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ACTIVATED SLUDGE ACTIVITY IN THE TREATMENT OF ANAEROBIC SLUDGE DIGESTER SUPERNATANT

The objective of the study was to examine the effect of the food/microorganisms (F/M) ratio on the respirometry of activated sludge treating anaerobic sludge digester supernatant at low oxygen concentration (DO) of 0.7 mg O_2/dm^3. Exogenous respiration rate ($SOUR_1$), endogenous respiration rate ($SOUR_3$) and the rate of oxygen uptake for nitrification ($SOUR_2$) were evaluated. Taking into consideration that BOD_5/COD in anaerobic digester supernatant was 0.53, it may be supposed that weak activity of heterotrophic bacteria (low $SOUR_1$) resulted from low DO concentration in the reactor, and not from limited accessibility of biodegradable substrate. At the F/M ratio of 0.05–0.26 g COD/(g TSS·d), $SOUR_2$ was from 2.1-fold to 20.6-fold higher than $SOUR_1$. Further increase in the F/M ratio induced a decline in $SOUR_2$ but the efficiency of nitrification enhanced.

1. INTRODUCTION

Measurement of the activity of microorganisms involved in wastewater treatment delivers information about biochemical processes responsible for substrate utilisation. Microbial activity is often determined by respirometric measurements that allow estimating oxygen depletion due to substrate consumption. Oxygen uptake rate (OUR), defined as the amount of oxygen consumed by the microorganisms per unit of time and volume, is an indicator of microbial activity in aerobic processes and is widely used in different areas of wastewater treatment [1–4]. Witzig et al. [5] considered the value of oxygen uptake rate to be equivalent to the overall metabolic activity of the sludge community. Moreover, the fraction of active biomass is proportionally related to the respirometric activity [3]. Benes et al. [6] proved that respiration rates of acti-

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vated sludge should be measured in order to predict and control the activated sludge process in wastewater treatment. If it is used as a routine measurement, respirometric analysis can indicate early changes in activated sludge of wastewater treatment plants.

Oxidation of organic compounds and ammonia are the key processes in wastewater treatment that require oxygen. The rate of oxygen uptake for oxidation of organic substrates present in wastewater (exogenous respiration rate) is defined as the oxygen consumption rate reached when all the individual substrates that can be oxidised by a heterogeneous microbial population are present in excess [7]. The applications of this measurement include characterizing of the activity of heterotrophs and assessment of easily biodegradable COD in wastewater [8]. In the case of the absence of substrate in wastewater, oxygen is utilized for the endogenous respiration that means the oxygen consumption for bacterial growth-decay cycle, maintenance energy production and protozoa respiration [7]. Respirometric measurements can be also used for monitoring the nitrification activity [9].

Because of the fact that simultaneous carbon and nitrogen removal from wastewater requires the occurrence of heterotrophic bacteria and autotrophic nitrifiers in activated sludge, it is necessary to distinguish the oxygen uptake for oxidation of heterotrophic substrate and for oxidation of ammonia. It is commonly known that the number and activity of heterotrophic and autotrophic bacteria in biomass are influenced by feeding conditions in the reactor, especially the COD/N ratio [10], and DO concentration. In conventional activated sludge systems operated at 2–3 mg O_2/dm^3, the specific growth rate of autotrophs is significantly lower than that of heterotrophs. However, in the case of wastewater with extremely high ammonia concentration (digester supernatant, landfill leachate) technological solutions with low oxygen concentrations are required, e g. partial nitrification [11] or the Sharon process [12] which allow limitations in the amount of oxygen supplied. The efficiency of these processes is improved mainly by low DO concentration. Until now, most of the research concerning the treatment of anaerobic sludge with digester supernatant concentrates on ammonia oxidation and/or nitrogen elimination, whereas there is still lack of data on the efficiency of organic compounds removal and heterotrophic bacteria activity under the conditions of limited DO. Therefore, preliminary respirometric measurements carried out in this study can give information about the activity of both mentioned groups of bacteria. The results of the research may constitute a base to choose the optimum mode of wastewater feeding to the reactor and as a result promote the effective treatment of anaerobic digester supernatant. The objective of the study was to determine the effect of the F/M ratio on biomass activity at low DO concentration while anaerobic digester supernatant was the substrate. The microbial activity was evaluated with the use of respirometric tests that were based on the calculation of the specific oxygen uptake rate (SOUR). Exogenous and endogenous respiration rates, as well as oxygen consumption for ammonia oxidation were determined.

