



## Biochemical conversions and biomass morphology in a long-term operated SBR with aerobic granular sludge

Agnieszka Cydzik-Kwiatkowska<sup>a,\*</sup>, Irena Wojnowska-Baryła<sup>a</sup>, Marcin Szatkowski<sup>a</sup>,  
Lech Smoczyński<sup>b</sup>

<sup>a</sup>Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn, Słoneczna 45G,  
10-709 Olsztyn, Poland

Tel. +48 895234194; Fax: +48 985234131; email: agnieszka.cydzik@uwm.edu.pl

<sup>b</sup>Department of Chemistry, University of Warmia and Mazury in Olsztyn, Plac Łódzki 4, 10-957 Olsztyn, Poland

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### ABSTRACT

The experiment was carried on for about one year (995 cycles) in a constantly aerated, column sequencing batch reactor with granular sludge fed with a mixture of anaerobic sludge digester supernatant and synthetic wastewater. The research proved the usefulness of granular sludge technology for the removal of ammonium from highly concentrated ammonium streams with an unfavorable chemical oxygen demand (COD)/nitrogen (N) ratio. In the period of stable reactor performance, all ammonium in the influent was oxidized and nitrification–denitrification via nitrite occurred with an efficiency of 26%. The calculated carbon (COD) and N balances showed how the substances were distributed in the reactor under the conditions of low organic accessibility and high N load. Despite the low organic availability, intracellular storage occurred and the yield of polyhydroxybutyrate (PHB) was  $Y_{\text{PHB}} = 0.206 \text{ g COD/g COD}$ . Detailed characteristics of the biomass operating under conditions of low COD/N was performed and showed that the wastewater composition and reactor operational parameters promoted the growth of dense, smooth granules with an average fractal dimension of  $2.75 \pm 0.15$ , characterized by settling velocity and equivalent diameter of  $11.6 \pm 7.9 \text{ mm/s}$  and  $2.13 \pm 1.29 \text{ mm}$ , respectively. The presented results can be used for the modeling of reactors with granular sludge for treating wastewater with the very low COD/N ratio.

*Keywords:* Granular sludge; Anaerobic sludge digester supernatant; Partial nitrification; Granule morphology; Mass balance; Fractal dimension

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### 1. Introduction

The supernatant from the anaerobic digestion of sludge is a highly concentrated ammonium stream with an unfavorable chemical oxygen demand (COD)/nitrogen (N) ratio for N removal by denitrification. This supernatant is most often introduced to the biological section of wastewater treatment plant (WWTP) without pretreatment [1] leading to 15–20%

increase in the influent nitrogen load. The application of anaerobic sludge digester supernatant treatment in the side stream of the plant increases the possibility to adjust the operational parameters and more efficiently oxidize the nitrogen compounds. The use of aerobic granule technology in a side stream of WWTP is a promising solution since the granular biomass concentration is 2–3 times higher than in a typical activated sludge process. Additionally, the percentage of nitrifiers in the reactor is higher because of the long retention time of biomass and the lowered affinity of

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\*Corresponding author.

this group of micro-organisms to unfavorable environmental conditions owing to immobilization in the granule structure.

It is particularly advantageous to optimize the process so the oxidation of ammonium is only conducted to nitrite. In the structure of the granules, anoxic zones are created which promote the reduction of oxidized forms of nitrogen in denitrification. As a result, in the reactor nitrification-denitrification via nitrite (SBNR-shortcut biological nitrogen removal) may occur which lowers the energy consumption for aeration, decreases the amount of organics and alkalinity for denitrification, and reduces the excess sludge production [2,3]. From the standpoint of anaerobic sludge, digester supernatant treatment is especially valuable to reduce organic consumption in denitrification, because of the low COD/N ratio of this wastewater.

Aerobic granular sludge technology is intensively studied because of the potential of this biomass for different pollutant removals. Granular sludge formation is exploiting the natural ability of micro-organisms to self-aggregate and create dense microbial consortia. The process is affected by many factors, including the type of substrate, organic loading, intensity of aeration, and the production of extracellular polymeric substances (EPS) [4]. Aerobic granular sludge was used for the treatment of synthetic wastewater with high ammonium concentrations [5,6] and for the treatment of real wastewater with a high nitrogen loading but a high COD/N ratio at the same time [7]. The information on the application of this technology for real wastewater treatment with a very low COD/N ratio supported by detailed biomass characteristic is still limited.

