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# BIODEGRADATION OF SODIUM DODECYL SULPHATE (SDS) BY ACTIVATED SLUDGE IN THE FLOW SYSTEM

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# BIODEGRADACJA DODECYLOSIARCZANU SODU (SDS) W UKŁADZIE CIĄGŁYM METODĄ OSADU CZYNNEGO

Syntetyczne substancje powierzchniowo czynne mogą oddziaływać na biologiczne oczyszczanie ścieków. Przyczyniają się one między innymi do zmian w strukturze kłaczków osadu czynnego. Celem niniejszej pracy było określenie wpływu dodecylosiarczanu sodu (SDS), zaliczanego do anionowych substancji powierzchniowo czynnych, na morfologię kłaczków osadu czynnego. Badania zostały przeprowadzone w skali laboratoryjnej w układzie ciągłym dla czterech różnych szybkości rozcieńczania. Stężenie SDS w dopływających ściekach było stałe i wynosiło 250 mg dm<sup>-3</sup>. Okazało się, że wraz ze zwiększaniem szybkości rozcieńczania od 0,029 do 0,192 h<sup>-1</sup> średnie pole powierzchni rzutu kłaczka zmniejszyło się z 50 000 do 15 000 μm<sup>2</sup>. Uzyskane wyniki wskazują, że istnieje korelacja między parametrami morfologicznymi kłaczków a innymi indykatorami biomasy. Stwierdzono liniową zależność pomiędzy średnim polem powierzchni rzutu kłaczków a zawiesiną organiczną.

#### Summary

Surfactants can interfere with the biological wastewater treatment processes. They contribute to the changes in activated sludge flocs structure. In order to quantify the influence of surfactants on sludge flocs morphology the series of experiments in the flow continuous system were conducted. Sodium dodecyl sulphate, which belongs to the most ubiquitous anionic surfactant in everyday use, was selected to be the object of investigations. The results of its biodegradation in continuous flow system at influent concentration of 250 mg dm<sup>-3</sup> are presented. It turned out that SDS diminished the mean projected area of flocs from 50 000 to 15 000  $\mu$ m<sup>2</sup> with the increase of dilution rate from 0.029 to 0.192 h<sup>-1</sup>. At the same time the obtained data confirmed that there was a correlation between the morphological parameters of flocs and other biomass indicators. The linear relation between mean projected area of flocs and volatile suspended solids was found.

#### INTRODUCTION

Synthetic surfactants are still one of the most often used chemicals [11]. Among all surfactants, anionics are produced in the largest quantity. This is due to the fact that, firstly, their production is generally more profitable than the other compounds, and secondly, anionics are applied in almost all kinds of cosmetics. They are usually considered to be the

"workhorse" in the detergency world [2].

The anionics concentration in domestic wastewater is about 20 mg dm<sup>-3</sup>. However, in industrial wastewater it often exceeds 300 mg dm<sup>-3</sup> [15]. It has been already proved that surfactants affect biological wastewater treatment processes. They are toxic to microorganisms; they may induce foam and reduce settling ability of sludge [13]. Especially settling properties of the sludge play an important role in wastewater treatment plant (WWTP) because they determine the quality of the effluent from the final clarifier. Settling ability of sludge was usually expressed by sludge volume index (SVI). Grijspeerdt and Verstraete showed that the settling properties of the sludge flocs [5]. These authors used image analysis techniques in order to quantify the size and the shape of activated sludge flocs. Moreover, Grijspeerdt and Verstraete indicated that there was a linear relation between the field area and volatile suspended solids in the certain range of concentration for the activated sludge [5]. Also da Motta *et al.* applied an automated image analysis procedure for the simultaneous characterization of flocs [9].

This study is the development of the previous batch shake flasks experiments. They showed that SDS in a wide range of concentrations (2.5–2500 mg dm<sup>-3</sup>) caused the decrease of sludge flocs dimensions [7].

The purpose of this study was to estimate the influence of sodium dodecyl sulphate on activated sludge flocs morphology and the settling ability of the sludge in the continuous flow system. Flocs morphology was investigated with the use of image analysis techniques and as a result, selected morphological parameters were obtained. Additionally, the application of some morphological parameters as biomass concentration indicators was tested.