2. EXPERIMENTAL

Managing of the experiment. In order to measure the microbial activity, the dissolved oxygen (DO) consumed by activated sludge was determined using a close respirometric unit OxiTop Control OC 110 (WTW, Germany). The respirometer comprised of a stirred vessel of the volume of 0.5 dm^3. During the analysis, oxygen was taken from the headspace of the vessel. The DO changes in the samples were determined based on the variations of partial pressure inside the measuring vessels. Carbon dioxide, formed during the microbial metabolism, was absorbed by NaOH placed in a small tube above the liquid level. Since the amount of used O_2 is proportional to the amount of formed CO_2, removal of CO_2 from the gas phase causes the drop of the pressure in the measuring vessel. Pressure decline is automatically converted to the changes of DO in the sample.

Before transferring into the respirometric unit, activated sludge was adapted to experimental conditions in the SBRs, equipped with stirrers and controlled air supply systems. Anaerobic digester supernatant was the substrate for biodegradation. Three reactors were supplied with the mixture of the anaerobic digester supernatant (30%) and synthetic media of the quality of municipal wastewater (70%) and the next three SBRs with anaerobic sludge digester supernatant being 100% of the influent. The reactors differed in volumetric exchange rate that was 0.1, 0.3 and 0.5 d^{-1}. The amount of air entering the SBRs was automatically adjusted to a stable set-point of 0.7 mg O_2/dm^3. The reaction in the SBRs was maintained at the level of about 8 pH. The reactors were operated at 20 °C. After the adaptation period, the mixed liquor samples of activated sludge were taken directly from the reactors at the beginning of the reactor cycle, rinsed with distilled water, and used for the respirometric measurements.

Table 1

Experiment management in series 1–6

Series	Contribution [%] of the sludge digester supernatant /synthetic wastewater	TKN in the wastewater [mg/dm^3]	COD/N of the wastewater	F/M [g COD/(g TSS·d)]
1	30/70	195	4.1	0.02
2				0.05
3				0.08
4	100/0	690	2.0	0.18
5				0.22
6				0.26

The respirometric tests were conducted in 6 series. To assure the same conditions as in the SBRs, in series 1–3, the mixture of the supernatant (30%) and synthetic wastewater (70%) was introduced into the respirometric units with activated sludge. In series 4–6, anaerobic sludge digester supernatant accounted for 100% of the wastewater (Table 1). The concentrations of total Kjeldahl nitrogen (TKN) and the

COD/N ratios in the wastewater placed in the measuring vessels are given in Table 1. The F/M ratios in series 1–3 and 4–6 were changed by introducing increased volumes of wastewater to the stable amount of biomass.

Respirometric analyses. The total oxygen uptake rate (OUR_{tot}) being the sum of the exogenous respiration rate (OUR_1) and the rate of oxygen uptake for nitrification (OUR_2), was determined as follows: the mixture of the anaerobic digester supernatant (30%) with synthetic media of the quality of municipal wastewater (70%) and activated sludge (series 1–3) or anaerobic digester supernatant and activated sludge (series 4–6) were placed into the measuring vessel.

Then the exogenous respiration rate (OUR_1) was determined. The mixture of the anaerobic digester supernatant (30%) with synthetic media of the quality of municipal wastewater (70%), activated sludge and allylthiourea (ATU) (series 1–3) or anaerobic digester supernatant, activated sludge and ATU (series 4–6) were placed in the measuring vessel; the concentration of ATU was 10 mg/dm^3, it is considered as an effective inhibitor of nitrifying activity [13].