The objective of the study was to investigate the carbon and nitrogen removal and mass balances during the long-term treatment of anaerobic sludge digester supernatant in SBR with aerobic granular sludge. Since morphology of aerobic granular sludge influences reactor performance and biomass settling ability, granule diameters and fractal dimensions were determined under the conditions of high nitrogen concentration and low COD/N ratio in wastewater.

## 2. Materials and methods

### 2.1. Wastewater characteristics

Anaerobic sludge digester supernatant was obtained from open fermentation basins of the Municipal WWTP in Olsztyn (Poland). The composition of the supernatant is given in Table 1. At the beginning

Table 1  
Composition of the anaerobic sludge digester supernatant

Parameter	Unit	Value
Total nitrogen	mg N <sub>tot</sub> /L	425
TKN	mg TKN/L	418
Ammonium nitrogen	mg N-NH <sub>4</sub> <sup>+</sup> /L	332
COD	mg/L	420
COD/N <sub>tot</sub>	–	1
alkalinity	mval/L	66

of the experiment, the supernatant was mixed with synthetic wastewater Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O (46.0 mg/L), NaCl (10.1 mg/L), KCl (4.7 mg/L), CaCl<sub>2</sub> (3.5 mg/L), MgSO<sub>4</sub>·7H<sub>2</sub>O (16.7 mg/L), FeCl<sub>3</sub>·6H<sub>2</sub>O, MnSO<sub>4</sub>·H<sub>2</sub>O, ZnSO<sub>4</sub>, and CuSO<sub>4</sub>·5H<sub>2</sub>O (0.2 mg/L). The participation of supernatant in the influent was gradually increased in order to adapt the biomass to a high ammonium concentration. In cycle 600, the influent was 100% comprised of supernatant and the total nitrogen (N<sub>tot</sub>) concentration in the influent reached 450 mg/L. Sodium acetate was added to the reactor and during the first 406 cycles, COD concentration in the influent was maintained at the level of 600 mg/L. In cycles 407–796, the COD concentration in the influent was increased to about 1,700 mg/L and from cycle 797, it was lowered back to about 600 mg/L. In order to maintain alkalinity, the theoretical NaHCO<sub>3</sub> required for nitrification was added to wastewater.

### 2.2. SBR operation

The experiment was carried on for about one year (995 cycles) in a sequencing batch reactor with a height of 1 m and a diameter of 100 mm. The working volume of the reactor was 3.25 L. As a seed sludge, granular sludge from a previous experiment was used [8]. The initial biomass concentration was about 1,600 mg volatile suspended solids (VSS)/L. The temperature in the SBR was 26 ± 1 °C, and the reaction was kept between 7–8 pH. The reactor was supplied with a constant air velocity of 3.75 L/min (superficial air velocity of 0.8 cm/s) by a perforated plate with 0.2 mm holes placed in the bottom of the reactor. A wastewater exchange ratio of 63% was employed. In each cycle, 2 L of fresh wastewater was introduced to the reactor. The SBR was operated in an 8 h cycle, with the following operating strategy: filling (2 min), aeration (471 min), settling (5 min), and decantation (2 min). The measurements of pollutant concentrations in the influent and effluent included: COD, N<sub>tot</sub>, total Kjeldahl nitrogen (TKN), ammonium nitrogen, nitrites,