### MATERIALS AND METHODS

#### Substrate and inoculums characterization

The culture medium was synthetic wastewater prepared according to the Polish Norm PN-72/C-04550 [12]. The synthetic wastewater contained sodium dodecyl sulphate (SDS) at a constant concentration equals 250 mg dm<sup>-3</sup>. Sodium dodecyl sulphate (SDS)  $C_{12}H_{25}OSO_3Na$  was purchased from POCh (Poland). SDS belongs to alkyl sulphates (AS) and is one of the most common anionic surfactant [16]. Its molecular weight is 288 g mol<sup>-1</sup>. A critical micellar concentration (cmc) is equal to 2310 mg dm<sup>-3</sup>.

Activated sludge was taken from aerated chamber at Wastewater Treatment Plant (WWTP) in Zgierz (Poland) and within one hour the inoculation was performed.

## Experimental setup and process conditions

All experiments were conducted parallelly in the continuous flow system. The main device of the experimental setup was the model of aerated chambers coupled with clarifiers, which simulated the large-scale biological wastewater treatment plant. Each chamber had the working volume of 7.8 dm<sup>3</sup>. Dissolved oxygen concentration, temperature and pH were controlled during biodegradation processes. Aeration flow rate was constant and identical in each experimental run (0.321 vvm). The experiments were conducted at ambient temperature ( $22\pm1^{\circ}$ C). Substrate was added to aerated chambers at four different dilution rates: 0.192, 0.128, 0.069, and 0.029 h<sup>-1</sup>. Steady state in the chamber was assumed when at least five residence times passed.

# ANALYTICAL TECHNIQUES

#### Physicochemical and biochemical analyses

Sodium dodecyl sulphate (SDS) concentration was determined according to methylene blue method [1]. SDS form ion pairs with methylene blue that are extracted by chloroform and determined spectrophotometrically at 652 nm.

Chemical Oxygen Demand (COD) was measured by standard dichromatic method [1]. Turbidity was measured using HACH spectrophotometer DR/2000 at 450 nm according to method no. 750 [6]. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured gravimetrically. Also sludge volume index (SVI) was determined. Dehydrogenase activity was determined according to Miksch with the use of 2,3,5-triphenyltetrazolium chloride (TTC) [8].

#### Image processing and analysis

The activated sludge flocs were observed under light microscope Olympus BX40 with blue filter under the magnification 100x. The RGB (Red, Green, Blue model of color) images were snapped, processed and analyzed with help of Micro Image 4.0 software (Media Cybernetics for Olympus). At least 40 images from each sample were snapped and stored. A single image was later processed as follows. Blue plane was extracted from each RGB image and segmented in greyscale in order to detect the flocs. This way the binary image was obtained. It was further subjected to detailed analysis. The following morphological parameters of the flocs were measured: mean projected area, perimeter, mean diameter and circularity index. Mean projected area is the basic image analysis parameter, easily found by pixel count and its multiplication by scaling factor. The perimeter is the length of the boundary of the object. A mean diameter was measured as the lengths of lines between two points on the boundary of the object going through its centroid. Finally, the circularity index is the shape factor that indicates to what extent the measured object is similar to the true circle. If it is equal to one, the object is the true circle. The higher it is, the less circular the object is [10, 14]. Additionally, the percentage field area was calculated. This parameter is defined as a ratio of total area of flocs to total area of the field of view [5].

#### **RESULTS AND DISCUSSION**

Changes of sodium dodecyl sulphate concentration and total substrate concentration expressed as COD with the dilution rate are presented in Figs 1 and 2. At dilution rates from 0.029 to 0.128 h<sup>-1</sup> SDS was practically removed from the system (over 99%). At the highest of the tested dilution rates about 64% of SDS was biodegraded. These results confirmed previous literature data that alkyl sulphates were readily bioconverted chemicals [3]. Nevertheless, the level of total organic substances removal (degree of COD removal) was lower than that obtained for SDS and achieved 93–96% for the range of dilution rates from 0.029 to 0.128 h<sup>-1</sup>. Furthermore, at the highest tested dilution rate only half of the whole COD pool was biodegraded. This significant difference may suggest that certain intermediates remained in the effluent.

