

ESTIMATION OF THE IMPACT OF ANIONIC SURFACTANTS ON  
ACTIVATED SLUDGE FLOCS MORPHOLOGY IN A BATCH SYSTEMEWA LIWARSKA-BIZUKOJC<sup>1</sup>, MARCIN BIZUKOJC<sup>2</sup><sup>1</sup> Technical University of Łódź, Department of Environmental Engineering  
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ul. Wólczańska 213, 90-924 Łódź, Poland**Keywords:** Activated sludge, anionic surfactants, biodegradation, flocs morphology.

**Abstract:** In this study the effect of anionic surfactants on the morphology of activated sludge flocs and biomass activity is quantitatively described. Three anionic surfactants: sodium dodecyl sulphate, sodium alkylbenzene sulphonate and sodium alkyltrioxyethylene sulphate were tested. The batch experiments were performed for a wide range of initial concentrations of anionics in wastewater from 2.5 to 2500 mg·dm<sup>-3</sup>. In spite of different chemical structure the action of all tested anionic surfactants resulted in the decrease of activated sludge flocs dimensions at the similar level. It occurred that in the range of anionics concentrations, which are typical for domestic wastewater (2.5–25 mg·dm<sup>-3</sup>), they contributed to the decrease of mean projected area of flocs by about 30%, whereas at the concentrations of 250 and 2500 mg·dm<sup>-3</sup> mean projected area decreased usually by 50–60%. Sodium alkylbenzene sulphonate exerted the strongest inhibition effect on dehydrogenase activity of activated sludge biomass. This effect coincided with the decreased degrees of removal for this surfactant and its biodegradation products, especially at its lower initial concentrations in wastewater.

## INTRODUCTION

Microbial systems due to their simplicity and relatively low costs have been employed for the decomposition of synthetic surfactants at different process configurations. Most of them are activated sludge systems [6, 7, 20]. The results obtained so far indicated that the removal efficiency of anionic surfactants was generally higher than 95% in the activated sludge wastewater treatment plants [6, 16, 19]. At the same time anionic surfactants can inhibit biochemical activity of bacteria as well as have an impact on surface properties of sludge flocs [3, 10]. The conventional microbiological methods were not well suited to the investigation of such microbial aggregates as flocs. Thus, during the last two decades various new techniques have been developed to estimate the morphological parameters of activated sludge flocs and reveal their internal structure [2, 4, 7, 18]. Digital image analysis is one of these techniques.

Petrovic and Barceló indicated that nowadays three classes of anionic surfactants are the most frequently used: linear alkylbenzene sulphonates (LAS), alkylether sulphates (AES) and alkyl sulphates (AS) [15]. Linear alkylbenzene sulphonates (LAS) make more than 41% of consumed anionics, whereas AES and AS represent 31% and 9% of them, respectively. Taking the contribution of these three classes of anionics in the synthetic

surfactants market into account, the compounds belonging to these groups were selected for tests in these investigations.

The digital image analysis procedures were applied in order to measure the morphological parameters of flocs. They can be divided into two groups. The first group comprises the parameters representing the size of flocs, i.e. mean projected area, diameter, perimeter and equivalent circle diameter, while the second group consists of the parameters describing the shape of flocs as circularity index, convexity or fractal dimension [2, 4].

The main aim of this study was to compare the influence of different anionic surfactants on the morphological parameters of the activated sludge flocs as well as the biochemical activity of microorganisms in a batch system. The experiments were conducted for a wide range of anionic surfactants concentrations from 2.5 to 2500 mg·dm<sup>-3</sup>.

## MATERIALS AND METHODS

### *The object of investigations*

Three anionic surfactants of different chemical structure and properties were tested. They were sodium dodecyl sulphate (A1) C<sub>12</sub>H<sub>25</sub>OSO<sub>3</sub>Na belonging to alkyl sulphates (AS), sodium alkylbenzene sulphonate (A2) C<sub>10-13</sub>H<sub>21-27</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>Na representing linear alkylbenzene sulphonates (LAS) and sodium alkyltriethoxyethylene sulphate (A3) C<sub>12-14</sub>H<sub>25-29</sub>O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>3</sub>SO<sub>3</sub>Na representing alkylether sulphates (AES). Two of them A2 and A3 are commercial products provided by one of Polish detergents manufacturers, whereas sodium dodecyl sulphate (A1) was purchased as a pure for analysis compound from POCh (Poland).