To determine the endogenous respiration rate (OUR_3), the samples of activated sludge taken directly from SBRs operating under experimental conditions were centrifuged for 10 min (4000 rpm), rinsed 2 times with a phosphate buffer (pH 7.2) and distilled water to remove the residual COD. Activated sludge was placed into the measuring vessel supplemented with distilled water to maintain the same sample volume as in tin two previous experiments. The rate of oxygen uptake for nitrification (OUR_2) was calculated as the difference:

$$OUR_2 = OUR_{tot} - OUR_1.$$

To keep the temperature of the vessels constant at the level of 20 °C, the vessels with samples were placed into the thermostatic chamber for 1 day (corresponding to the reaction time of 1 d in SBRs). In every series, the respirometric measurements were repeated twice. The concentrations of DO used to calculate the OUR were the the mean values obtained during the two measurements.

Calculation methods. Non-linear regression from the slope of the plot of dissolved oxygen concentration versus time gave the values of the oxygen uptake rates (OUR_1, OUR_2 and OUR_3). Statistica 8 software was used. The oxygen uptake rates (OUR_1, OUR_2 and OUR_3) described by the first order kinetics were defined by the equation:

$$OUR = kL_0 \qquad [mg\,O_2/(dm^3 \cdot h)] \tag{1}$$

which after solving gives:

$$L = L_0\,(1 - e^{-k \cdot t}) \qquad [mg\,O_2/dm^3] \tag{2}$$

where: k is the constant of the reaction rate (h^{-1}), L_0 is the maximum concentration of used oxygen, L is the concentration of used oxygen after time t.

To determine the activity of microbial communities in the activated sludge, the specific oxygen uptake rates for exogenous respiration, nitrification and endogenous respiration, $SOUR_1$, $SOUR_2$ and $SOUR_3$ respectively, were calculated [14, 15]:

$$SOUR_{1-3} = \frac{OUR_{1-3}}{VSS} \qquad [mg\ O_2/(g\ VSS \cdot h)] \tag{3}$$

where OUR_{1-3} are the oxygen uptake rates [mg O_2/(dm^3·h)] and VSS is the volatile suspended solids concentration of activated sludge [g VSS/dm^3]. The average value of VSS was 3.5 g/dm^3 and comprised 65% of TSS.

3. RESULTS

The evaluation of the activity of biomass fed with anaerobic digester supernatant was based on respirometric measurements. The rates of oxygen utilization for oxidation of organic substrates, ammonia and for endogenous respiration of activated sludge were investigated. The concentrations of used oxygen (in mg O_2/dm^3), obtained from respirometer system, in every measuring moment, were divided by time (in hours) and presented as OUR profiles in Figs. 1 and 2. Total areas under the curves indicate the amount of oxygen that must be supplied to obtain full oxidation.