and nitrates. The activated sludge was analyzed for total suspended solids (TSS), VSS, and sludge volume index (SVI). The analyses were performed according to APHA [9]. Polyhydroxyalkanoates were extracted from biomass, hydrolyzed, esterified to 3-hydroxyacyl methyl esters, and determined by gas chromatography using method described by Braunegg et al. [10] and Comeau et al. [11] with minor modification. An oxygen use for endogenous respiration was measured using OxiTop Control OC 110 (WTW, Weilheim, Germany) as described in Zielińska et al. [12]. Nitrification efficiency was calculated on the basis of ammonium removal, taking into consideration ammonium uptake for biomass synthesis. Biomass production was expressed as the observed coefficient of biomass production ( $Y_{obs}$ ). In cycle 990, the research was carried out to determine the COD removal rate, ammonia removal rate, and nitrification rate. In the working cycle of the reactor, periodic sampling and measurements of COD and nitrogen compounds were performed. In cycle 990, granules were investigated using the free settling test procedure as described in Cydzik-Kwiatkowska et al. [13]. In short, the granules were placed in a column filled with tap water and their settling was illuminated with photo flash lamp controlled by an electronic system ensuring flashes every 3 s. The digital camera (Olympus, resolution 10Mpic.) shutter was kept open for five flashes for every photo. During the experiment, 120 granules from 12 photos were examined. The equivalent diameter, granule radius, and settling velocity of granules were measured. According to the Stoke's law, density of granules, granule volume, granule mass, Reynolds number, and fractal dimension of granule were determined.

### 2.3. Calculation methods

All constants of reaction rates were determined based on the experimental data by nonlinear regression with the use of Statistica 7 (StatSoft, Tulsa, USA). The  $r$  values were expressed per unit of biomass. The concentration of free ammonia (FA) (mgN/L) and free nitrous acid (FNA) (mgN/L) was calculated as a function of pH, temperature and total ammonium nitrogen (TAN), for FA, or total nitrite (TNO<sub>2</sub>), for FNA [14].

## 3. Results and discussion

The research focused on anaerobic sludge digester supernatant treatment in constantly aerated SBR with granular sludge. At the beginning of the experiment,

the supernatant was mixed with synthetic wastewater, then the supernatant share was gradually increased and from cycle 600 only the supernatant was introduced to the reactor (Fig. 1(a)). The organic carbon in the form of sodium acetate was added to the influent. Initially, the COD concentration in the influent averaged 600 mg/L at VSS of 1,600 mg/L (Fig. 1), then it was increased to about 1,700 mg/L in order to increase the biomass concentration in the reactor. This resulted in a gradual growth of the biomass amount to 5,200 mg VSS/L in cycle 796. Until this moment, an accumulation of  $N_{tot}$  and lack of oxidized forms of nitrogen in the effluent was observed (Fig. 1(a)). Reduction of COD to the level of 600 mg/L in the 797 cycle stimulated ammonium oxidation, which resulted in a sharp decrease of TKN concentration in the effluent and simultaneously a sharp increase in  $N-NO_2^-$  concentration in the effluent (Fig. 1(a)). The concentration of nitrates was very low during the experiment and did not exceed 5 mg/L. The reduction

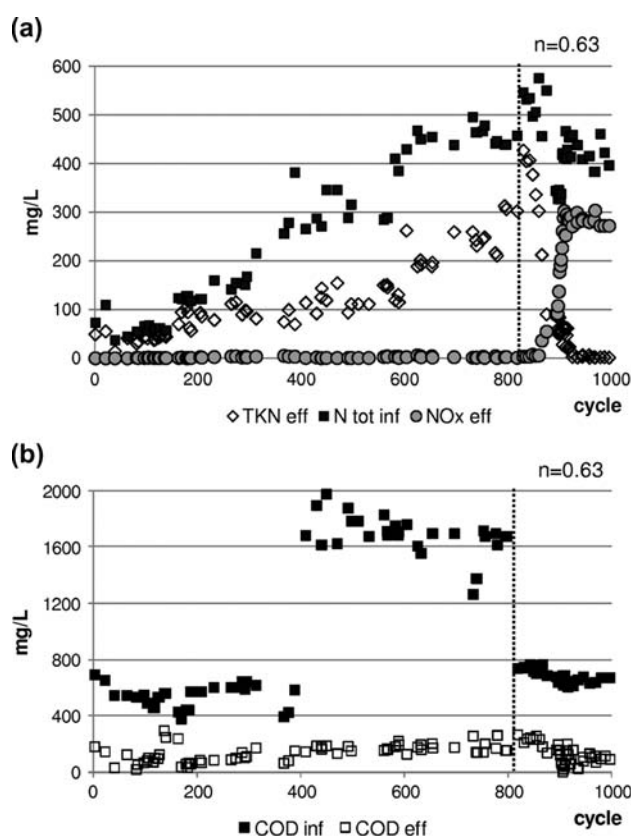


Fig. 1. Performance of the SBR: (a)  $N_{tot}$  in the influent (black squares) and TKN (white diamonds) and oxidized nitrogen forms (gray circles) in the effluent, (b) COD in the influent (black squares) and in the effluent (white squares). The vertical dotted lines highlight the moment of organic carbon load reduction and the beginning of effective ammonium oxidation in the reactor.

of COD amount in the influent resulted in a reduction of the biomass amount to the level of 2,900 mg VSS/L at which it remained until the end of the experiment (data not shown). The SVI of biomass did not exceed 50 mL/g TSS (Fig. 5) indicating that granular sludge dominated in the reactor throughout the experiment [4].