### *Substrate*

Synthetic wastewater prepared with or without addition of proper amount of anionic surfactants was used as the substrate in the experiments. Synthetic wastewater contained 300 mg peptone, 100 mg sodium acetate, 50 mg potassium monophosphate, 50 mg sodium hydrocarbonate, 50 mg ammonium hydrophosphate, 5 mg magnesium sulphate and 5 mg sodium chloride per dm<sup>3</sup>. The experiments were carried out for the following concentrations of anionic surfactants expressed as mg MBAS (methylene blue active substances) per dm<sup>3</sup>: 2.5, 25, 250 and 2500. In each surfactant run only one anionic surfactant was added to the synthetic wastewater. The properties of all substrates used are presented in Table 1.

### *Inoculum and cultivation procedure*

The activated sludge taken from the aerated chamber at Wastewater Treatment Plant (WWTP) in Zgierz (Poland) played the role of the inoculum in the experiments performed. Its characteristics was as follows. The content of total suspended solids (TSS) was between 3.3 and 3.7 g·dm<sup>-3</sup> and volatile suspended solids (VSS) were in the range from 2.4 to 3.3 g·dm<sup>-3</sup>. Dehydrogenase activity of the activated sludge microorganisms changed in the range from 4.97 to 6.88 mg TF·g·VSS<sup>-1</sup>·h<sup>-1</sup>, where TF is 1,3,5-triphenyltetrazolium formazan. Also the morphological parameters of the activated sludge flocs were measured: mean projected area of flocs (from 26417 to 27247 μm<sup>2</sup>), mean diameter (from 163 to 165.2 μm), circularity index (from 2.57 to 2.62) and convexity (from 0.745 to 0.761).

Table 1. Physicochemical properties of the substrates (synthetic wastewater and proper surfactant)

Surfactant	Surfactant code	MBAS [mg·dm <sup>-3</sup> ]	COD [mg O <sub>2</sub> ·dm <sup>-3</sup> ]	BOD <sub>5</sub> /COD [-]	TS [mg·dm <sup>-3</sup> ]	VS [mg·dm <sup>-3</sup> ]
Sodium dodecyl sulphate	A1	2.5	425	0.605	520	235
	A1	25	484	0.600	590	364
	A1	250	910	0.588	900	600
	A1	2500	5790	0.142	2770	1720
Sodium alkylbenzene sulphonate	A2	2.5	475	0.520	595	390
	A2	25	596	0.437	670	490
	A2	250	1170	0.411	990	730
	A2	2500	5370	0.109	2990	1430
Sodium alkyltrioxyethylene sulphate	A3	2.5	503	0.515	598	420
	A3	25	571	0.490	820	540
	A3	250	1130	0.469	1005	735
	A3	2500	6100	0.148	3560	2410
Synthetic wastewater	–	0	420	0.608	385	190

All experiments were conducted in shake flasks under aerobic conditions. 15 cm<sup>3</sup> of activated sludge (inoculum) was transferred to each 300 cm<sup>3</sup> Erlenmeyer flask, containing 135 cm<sup>3</sup> fresh synthetic wastewater with or without anionic surfactant. The flasks were incubated at 22 ± 1°C in a thermostated bath shaker at speed of 110 min<sup>-1</sup> for 24 hours. The choice of 24 hour incubation time was justified by the previous investigations revealing that the substantial degradation of surfactants as well as microbial growth was observed in these conditions within this period of time [9, 18]. All experiments: surfactant runs as well as control runs (without surfactants) were conducted according to this procedure. For each anionic surfactant and for control run the experiments were made in triplicate. In these shake flask tests the contribution of biodegradation and sorption processes for the surfactants removal was not tested. As it is widely known, the biodegradation processes are the main way to remove anionic surfactants from wastewater [6, 15, 19].