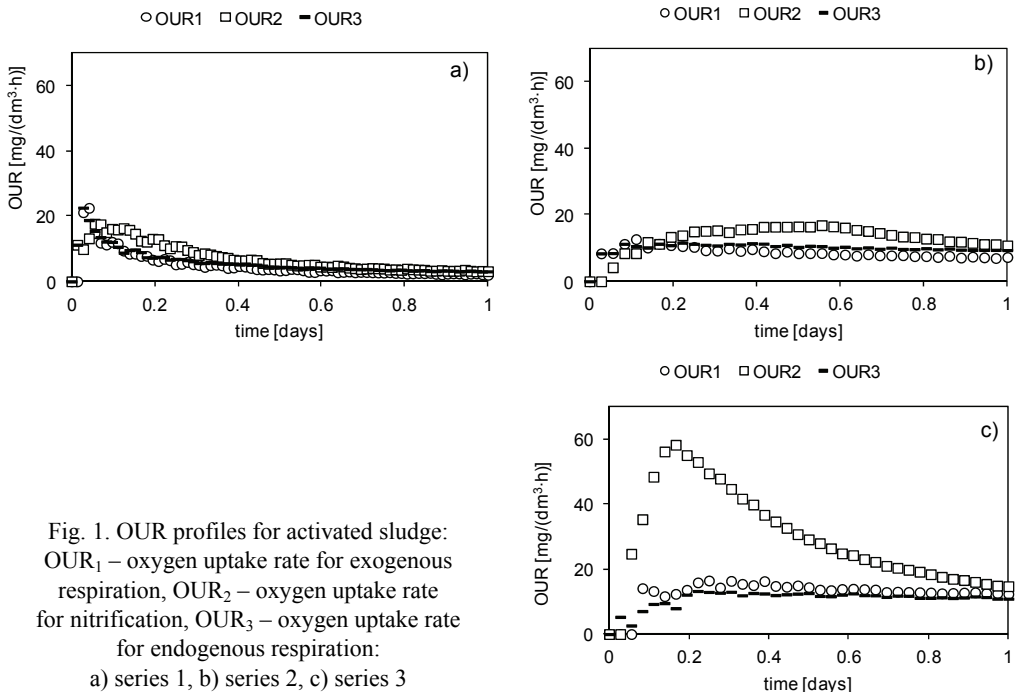

Fig. 1. OUR profiles for activated sludge: OUR_1 – oxygen uptake rate for exogenous respiration, OUR_2 – oxygen uptake rate for nitrification, OUR_3 – oxygen uptake rate for endogenous respiration: a) series 1, b) series 2, c) series 3

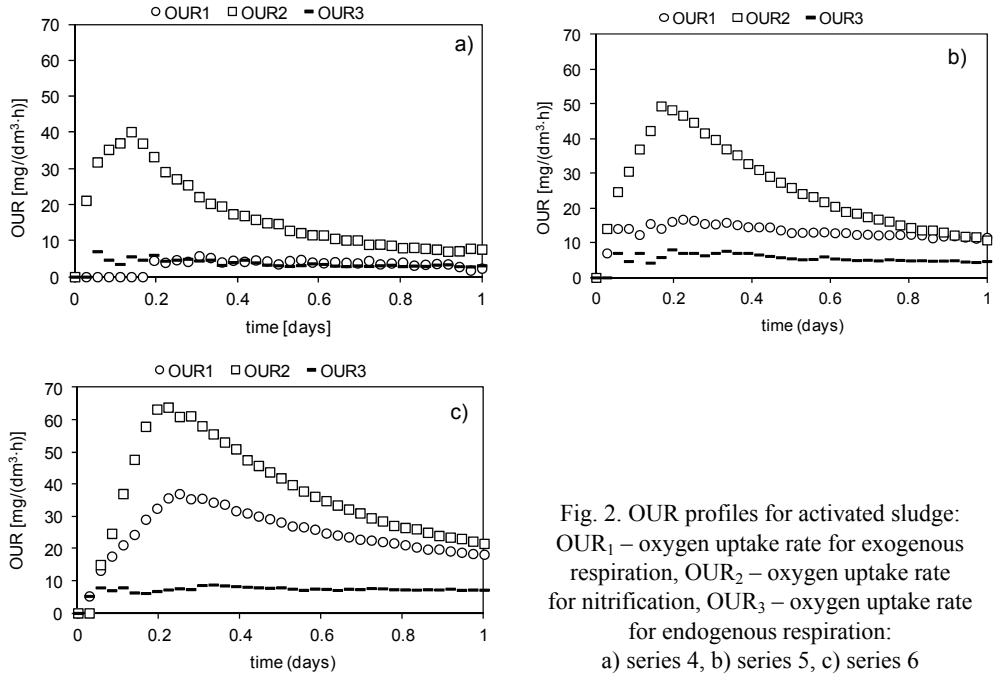

Fig. 2. OUR profiles for activated sludge: OUR_1 – oxygen uptake rate for exogenous respiration, OUR_2 – oxygen uptake rate for nitrification, OUR_3 – oxygen uptake rate for endogenous respiration: a) series 4, b) series 5, c) series 6

At the COD/N ratio of 4.1, with 30% portion of anaerobic digester supernatant in wastewater, and the F/M ratio of 0.02–0.05 g COD/(g TSS·d) (series 1, 2), the rates of oxygen uptake for exogenous respiration (OUR_1), for nitrification (OUR_2) and endogenous respiration (OUR_3) were similar on the maximum level of ca. 20 mg/(dm^3·h) (Fig. 1). Moreover, in every experimental series (1–3), there were no periods with zero OUR for endogenous respiration indicating that organic concentration in wastewater (exogenous carbon source) was too low for heterotrophic bacteria that had to use endogenous carbon. Surprisingly, at the highest F/M ratio (0.08 g COD/(g TSS·d)), the oxygen uptake for ammonia oxidation (OUR_2) was above 3-fold higher than OUR_1 and OUR_3 and reached 60 mg/(dm^3·h) (Fig. 1c).