Stable nitrogen and carbon removal was noted during the last 100 cycles of the experiment (the stabilization period). The COD/N ratio of wastewater was very low and equaled 1.3. The reactor was operated at an organic carbon load at the beginning of the aeration phase ( $r_C$ ) of 0.36 g COD/(g VSS d), carbon removal efficiency of  $77 \pm 12\%$  was achieved. The COD removal constant was  $1.5 \text{ h}^{-1}$  and the COD removal rate was 260 mg COD/(g VSS h). The nitrogen load was high and equaled 0.216 g N/(g VSS d). The TKN removal efficiency in the reactor, calculated after taking into consideration N uptake for biomass synthesis, was  $94.5 \pm 7.4\%$ . The ammonium removal rate was  $16 \text{ mg N} - \text{NH}_4^+ / (\text{g VSS h})$ , the increase rate for oxidized forms of nitrogen ( $\text{NO}_x$ ) was  $10.1 \text{ mg N} - \text{NO}_x / (\text{g VSS h})$ . These results indicate very good conditions for nitrification. For the biomass operated in this substrate condition, i.e. under a low COD/N and a high nitrogen concentration in the influent, the coefficient of biomass production was low and equaled 0.29 g VSS/g COD.

In the stabilization period, nitrite accumulation rate (NAR), calculated as percentage of  $\text{N} - \text{NO}_2$  to  $\text{NO}_x$  in the effluent, was over 99% and the nitrification was considered as proceeding via nitrite [15]. The domination of nitrites as a nitrification product shows that ammonia oxidizing bacteria (AOB) activity exceeded the activity of nitrite oxidizing bacteria (NOB) which can be promoted by a number of factors such as low oxygen levels [16] or long sludge age [17]. In our research, the dissolved oxygen (DO) concentration was not limited, so this factor can be excluded; however, the granular biomass was characterized by sludge age of 27 days which promoted shortened nitrification. At higher temperatures, the growth rate of AOB is higher than NOB. In our experiment, the temperature of  $26^\circ\text{C}$  was applied. The applied temperature was only  $1^\circ\text{C}$  higher than the optimal for two-stage nitrification; however, in a long-term perspective, it may have favored the AOB growth over NOB. Nitrite oxidation is also inhibited by a high concentration of FA. The calculation of FA concentration indicated that in our research at the beginning of aeration phase, FA equaled 29.5 mg FA/L. According to the published data, the FA inhibition threshold is 10–150 mg FA/L and 0.1–4.0 mg FA/L for *Nitrosomonas* in phase I and *Nitrobacter* in phase II of nitrification, respectively

[18,19]. The FA concentration observed in the experiment is, therefore, higher than the inhibition threshold for *Nitrobacter* and lower than FA inhibition threshold for *Nitrosomonas*, most likely posing the main reason for nitrite accumulation in the effluent.

Yang et al. [20] reported that aerobic granules formed only when the FA concentration was lower than 23.5 mg/L. The presented research showed, however, that FA concentration up to 29.5 mg  $\text{N} - \text{NH}_4^+ - \text{N} / \text{L}$  still did not inhibit the granule formation and efficient nitrification in the reactor. This result can be explained by the very long period of reactor operation under high ammonium concentrations which enabled the adaptation of biomass to high FA concentrations.