### ANALYTICAL TECHNIQUES

The concentration of anionic surfactants was determined according to the methylene blue method [1]. Chemical Oxygen Demand was measured by a standard dichromatic method [1]. Dehydrogenase activity was determined according to Miksch with the use of 2,3,5-triphenyltetrazolium chloride (TTC) [13]. It was expressed as a ratio of the activity of the actual sample to the activity of the inoculum. Biochemical Oxygen Demand (BOD<sub>5</sub>), total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS), were measured according to standard methods [1].

The vital unstained slides of activated sludge sample were prepared. The activated sludge flocs were observed under Olympus BX40 light microscope with blue filter at the magnification 100x. The RGB (Red, Green, Blue model of color) images were snapped, processed and analyzed with the help of Micro Image 4.0 software (Media Cybernetics for Olympus). At least 40 images from each sample were snapped and stored for further processing. These images were subjected to a detailed analysis. The following morphological parameters of the flocs were measured: mean projected area, perimeter, convex

perimeter, mean diameter, circularity index and convexity [14, 17]. Mean projected area is the basic image analysis parameter and is found easily by pixel count and its multiplication by scaling factor. The perimeter is the length of the object's outline. The convex perimeter is the perimeter of the convex outline of the object. 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. Additionally, another shape factor, convexity ( $F_p$ ) was used in this study. It is defined as the ratio of taut string perimeter (convex perimeter) to perimeter. This ratio is equal to 1 for the boundary case, when an object is ideally convex, i.e. circle, square or polygon. For other cases, the lower the convexity, the more protrusions are present in the object [17]. Image analysis procedures as well as the definitions of measured morphological parameters were described in detail elsewhere [10].

The relative degree of flocs saponification was used in order to evaluate changes of activated sludge flocs size. It was defined as follows:

$$D_s = \frac{X_I - X_p}{X_I} \cdot 100$$

where:

$D_s$  is the relative degree of flocs saponification [%];

$X_I$  is the value of a morphological parameter related to the flocs size i.e. mean projected area, diameter or perimeter in the inoculum;

$X_p$  is the value of a morphological parameter related to the flocs size in the actual sample.

The relative degree of flocs saponification was estimated after 24 hours in each shake flask experiment. If the saponification did not occur, i.e. the growth of flocs dominated, the relative degree of flocs saponification reached negative values, which is in agreement with the formula presented above [10].

## RESULTS AND DISCUSSION

Despite the fact that the surfactants are widely used because of their surface active properties, and that they were thoroughly investigated in terms of their biodegradation and fate in the aquatic and soil environment [15, 19], the influence of these chemical compounds on activated sludge flocs morphology was rarely the object of investigations and hardly any references can be found.

The obtained results showed that anionic surfactants changed the morphology of activated sludge flocs in relation to the size and shape alike. The changes of flocs size were expressed with the use of the relative degree of flocs saponification, whose definition is given in Materials and methods, while the shape of flocs was analyzed with regard to the circularity index and convexity.

In Figure 1 the changes of the relative degree of flocs saponification for the mean projected area and mean diameter of flocs are presented. In all surfactant runs the size of activated sludge flocs exposed to anionic surfactants decreased, while in the control run the growth of activated sludge flocs was observed. Therefore, the relative degree of flocs saponification reached the negative values in the control run. The mean projected area of

sludge flocs here increased on average by about 10.1%, whereas the mean diameter was higher by 8.4% in comparison with the diameter of flocs in the inoculum.