When anaerobic digester supernatant accounted for 100% of the feeding substrate (series 4–6), nitrification was the process that induced the highest oxygen uptake (Fig. 2). Upon the growth of the F/M ratio from 0.18 to 0.26 g COD/(g TSS·d) nitrifier activity increased, and OUR_2 reached almost 70 mg/(dm^3·h) at the highest investigated F/M. Similarly, the increase in the F/M ratio resulted in the rise of oxygen uptake for exogenous respiration, OUR_1 achieved the value of almost 40 mg/(dm^3·h) in series 6 (Fig. 2c). Endogenous activity did not vary significantly in series 3–6 and required the amount of oxygen at the level of about 10 mg/(dm^3·h).

The specific oxygen uptake rates (SOUR) for every series are presented in Fig. 3. In series 1, at the F/M ratio of 0.02 g COD/(g TSS·d), activated sludge was characterised by the lowest respirometric activity. The rates of oxygen uptake for exogenous

($SOUR_1$) and endogenous ($SOUR_3$) respiration, as well as for ammonia oxidation ($SOUR_2$) were on similar level of about 1.0 mg O_2/(g VSS·h).

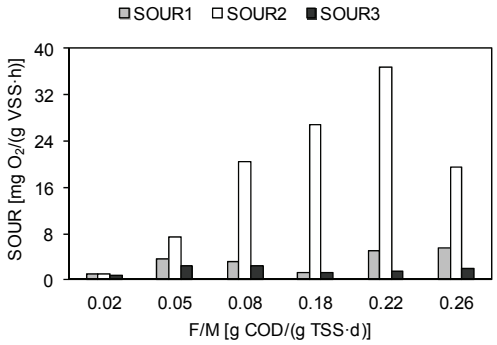

Fig. 3. Specific oxygen uptake rates for exogenous respiration ($SOUR_1$), nitrification ($SOUR_2$) and endogenous respiration ($SOUR_3$)

The increase in the F/M ratio resulted in a growth in microorganisms' activity. However, in series 2 and 3, there were no differences in the amount of oxygen utilized for oxidation of exogenous and endogenous substrates per g of VSS. $SOUR_1$ achieved the average value of 3.4 mg O_2/(g VSS·h), $SOUR_3$ – 2.45 mg O_2/(g VSS·h). In series 4–6, with anaerobic digester supernatant as a sole substrate for microorganisms, an increase in the F/M ratio from 0.18 g COD/(g TSS·d) to 0.26 g COD/(g TSS·d) resulted in a small increase in the specific oxygen uptake rates for exogenous and endogenous respiration. Generally, the growth of the F/M ratio from 0.02 to 0.22 g COD/(g TSS·d) resulted in 36-fold increase in the specific rate of oxygen utilized for ammonia oxidation. The growth of the F/M ratio above 0.22 g COD/(g TSS·d) limited the amount of oxygen used for nitrification to 19.5 mg O_2/(g VSS·h).

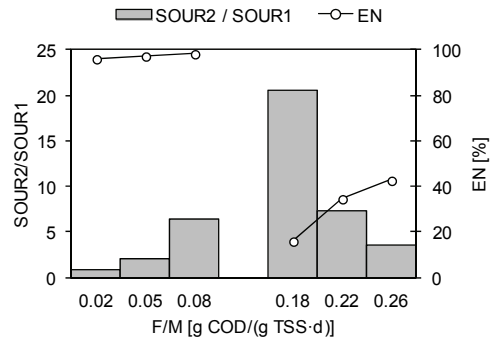

Fig. 4. Ratio of nitrification activity to exogenous respiration and nitrification efficiency (E_N) for series 1–6

Figure 4 shows the abundance of a nitrifying activity over a heterotrophic activity in activated sludge as the ratio between specific oxygen uptake rates for nitrification and for exogenous respiration ($SOUR_2/SOUR_1$), according to Yang et al. [10]. In series 1–3 with 30% of anaerobic digester supernatant in the wastewater mixture