Observation of changes of pollutant concentrations during the aeration cycle enabled carbon and nitrogen balances to be created. The nitrogen balance is shown in Fig. 2(a). The main nitrification products were nitrites, which comprised over 68% of the  $\text{N}_{\text{tot}}$  pool in the reactor (nitrates posed slightly over 1%). Nitrogen uptake for biomass synthesis was  $11.4 \text{ mg N}_{\text{tot}} / \text{L}$ , contributing to 4% of  $\text{N}_{\text{tot}}$  removed in SBR cycle. The nitrogen loss in the SBR cycle that not result from the biomass synthesis was taken as removed in denitrification. It can, therefore, be calculated that about 26% of  $\text{N}_{\text{tot}}$  was removed from wastewater as a result of simultaneous nitrification–denitrification (SND) activity in the three-dimensional structure of the granule. The efficiency of denitrification is strongly related to the granule diameter [21]. Since the granule average diameter was  $2.13 \pm 1.29 \text{ mm}$ —and oxygen concentration is limited at the depth of about  $600 \mu\text{m}$  [22]—in the interior of the granules, anoxic or anaerobic zones occurred which enabled nitrite reduction.

The COD balance is presented in Fig. 2(b). The use of oxygen for endogenous respiration designated in

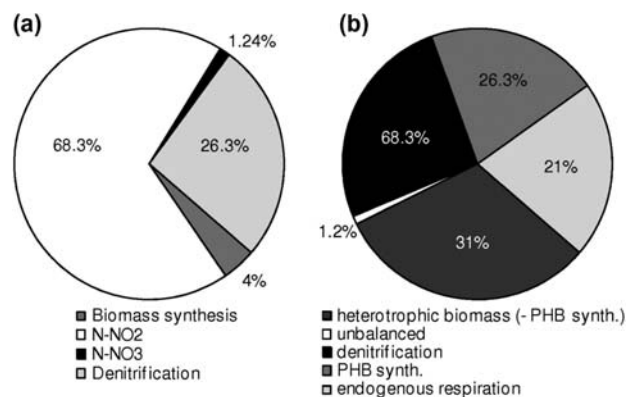


Fig. 2. The (a)  $\text{N}_{\text{tot}}$  and (b) COD balances in cycle 990 in the period of stable reactor performance.

respirometric measurements was 15 mg O<sub>2</sub>/(L cycle). Since the oxidation of 1 g of COD requires 1.42 mg O<sub>2</sub> [23], it was calculated that for endogenous respiration 84.5 mg COD/(L cycle) was used, which comprised about 21% of the entire pool of organics introduced with the influent.

In the calculation of oxygen consumption in denitrification, it was assumed that it proceeded from nitrites. The literature data [24] report that acetate consumption in denitrification is approximately 1.6 times lower if the acceptor of electrons is nitrite rather than nitrate. Assuming that the consumption of easily degradable carbon by activated sludge for denitrification is 2.3 g COD/g N–NO<sub>3</sub><sup>-</sup> [25], it was calculated that for the denitrification from nitrite granular sludge used approximately 1.4 g COD/g of NO<sub>2</sub><sup>-</sup>. At this assumption, about 26% of easily degradable COD introduced with wastewater has been used for SND in the granule structure.

The SND in the granule structure involves heterotroph and nitrifier activities. It can be stimulated by lowering the oxygen concentration in the reactor [26] or by the proper choice of organic carbon load. Nitrification and denitrification require different organic carbon conditions; nitrification is stimulated by depletion and denitrification by the presence of organic carbon in wastewater. It is, therefore, necessary to balance the organic load to enable nitrification and in the same time ensure a sufficient amount of COD for denitrification. For example, in research by Yang et al. [27], the increase in COD/N<sub>tot</sub> from 5.6 to 12.9 denitrification efficiency increased from 82.6 to 95.6%. In the mentioned study, the organic load and COD/N<sub>tot</sub> were significantly higher than this characterizing wastewater (0.36 g COD/(g VSS d), COD/N = 1.3). For efficient denitrification, the total organic carbon-to-N<sub>tot</sub> ratio (TOC/N<sub>tot</sub>) should theoretically be 2.86 [15], while in presented research it was only 0.39. It can be concluded that in our experiment, denitrification was limited by the organic carbon availability and for the improvement of N<sub>tot</sub> removal the amount of acetate in the influent should be increased. In this study, however, we concentrated on efficient ammonium oxidation, assuming that the denitrification can be easily achieved in the mainstream of the plant. From this perspective, the results are promising since excellent ammonium removal was observed from concentrated wastewater. Moreover, the 26% reduction of nitrogen content and the presence of nitrite as the main product of nitrification in the effluent would significantly improve the COD/N ratio in the mainstream of WWTP and reduce the COD requirements.