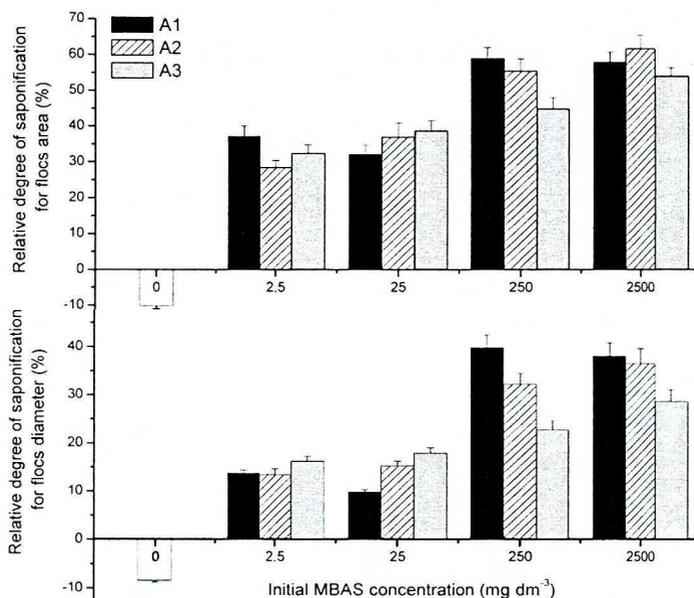


Fig. 1. Comparison of the relative degree of flocs saponification estimated for mean projected area and diameter of flocs in the batch experiments

Analyzing the changes of relative degree of flocs saponification in the surfactant runs, two ranges of initial concentrations of anionic surfactants can be discriminated: 2.5–25 mg·dm<sup>-3</sup>, which is typical for the domestic wastewater, and 250–2500 mg·dm<sup>-3</sup>, which can be met in the industrial wastewater [15, 20]. In the first range of concentrations the mean projected area decreased on average from 30.3 to 38.7% in comparison with the mean projected area of flocs introduced to the system with the inoculum. The decrease of mean projected area of sludge flocs is at the similar level for both tested concentrations 2.5 and 25 mg·dm<sup>-3</sup>. What is more important, it is difficult to equivocally indicate, which of three investigated anionics had the strongest effect on flocs size in these range of concentrations.

At the surfactant concentrations 250 and 2500 mg·dm<sup>-3</sup> the decrease of flocs size exceeded even 60%, when the mean projected area was considered. At the same time the decrease of diameter varied from 22.6 to 39.7% at these concentrations dependently on the surfactant tested. The scatter of the relative degree of flocs saponification calculated for the diameter was higher than for the mean projected area, independently of what concentration of anionic surfactants was taken into account. This observation confirmed the fact that the mean projected area as a direct measure better expressed the size of flocs, even if the diameter is easier to grasp. The fact, that the projected area of any object is more accurately estimated, is connected with a discontinuous nature of a digital image and was discussed by Russ [17].

At the higher surfactant concentrations 250 and 2500 mg·dm<sup>-3</sup> the effect of anionics on flocs dimensions was slightly dependent on the chemical structure of surfactants. Sodium dodecyl sulphate (A1) influenced the size of flocs to the greatest extent. Sodium alkylbenzene sulphonate (A2) had a similar effect on the size of flocs as sodium dodecyl sulphate (A1), especially if mean projected area of flocs was taken into consideration (Fig. 1). The mildest of anionics tested in relation to the size of flocs was sodium alkyltrioxyethylene sulphate (A3).

Due to differences in the supply of substrates and operation conditions between batch and continuous systems, it is difficult to directly compare the effect of anionics on flocs morphology in them. However, it must be stated that the decrease in size of activated sludge flocs exposed to anionics was higher in the batch tests than it was observed previously in the continuous experiments [12]. It is most probably caused by the accumulation of non-biodegraded surfactant or/and its biodegradation products in the batch system.

Anionic surfactants also acted on the shape of activated sludge flocs. In Figure 2 the circularity index and convexity in the control and surfactant runs are compared. The changes of convexity in the surfactant runs behaved inversely compared with the circularity index curves (Fig. 2).

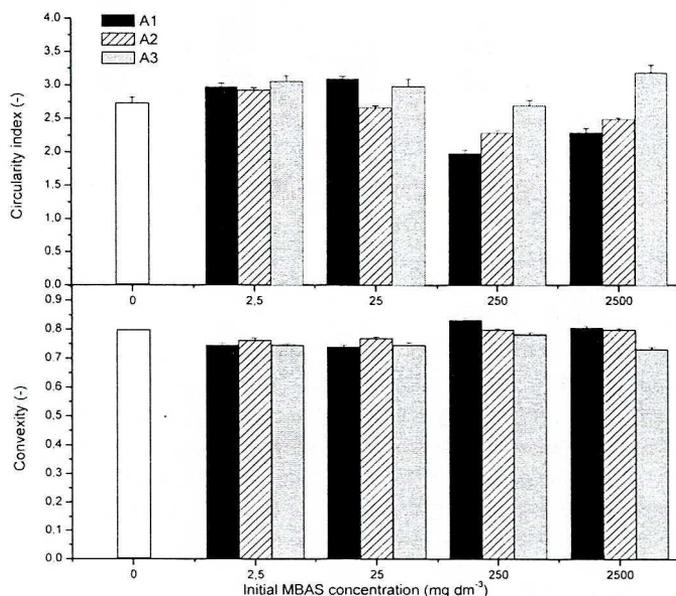


Fig. 2. Comparison of convexity and circularity index of activated sludge flocs in the batch system