(COD/N ratio of 4.1), the rate of $SOUR_2/SOUR_1$ increased from 0.9 to 6.4 upon the growth of the F/M ratio. Based on the concentrations of ammonia nitrogen in wastewater, it was calculated that nitrification effectiveness (E_N) was on the similar level, averagely 97% (ammonium used for heterotrophic assimilation was accounted). By contrast, at the COD/N ratio of 2.0, the highest nitrifiers' activity comparing to heterotrophic activity was obtained at the F/M ratio of 0.18 g COD/(g TSS·d) (series 4). At the highest F/M ratio of 0.26 g COD/(g TSS·d), the $SOUR_2/SOUR_1$ was almost 6-fold lower. However, the results revealed the increase in nitrification efficiency from 16.1 to 42.8%.

4. DISCUSSION

Respirometric activity of heterotrophic bacteria was described by the specific heterotrophic oxygen uptake rate ($SOUR_1$), called exogenous oxygen uptake rate, whereas the activity of autotrophic bacteria was quantified by the specific rate of oxygen used for ammonia oxidation ($SOUR_2$). Besides, the amount of oxygen used for oxidation of endogenous substrates was described as $SOUR_3$.

The specific exogenous oxygen uptake rate ($SOUR_1$) indicates the physiological state of activated sludge and, according to Kosińska [16], depends on the amount of readily biodegradable substrate and the presence of toxic substances. In our research, in series 1–3, with 30% of anaerobic digester supernatant in the wastewater, $SOUR_1$ equalled 1.1–3.6 mg O_2/(g VSS·h), in series 4–6, with 100% of supernatant, $SOUR_1$ accounted for 1.3-5.5 mg O_2/(g VSS·h). Numerous research give the values of exogenous respiration in industrial wastewater or in municipal wastewater with the addition of industrial one. In the mixture of food industry and municipal wastewater exogenous respiration was at the level of 15.9 mg O_2/(g VSS·h) that was even higher than in control samples (without industrial sewage) [16]. Krzanowski, Wałęga [17] observed the treatment of sugar industry wastewater and obtained the exogenous SOUR of 7.77 mg O_2/(g VSS·h) that points on low activity of activated sludge. Authors explain such results by the stabilization of activated sludge confirmed by sludge age of 17 d. In our research, activated sludge adapted to experimental conditions and used for respirometric measurement was characterized by sludge age of about 30 d. Besides, the endogenous respiration rate on the level of 0.9-2.5 mg O_2/(g VSS·h) could have indicated the stabilization of activated sludge. According to Henze et al. [18], the exogenous respiration rates of 20–40 mg O_2/(g VSS·h) indicate active biomass, containing numerous alive microorganisms and sufficient amount of organic substrate. In turn, low exogenous respiration rates (5–10 mg O_2/(g VSS·h)) informs that activated sludge is dead, there is lack of readily biodegradable organic substances or biomass is stabilized. In our study, the percentage of readily biodegradable fraction of the influent accounted of ca 50% of total COD. Therefore, the low rate of oxygen uptake for exogenous respiration can be explained rather by weak activity of heterotrophic bacteria at

limited DO concentration. According to Sözen et al. [19], the maximum specific growth rate of heterotrophs may be reduced under anoxic conditions. This can be confirmed by low effectiveness of COD removal beings approximately 75% in series 1–3 and 10–42% in series 4–6. Such microbial activity at low DO of 0.7 mg/dm^3 may result from the presence of different microoenvironments in activated sludge flocs. Li and Bishop [20] observed that the aerobic zone was limited to the surface layer (0.1–0.2 mm) of the floc. The anoxic zone in the centre of the floc disappeared when DO in the bulk liquid was higher that 4 mg/dm^3.