In this research, VSS equaled 0.65 TSS. With a yield coefficient for AOB of 0.21 g COD per g of

removed N [28] and the conversion factor of g COD/g VSS at the level of 1.5 [29], it was calculated that the AOB growth in the SBR cycle was 37.9 mg VSS/L. The analysis of VSS increase in SBR cycle showed that after taking into account the autotrophic growth, 52% of COD in the reactor was built into the heterotrophic biomass as polyhydroxybutyrate (PHB), EPS, or cells.

According to Zhang et al. [30], micro-organisms in granules do not use the whole organic carbon from wastewater because the cell divisions in mature granules occur with much lower frequency than in the activated sludge. The excess organics are stored or used for EPS synthesis, which is exploited as an alternative energy source in a famine period [31]. According to Simon et al. [32], EPS in granular sludge may comprise from 40 to 70% of whole organic fraction, while in activated sludge it comprises no more than 20–30% [33]. Since the EPS production is positively correlated to organic carbon load [30], it can, therefore, be assumed that in the tested reactor under the conditions of low organics availability, expressed by the COD/N<sub>tot</sub> of 1.3 and organic carbon load at the beginning of the aeration phase of 0.36 g COD/(g VSS d), EPS fraction in the biomass was relatively low.

Measurements of the amount of PHB, both in the form of 3-hydroxyvaleric acid (3-HV) and 3-hydroxybutyric acid (3-HB), in biomass have shown that about 21% of the total pool of COD has been stored in the form of PHB. In calculating the amount of PHB to COD, the conversion factors of 1.38 mg COD/mg and 1.63 mg 3HB COD/mg of 3HV, as given by Dionisi et al. [34], were adopted. The obtained result shows that despite the very low organic load, the polymer storage occurred in the granule structure. The determined value of Y<sub>PHB</sub> was 0.206 g COD/g COD. A slightly higher value was reported for granular sludge by Ni and Yu [35]; however, the coefficient given by the authors refers to all the substances stored (Y<sub>STO</sub>), while in the presented research we focused on the PHB only. Approximately, 1% of the incoming COD was not balanced.

In cycle 990, an analysis of the physical properties of granular sludge was carried out by the free settling test in order to determine the granule morphology at a COD/N<sub>tot</sub> ratio of 1.3 and at a high concentration of ammonium nitrogen in the influent. A microscopic image of the granule as well as an example photograph taken during the free settling test procedure are shown in Fig. 3(a) and 3(b), respectively. The granule structure was densely packed with micro-organisms, the granules possessed a clear outer shape and exhibited excellent settling ability with a SVI of 35 ± 10 mL/g TSS. Granules with the diameters below

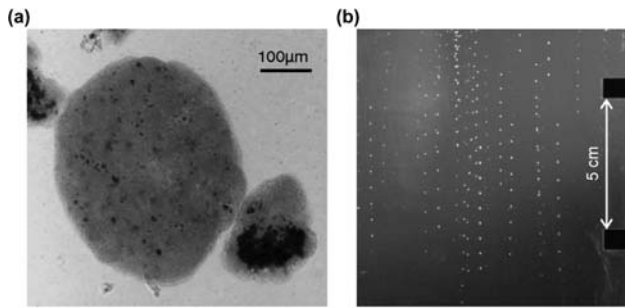


Fig. 3. A microscopic image of the granule (a) and an exemplary photograph taken during the free settling test procedure (b).

1 mm, and in the ranges from 1 to 2 mm, from 2 to 4 mm, and over 4 mm comprised 21, 30, 30, and 19% of all granules, respectively.