At the concentrations 2.5 and 25 mg·dm<sup>-3</sup> the small increase of circularity index in relation to the inoculum was observed. Also in the control runs circularity index slightly increased from 2.6 in the inoculum to 2.7 after 24 h. The influence of anionics on the shape of flocs was mainly observed at higher concentrations 250 and 2500 mg·dm<sup>-3</sup> because activated sludge flocs exposed to anionic surfactants at these contents in wastewater became more circular than flocs introduced with the inoculum. This effect was evident also for flocs exposed to sodium dodecyl sulphate (A1) and sodium alkylbenzene sulphonate

(A2). The circularity index for these substances was in the range from 1.98 to 2.48 at concentrations 250 and 2500  $\text{mg}\cdot\text{dm}^{-3}$ , respectively. Activated sludge flocs subjected to sodium alkyltrioxyethylene sulphate (A3) had the circularity index at the level from 2.69 to 3.18, independently of the concentration of surfactant A3 in wastewater. These observations might suggest that the ability of the surfactants to change the flocs morphology (shape), especially in the higher range of concentrations, is connected with their chemical structure. Nevertheless, it must be also stated that even the lowest values of circularity index obtained (about 1.9) exceeded the boundary value found in the literature for ideally circular shape, which should not be higher than 1.2 [14]. The literature data obtained so far have indicated that the ideally circular flocs are rarely found in the activated sludge systems [5, 10]. Generally, activated sludge flocs are classified as encircled or irregular with regard to their shape [5]. Therefore, the values of circularity index in the range from 1 to 1.2 are not typical for activated sludge flocs. However, another fact is important here. The presence of anionics, especially A1 and A2 at higher concentrations 250 and 2500  $\text{mg}\cdot\text{dm}^{-3}$ , made activated sludge flocs more circular than it was observed at lower surfactant concentrations and in the inoculum. It was also confirmed by the values of convexity (Fig. 2). The lower circularity index was accompanied with the higher convexity. The closer to 1 the values of convexity were, the less protrusions the flocs had.

In order to evaluate the influence of the investigated anionic surfactants on activated sludge biomass activity, the dehydrogenase test in each run was performed. The results of these tests for control and surfactant runs are shown in Figure 3. In the run without surfactants dehydrogenase activity increased by about 40% in comparison with the inoculum. The increase of biomass activity is typical for batch systems containing the substrate, which is easily assimilated and does not inhibit the biomass growth [9]. In all surfactant runs the dehydrogenase activity decreased with the increase of anionics concentration. For example, in A1 run the dehydrogenase activity ratio decreased from 0.46 at 2.5  $\text{mg}\cdot\text{dm}^{-3}$  to 0.29 at 2500  $\text{mg}\cdot\text{dm}^{-3}$ . Among tested anionics sodium alkylbenzene sulphonate

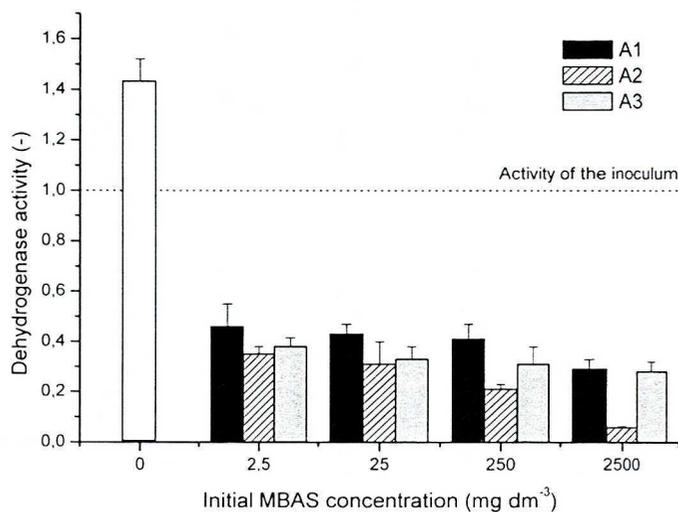


Fig. 3. Comparison of dehydrogenase activity of activated sludge biomass in the batch experiments