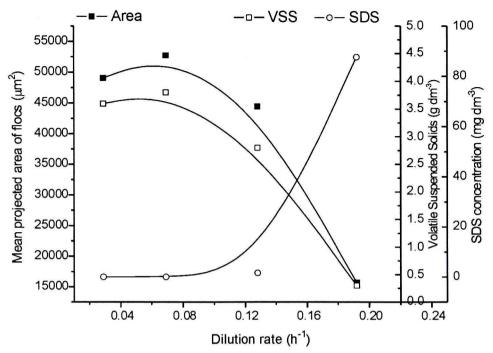


Fig. 1. Changes of SDS, VSS and mean projected area with the dilution rate in the continuous flow system

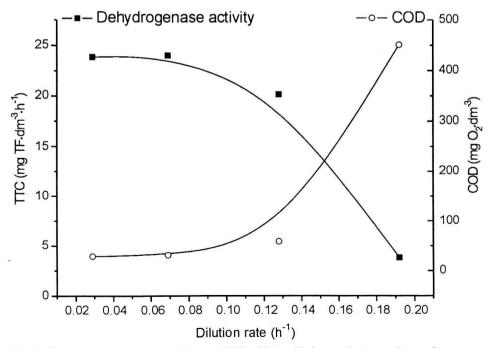


Fig. 2. Changes of dehydrogenase activity and COD with the dilution rate in the continuous flow system

#### BIODEGRADATION OF SODIUM DODECYL SULPHATE ...

In the conducted experiments biomass was expressed in three different ways: firstly as biomass concentration by measuring volatile suspended solids, secondly, as biomass activity by dehydrogenase activity, and thirdly as mean projected area of flocs. What is here the most important, the shape of curves for these three different biomass indicators are similar, which may suggest some correlations between them. This aspect will be thoroughly discussed further. Generally, all three indicators confirmed that biomass concentration as well as its activity is higher at longer residence times (Figs 1 and 2). Additionally, it is well seen that the shapes of the obtained biomass indicators and SDS and COD curves go well with the typical changes of biomass and substrate in chemostat.

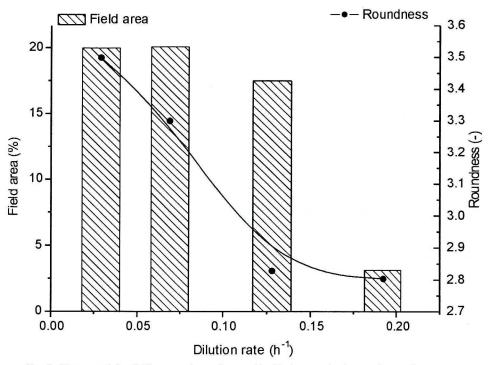


Fig. 3. Changes of the field area and roundness with dilution rate in the continuous flow system

SDS had a strong influence on activated sludge flocs morphology (Figs 1 and 3). Out of all calculated morphological parameters, the strongest effect of SDS on mean projected area of flocs and its derivatives as diameter, perimeter was observed. The mean projected area of flocs increased with retention time from 15 000  $\mu$ m<sup>2</sup> to about 50 000  $\mu$ m<sup>2</sup>. The percentage field area for the highest dilution rate was only 3.1%, while at 0.029 and 0.069 h<sup>-1</sup> it achieved about 20%. The decrease of mean projected area of flocs and the field area univocally indicated that flocs saponification processes play an important role at higher dilution rates. Concerning their shape, the flocs exposed to higher SDS load are more regular than flocs aggregated at lower dilution rates. Their circularity index was about 2.8, while at dilution rates from 0.029 to 0.069 h<sup>-1</sup> it was equal to 3.4. The observations of activated sludge flocs morphology made during the experiments in the continuous flow system are in agreement with the previous results achieved in batch shake flasks [7].

The significant reduction of flocs area, which was the effect of saponification, resulted in the decrement of sludge volume index (SVI) with the increase of dilution rate even to about 18 cm<sup>3</sup> g<sup>-1</sup> TS for the shortest residence time (Fig. 4). At the same time the turbidity of the effluent increased with dilution rate (Fig. 5). This phenomenon was most probably caused by the growing number of freely suspended bacteria. The microscopic observations showed that not only size and structure of flocs had been changed with the dilution rate, but also the biomass diversity. The number of freely suspended bacteria increased at higher dilution rates in comparison to the lower dilution rates and inoculums. At high SDS loads rod bacteria seemed to dominate over cocci and vibrios, which were present in the inoculums and at dilution rates below  $0.069 \text{ h}^{-1}$ . High concentration of SDS in the system also caused death of practically all higher microorganisms as protozoa. It is strong evidence that the simpler organisms easier adapt to new conditions [4].