Based on the free settling test, the physical properties of granular sludge were measured [36]. The relationships between the obtained parameters are presented as supplementary material and discussed in detailed below. In general, biomass growth and the average granule diameter depend on the nitrite-to-organic carbon ratio in the feeding. The increase in organic loading induces changes in the physical properties of granules, particularly in the gradual growth of their diameter. Liu et al. [37] showed that the increase in volumetric load rate from 3 to 9 kg COD/(m<sup>3</sup>d) resulted in an increase in the average granule diameter from 1.6 to 1.9 mm. With increasing organic load, the stability of granule structure deteriorates since high accessibility of organic substrate promotes an intensive microbial growth which lowers the density of the three-dimensional structure and granule mechanical stability [38]. In our experiment, the range of granule diameters in the period of stable reactor operation varied from 0.21 to 6.6 mm. The granule equivalent diameter averaged  $2.13 \pm 1.29$  mm and was within the range of 1–3 mm reported as optimal for granular sludge [22].

It was observed that the size of the three-dimensional structure of the granules translated to settling velocity. A high settling velocity allows a higher amount of biomass to remain in the reactor after the settling phase which, in the case of granular sludge cultivation, is usually very short (up to 5 min). The average settling velocity of granules was  $11.6 \pm 7.9$  mm/s. The range of settling velocities was from 0.47 mm/s to 28 mm/s and was comparable with the value ranges (0.38–3.21 mm/s) reported for granular sludge in the literature [39]. The correlation between the diameter and settling velocity had a linear character and an increase in diameter by 1 mm resulted in an increase in settling velocity by 5 mm/s.

Granules with a diameter below 1 mm were characterized by a high density, reaching up to 30 mg/mL. With the increasing diameter, the granule density decreased and for the granules with diameters over 4 mm, it stabilized at 2 mg/mL. The results show that the highest micro-organism concentration in the granule structure was obtained in small granules with diameters no higher than 1 mm. The processes occurring in the reactor, i.e. nitrification and SND, promote the high density of granule because nitrifiers create tightly packed consortia in biomass and during nitrification electron acceptors are produced which favor the bacteria growth inside the granules [21].

An increase in granule diameter translated to a mass increase of granules. The average mass of granules was  $0.038 \pm 0.044$  mg. On average, an increase in granule diameter of 1 mm resulted in a biomass increase by 0.03 mg. The settling velocity increased with increasing mass.

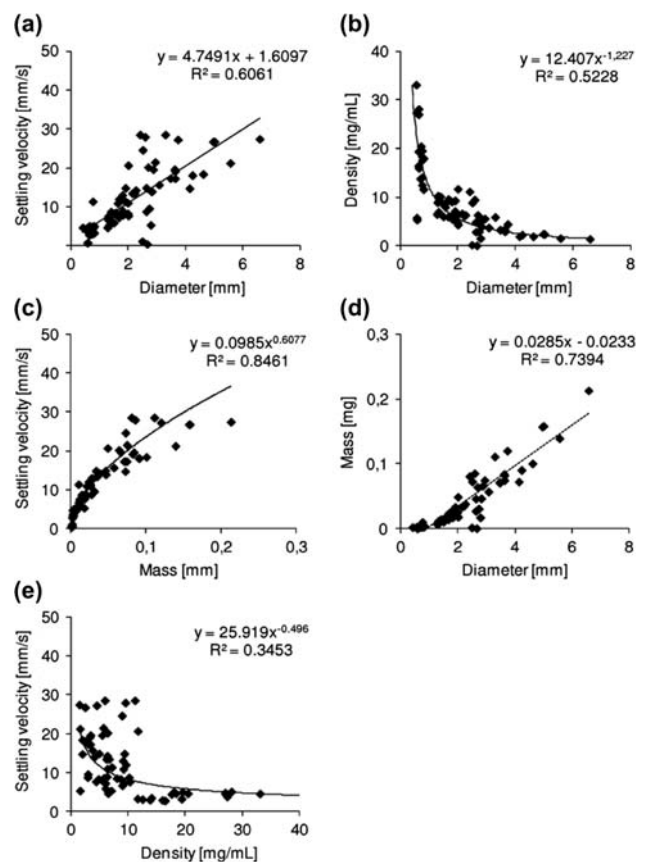


Fig. 4. The relationships between (a) diameter and settling velocity, (b) diameter and density, (c) mass and settling velocity, (d) mass and diameter, and (e) density and settling velocity observed for aerobic granular sludge at a low COD/N ratio of 1.3 and a high nitrogen loading of 0.216 g N/(g VSS d).