Generally, in conventional systems of activated sludge, large concentrations of organic compounds prevent oxygen being used for ammonia oxidation and autotrophic activity decreases due to a relative slow growth rate of nitrifiers. In the study of Ni et al. [21], in granule-based SBR supplied with a fatty acids-rich wastewater at COD of c.a. 800 mg/dm^3 the heterotrophs accounted for 61% of the total oxygen consumption of the reactor, while the autotrophs consumed only 39%. The relative activity of nitrifying population against heterotrophic population evolved until a balance between two populations was reached in the biomass [10]. In our research, the amount of oxygen used by microorganisms for ammonia oxidation was higher than the amount utilized during other processes requiring oxygen. The abundance of nitrifiers over heterotrophs can be particularly seen in the series with anaerobic digester supernatant as a sole substrate for activated sludge microorganisms. Effluents derived from digested municipal sewage sludge can contain up to 1000 mg N/dm^3, and they increase nitrogen loading in the influent of wastewater treatment plant even by 30% [22]. In our research, under the conditions of ammonia concentration in wastewater of ca 585 mg/dm^3, relatively high nitrification activity was observed suggesting that the biomass was rich with nitrifiers. At the F/M ratio of 0.02 and 0.05 g COD/(g TSS·d), relatively low autotrophic activity over heterotrophic activity could be caused by the competition between nitrifiers and heterotrophic bacteria that is supposed to proceed at higher dissolved oxygen concentration. Better aerobic conditions, caused by lower organic loading, may favour the development of heterotrophs with a higher growth rate and result in a decline in the number of autotrophs characterized by a slower growth rate [23]. The increase in the F/M ratio from 0.02 to 0.22 g COD/(g TSS·d) resulted in the growth of nitrification activity measured as the rate of oxygen uptake for ammonia oxidation ($SOUR_2$) to 36.7 mg O_2/(g VSS·h). It can be supposed that under these conditions autotrophic nitrifiers predominated. Further growth of the F/M ratio to 0.26 g COD/(g TSS·d) caused a decline of specific rate of oxygen used for ammonia oxidation to 19.5 mg O_2/(g VSS·h). It can be observed that in series 1–3 nitrification efficiency was stable at the level of about 97%. Under experimental feeding conditions, there was no inhibition of autotrophic ammonia oxidation that can be also confirmed by our unpublished data concerning the amount of ammonia-oxidizing bacteria (AOB) in activated sludge. In series 1–3, AOB content in the biomass was 2.6, 1.7 and 1.05%, respectively. By contrast, in series 4–6, nitrification effectiveness was on sig-

nificantly lower level of 16.1, 34.7 and 42.8%, respectively. Almost two-fold decrease in $SOUR_2$ in series 6, in comparison with series 5, went along with 1.2-fold increase in nitrification efficiency, which may suggest that under these conditions, heterotrophic bacteria were responsible for ammonia oxidation. The amount of AOB in activated sludge was merely on the level of 0.08–0.24% that can indicate the limitation of autotrophic nitrifiers' activity. Heterotrophic nitrification is considered as a process of significant participation in ammonia removal from wastewater at the presence of organic compounds [24].

5. CONCLUSIONS

Respiration measurements allowed the estimation of the rate of the biomass oxygen uptake at various environmental factors, particularly feeding conditions. Such evaluation can be useful in treating anaerobic digester supernatant that is characterized by lower biodegradability than municipal wastewater. Analysis of the oxygen uptake by activated sludge enables assessing the variables affecting physiological state of biomass, e.g. DO concentration and F/M ratio.

The measurement of respiration activity of activated sludge fed with anaerobic digester supernatant revealed that the rate of oxygen uptake for ammonia oxidation was higher than the other oxidation processes. In the range of the F/M ratio from 0.02 to 0.22 g COD/(g TSS·d), the specific oxygen uptake rate by autotrophic nitrifiers increased. At 0.26 g COD/(g TSS·d) SOUR of nitrifiers decreased almost twice. However, the efficiency of ammonia oxidation increased, suggesting nitrification carried out by nitrifiers other than autotrophic. Under the applied experimental conditions, the exogenous respiration rates indicate the weakened physiological state of heterotrophic bacteria probably resulted from low DO.

The research allowed the examination of the activity of key microorganisms in activated sludge: heterotrophs and autotrophic nitrifiers with the use of respirometric test. The results inform about the activity of biomass involved in technological systems treating wastewater with a low COD/N ratio under the conditions of low DO. The conducted method can be helpful in a full-scale wastewater treatment plant where the purification of reject supernatants in the main technological line is designed. Respirometric measurements can confirm the impact of additional flow of, i.e. anaerobic digester supernatant on the activated sludge condition, and can be helpful for designing technologies of anaerobic digester supernatant treatment.

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