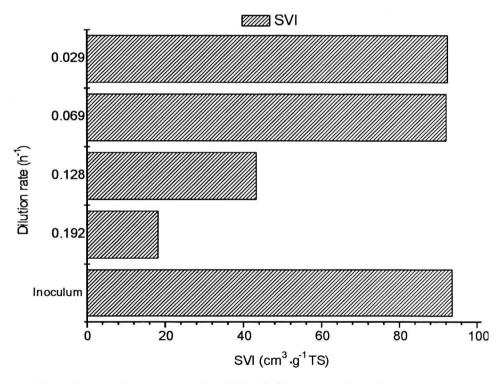


Fig. 4. Changes of sludge volume index (SVI) with dilution rate in the continuous flow system

Grijspeerdt and Verstraete reported that the field area was correlated with biomass concentration for activated sludge [5]. Also the results obtained in these experiments indicated that selected morphological parameters are constrained with biomass concentration. The linear relation between mean projected area as well as the field area and volatile suspended solids was found. For both parameters, the correlation coefficients were above 0.99 (Figs 6 and 7). Similar correlations were also observed for SDS run and control run (without SDS) in batch shake flasks experiments [7]. Nevertheless, the relation between mean projected area and SVI was not observed.

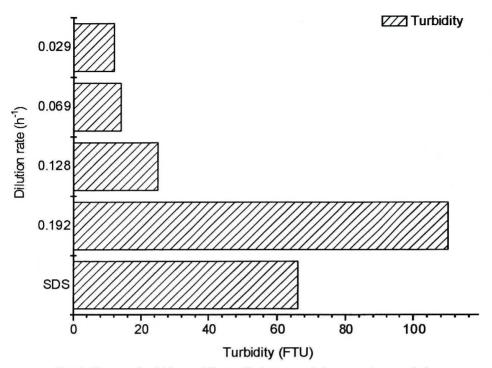


Fig. 5. Changes of turbidity at different dilution rate and for pure substrate solution

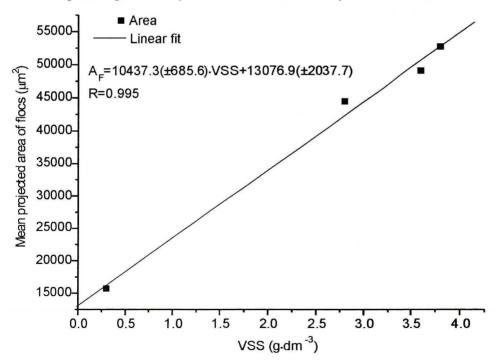
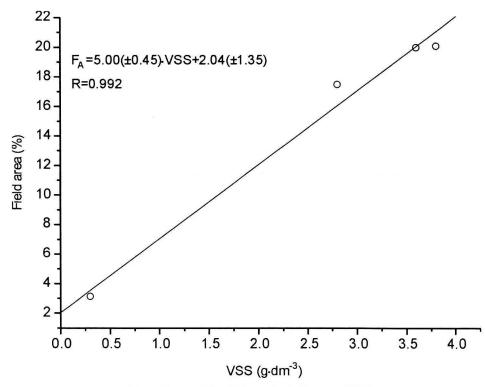


Fig. 6. Linear relation between mean projected area and VSS

Also da Motta *et al.* who developed an automated image analysis procedure, did not establish any correlation between flocs morphological characteristics and SVI [9].





#### CONCLUSIONS

SDS causes the significant destruction of activated sludge flocs, which is quantitatively expressed by morphological parameters. Its presence in wastewater resulted in substantial diminish of mean projected area of flocs and the field area with the increase of dilution rate. As a result, sludge volume index may become very low. At the same time, turbidity, due to the growing number of freely suspended bacteria, increased with the increase of dilution rate.

SDS also inhibits dehydrogenase activity of microorganisms and induces changes in biomass diversity. In spite of these unfavorable changes in sludge flocs structure, the high degree of SDS biodegradation is achieved. Nevertheless, it does not correlate with the level of COD removal, which indicates that certain intermediates remain in the effluent.

The linear relation between mean projected area of flocs, the field area and volatile suspended solids exists. It confirms the previous literature data according to which some morphological parameters can be good biomass indicators in the activated sludge processes.

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