An analysis of the relations between granule density and settling velocity showed that for granules with a density in the range of 0.022 to 10 mg/mL, the settling velocity varied from 5 to about 30 mm/s. For granules with densities over 10 mg/mL, the settling velocity stabilized at the level of 5 mm/s. In general, the dense structure of granules favors their quick settling. The slight opposite tendency observed in our experiment may have resulted from the fact that the main factors determining the settling velocity were mass and diameter of the granules (Fig. 4(a) and (c)) and both of them were negatively correlated to granule density (data not shown, Fig. 4(b), respectively). The values of Reynolds number ( $Re$ ) calculated for the granules in the experiment varied in the range from 0.36 to 146.5 with an average of  $26.4 \pm 11.5$ , indicating that the granule sedimentation in the reactor had a laminar character ( $Re$  value below 2,300).

The last investigated parameter was the fractal dimension of the granule ( $D$ ). The value of the fractal dimension depends on the way the microbial consortia are packed in the granule. The fractal dimension of an object is a quantitative measure of how the primary particles occupy the floc interior space. It allows the mechanism of bioaggregate formation and their structural properties to be defined. According to Jiang and Logan [40],  $D$  values close to 1.5 suggest a loose and porous biomass structure, while values of about 2.25 indicate a dense structure of the biomass. Cydzik-Kwiatkowska et al. [13] showed that the fractal dimension of granules feeding on glycerine from biodiesel production was very low i.e. 1.46 and the granules possessed a loose structure with many filamentous micro-organisms on the granule surface. Xiao et al. [39] observed that at a low pH of 3, the granules possessed a mushroom-like loose structure and the fractal dimension was  $2.23 \pm 0.06$ . At a pH of 8.1, the granules possessed a dense bacterial structure with a

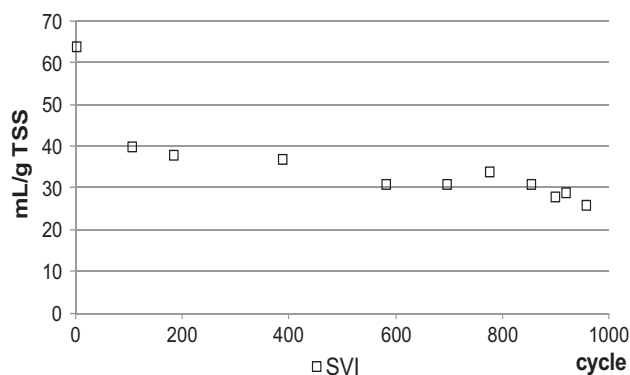


Fig. 5. Changes of SVI of granular sludge during the experiment.

fractal dimension of  $2.42 \pm 0.07$ . The average fractal dimension in the presented research was  $2.75 \pm 0.15$  and it was the highest in comparison with the cited studies. It indicates a very dense packing of micro-organisms in granule structures at low COD/ $N_{tot}$  which may be related to the high amount of nitrifiers in the total bacteria population.

#### 4. Conclusions

Aerobic granular sludge technology is developing dynamically; however, the use of the technology in technical-scale objects requires further studies into the efficiency of biochemical processes in the granule structure and granule morphology under different operational conditions. This study analyzed the carbon and nitrogen balances and aerobic granular sludge morphology during the treatment of anaerobic sludge digester supernatant with a very low COD/ $N$  ratio of 1.3 and a high nitrogen loading of 0.216 g N/(g VSS d) in SBR.

The ammonia oxidation in the reactor was initiated by a sharp reduction in the concentration of organics in the influent from about 1,700 to 600 mg COD/L. The main nitrification product was nitrite and the N removal proceeded via SBNR with an efficiency of 26%. The yield of PHB under the conditions of low organics' availability was  $Y_{PHB} = 0.206$  g COD/g COD. Under the operational conditions applied, granules densely packed with micro-organisms and characterized by a high settling velocity ( $11.6 \pm 7.9$  mm/s) were obtained in the reactor. The fractal dimension of granular sludge was very high ( $2.75 \pm 0.15$ ), which suggests a smooth granule structure.

The results show that aerobic granular sludge technology can be successfully used for ammonium nitrogen removal from anaerobic sludge digester supernatant, enabling a one-fourth reduction in nitrogen content and partial nitrification which reduces the COD requirements for denitrification in further stages of the treatment.

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