te (A2) exerted the strongest effect on the microbial activity. Its presence in wastewater resulted in the highest decrease of dehydrogenase activity, even by 93% at the highest of the studied surfactant concentrations. This remains in agreement with our previous findings concerning the assay of anionic surfactants toxicity by means of the dehydrogenase activity test. The inhibitive concentration  $IC_{50}$  was also the lowest for A2 [11].

In Figure 4 the degrees of Methylene Blue Active Substances (MBAS) and Chemical Oxygen Demand (COD) removal are presented. At the concentrations 2.5 and 25  $mg \cdot dm^{-3}$  the degree of MBAS removal was always above 90% independently of the surfactant. At higher concentrations it was lower: about 80% for 250  $mg \cdot dm^{-3}$  (apart from A1) and even below 60% at 2500  $mg \cdot dm^{-3}$  for all surfactants. The degree of COD removal exceeded 90% in control run and in surfactant runs at 2.5 and 25  $mg \cdot dm^{-3}$  excluding A2, which was worse degraded. At higher concentrations (250  $mg \cdot dm^{-3}$  and 2500  $mg \cdot dm^{-3}$ ) COD removal was usually lower than 60%.

The obtained results confirmed the fact that the contemporarily used anionics are easily biodegraded in the activated sludge systems [15, 19], especially in relation to the surfactant concentrations typical for domestic wastewater. As mentioned above, the degrees of COD removal as well as MBAS removal were high and usually exceeded 90% at concentrations 2.5 and 25  $mg \cdot dm^{-3}$ . However, MBAS removal degrees obtained in these experiments were slightly lower than the removal of anionic surfactants in the wastewater treatment plant reported by Fauser *et al.* [6]. As it was mentioned above it is connected with the different growth conditions of the microorganisms in the batch and continuous systems [8]. Although continuous systems are more common than batch systems in wastewater treatment, sequencing batch reactors (SBRs) are used for this purpose, too [8]. Therefore, the results presented in this work can support the proper operation of SBRs, especially the ones aimed at anionics removal.

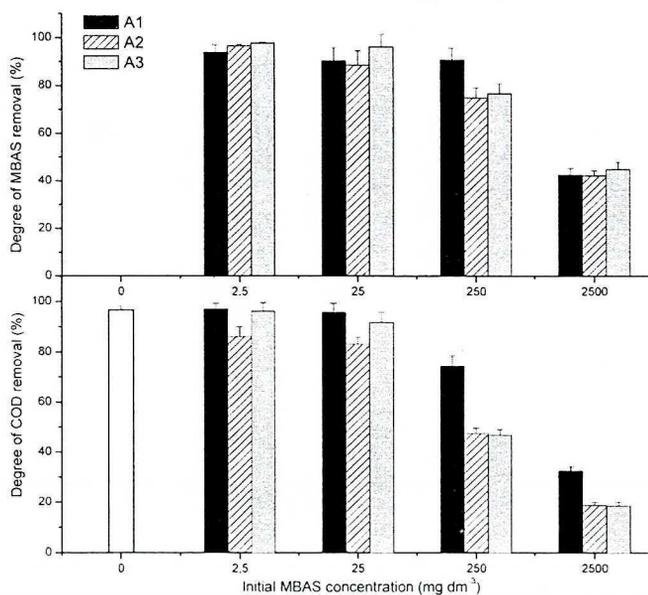


Fig. 4. Comparison of degree of MBAS and COD removal in the batch experiments

Comparing both degrees of removal in terms of surfactant type, sodium alkylbenzene sulphonate (A2) and its biodegradation products were more difficult to remove than organics in A1 and A3 runs, especially at lower initial concentrations 2.5 and 25 mg·dm<sup>-3</sup>. It coincided with the dehydrogenase activity, which was the lowest for the activated sludge exposed to A2 (Fig. 3). At the same time sodium dodecyl sulphate (A1) was the easiest biodegradable among the anionics tested (Fig. 4) and dehydrogenase activity in A1 runs was higher than in A2 and A3 runs (Fig. 3).

