastewaters. As a general trend, reduction of total count was observed as direct effect of the dilution.

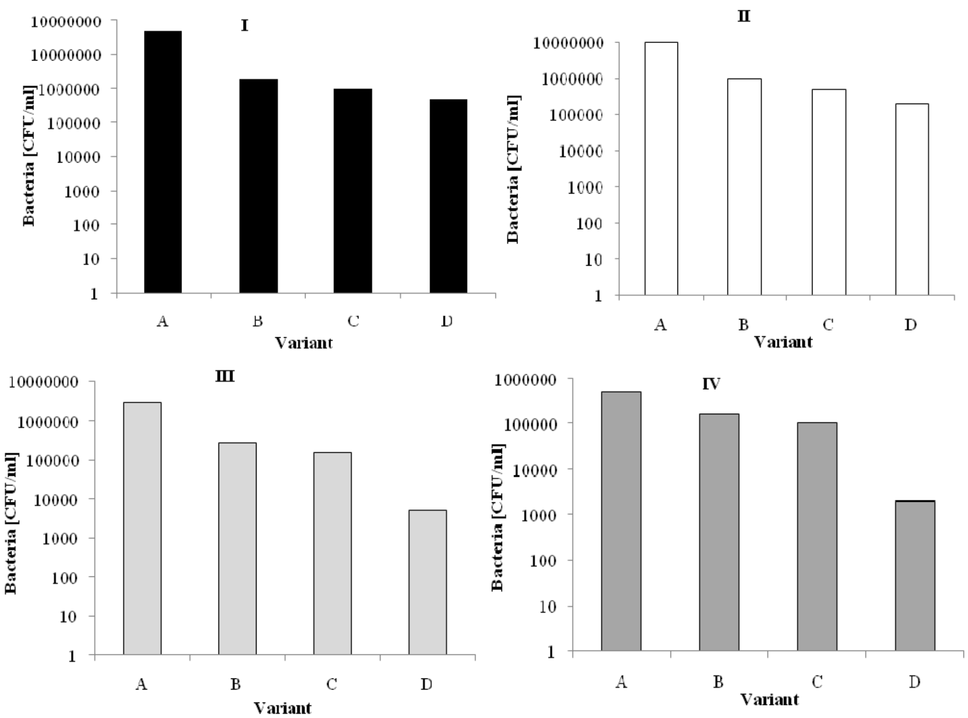


Fig. 3. Evolution over time of total count: I – Initial stage (phase) ($T_0$); II – 3 days; III- 8 days; IV – 14 days

Thus, in the control sample (variant A) at the beginning of the experiment, the density of bacteria was equal to $50x10^6$ CFU/ml. Following the dilution, the bacterial density has dropped to $2x10^6$ (variant B), $1x10^6$ (variant C), and to $5x10^5$ (variant D). Bacterial abundance has progressively decreased in all variants at the end of the experiment as the time lapse increased. Thus, bacterial number decreased in the control sample (variant A) from $5x10^7$ to $5x10^5$ after two weeks (Fig. 3 I and 3 IV). The highest rate of density reduction was recorded in variant D, the one having the highest dilution. Number of bacteria decreased from an initial value of $5x10^5$ CFU/mL to only $2x10^3$ after two weeks (Fig. 3 IV). In the case of the other samples, intermediate levels of density reduction were recorded due to the increased starvation period (Fig. 3 II and 3 III). It is worth to mention that the microbial number could be higher than the one detected. The recorded density represented only the viable fraction from total microbial population able to produce colonies on nutrient agar. It is well known that the cells exposed to long nutritional stress might lose the ability to generate colonies when they are transferred on rich-nutrient laboratory media. It has been observed that samples taken from control and diluted variants at the beginning of the experiment comprised bacteria able to produce colonies after 18-24 h of incubation. As time of incubation and starvation period increased, most of colonies developed slower, and only after 48-72 h. This fact is in accordance with most observations showing that the transfer of nutrient-deprived cells needs a period of adaptation, depending on the species, before they can generate colonies on rich nutrient media. As a result, changes in the normal hydrological regime of the majority of WWTPs, such as the increased water inflow during rainfall and storms, might cause not only the reducing of activated sludge biomass, but also the lowering of microbial activity with negative impact on the removal of wastes.

## 4. Conclusions

Dilution, as the main consequence of high hydraulic load, has affected the structure of activated sludge mainly on two aspects. First, a direct effect of dilution was the decrease of sludge biomass and disaggregation of larger flocs. Indirectly, dilution has caused a downshift in nutrient availability and induced changes in the microbial cell physiology as a result of nutrient deprivation. Therefore, the number of viable bacteria decreased at the end of experiment as well as their ability to grow on laboratory media. The area of activated sludge flocs has decreased as a result of the initial dilution, attributable mainly to the mechanical disaggregation of large flocs. At the end of experiment (after two weeks), the level of flocs disintegration was higher, but the cause was different and related to the massive cellular lysis, as a result of nutrient deprivation. Perimeter followed a similar pattern at the end of the experimental research period. The most important changes of flocs' structure were observed in the samples with the highest degree of dilution. Along with the decrease in size of flocs, a reduction in viability of heterotrophic microorganisms was also observed.

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