Zhang *et al.* performed the biodegradation experiments with sodium dodecyl sulphate (SDS) and sodium dodecylbenzene sulphonate (LAS as A2 in our study but possessing exactly 12 carbon atoms in the alkyl chain) in the activated sludge batch system at the initial concentration of 500 mg·dm<sup>-3</sup> for both surfactants [20]. They observed that SDS concentration significantly decreased within 24 h by about 80%, whereas sodium dodecylbenzene sulphonate was hardly removed within three weeks of the experiment. It is in agreement with the results obtained in this study that linear alkylbenzene sulphonates due to benzene ring in its structure demand longer time for biodegradation and removal from wastewater [20].

## CONCLUSIONS

1. Anionic surfactants exerted the decrease of activated sludge flocs size even at the low concentrations 2.5 and 25 mg·dm<sup>-3</sup>. The relative degree of flocs saponification is dependent on the concentration of anionic in wastewater. In the range of concentrations typical for domestic wastewater anionic surfactants contributed to the decrease of mean projected area of flocs by about 30%, whereas at the concentrations 250 and 2500 mg·dm<sup>-3</sup> the mean projected area decreased usually by 50–60%.
2. Sodium dodecyl sulphate and sodium alkylbenzene sulphonate present in wastewater at concentrations 250 and 2500 mg·dm<sup>-3</sup> made activated sludge flocs more circular and more convex in comparison with sodium alkyltrioxyethylene sulphate and wastewater without anionics added.
3. Anionic surfactants inhibited the dehydrogenase activity of activated sludge microorganisms. Sodium alkylbenzene sulphonate (LAS) exerted the strongest effect on the dehydrogenase activity of activated sludge biomass. This effect coincided with the decreased degrees of removal for this surfactant and its biodegradation products, especially at its lower initial concentrations in wastewater.
4. The degrees of organics removal obtained in the batch system are high (above 90%) at anionics concentrations typical for domestic wastewater (2.5 and 25 mg·dm<sup>-3</sup>). The higher amounts of anionics in wastewater influent may deteriorate not only activated sludge flocs structure but also the removal efficiency of the contaminants.

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#### OKREŚLENIE WPŁYWU ANIONOWYCH SURFAKTANÓW NA MORFOLOGIĘ KLACZKÓW OSADU CZYNNEGO W UKŁADZIE OKRESOWYM

W niniejszej pracy przedstawiono w ujęciu ilościowym wpływ anionowych środków powierzchniowo czynnych (ASPC) na morfologię klaczków osadu czynnego i aktywność biomasy. Obiekt badań stanowiły trzy anionowe surfaktanty: dodecylsulfian sodu, alkilobenzenosulfonian sodu i alkilotrioksyetylenosulfian sodu. Doświadczenia przeprowadzono w układzie okresowym w szerokim zakresie stężeń ASPC w ściekach od 2,5 do 2500 mg·dm<sup>-3</sup>. Wszystkie trzy badane surfaktanty przyczyniały się do zmniejszenia wielkości klaczków osadu czynnego na podobnym poziomie, pomimo odmiennej budowy chemicznej. Średnia powierzchnia rzutu klaczków zmniejszała się pod wpływem surfaktantów o około 30%, kiedy stężenie ASPC w ściekach wynosiło od 2,5 do 25 mg·dm<sup>-3</sup> i o 50–60% przy stężeniu ASPC w ściekach 250–2500 mg·dm<sup>-3</sup>. Alkilobenzenosulfonian sodu najsilniej inhibitował aktywność dehydrogenazową biomasy osadu czynnego. Towarzyszył temu niższy, w porównaniu do pozostałych badanych ASPC, stopień usunięcia tego surfaktanta i produktów jego rozkładu ze ścieków, zwłaszcza w niższym zakresie jego początkowych stężeń w ściekach.