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Nitrogen Removal with Aerobic Granules – Effect of Dissolved Oxygen and Carbon/Nitrogen Ratio

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Abstract
Nitrogen removal efficiency related to the dissolved oxygen (DO) concentration (DO 1–7 mg l^{-1}), carbon/nitrogen ratio (COD/N 1–14), and the effect of airflow (0.4–2.9 l min^{-1}) related to the granule size were investigated. The average chemical oxygen demand (COD) removal of ≥ 90 % was achieved at COD/N ≥ 11, but the satisfactory N values in effluent for discharge into the surface waters were almost achieved at COD/N 14. DO of 2 mg l^{-1} is recommended for efficient removal of N and COD with mature granules. The size (diameter) of the granules decreases with increased airflow.

Keywords
Aerobic granules, COD removal, N removal, sodium acetate, synthetic wastewater

1 Introduction

Aerobic granular sludge technology is a promising technology for the treatment of municipal and industrial wastewater. For the long-term stable operation of biological wastewater treatment plants in terms of the nutrients removal efficiency, microorganisms' growth and composition, and for the integrity and stability of the granules, the appropriate chemical oxygen demand/nitrogen (COD/N) ratio is important.[1–5] The ratio COD/N 7.5 is reported as favourable with respect to the COD and N removal efficiency, as well as granule stability.[6] Lower COD/N ratios, such as 3.3 and lower, are beneficial for nitrifiers, while higher COD/N ratios, such as 20, are beneficial for heterotrophs.[5–7] Granule disintegration during long-term bioreactor operation is associated with low COD/N, typical for municipal wastewater, such as COD/N 1,[8] COD/N 2.5,[9] COD/N 5.[6,7] Within the granules, dense and compact structures of activated sludge there exist dissolved oxygen (DO) and substrate gradient. Therefore, aerobic processes such as degradation of organics and nitrification take place on the surface of the granule, while anoxic processes such as denitrification occur inside the granule (simultaneous nitrification and denitrification).[10,11] The structure of the aerobic granule is assessed by granule size, morphology, and microscopic observations.[12]

The intensity of aeration is an important factor of activated sludge granulation, and has a dual role: it provides DO and hydraulic shear force.[13–15] Additionally, the intensity and duration of hydraulic shear forces on granules lead to changes in pore structure, microbial species distribution, and in microbial metabolism, morphology, and granule size.[13] The stability of the aerobic granule, and the efficiency of COD and N removal are both affected by DO concentration.[10,16] Saturated DO concentration is often used to cultivate stable aerobic granules,[17] and at moderate DO concentration (40 % oxygen saturation), the granules disintegrate and stable granulation is not achieved.[18] Nevertheless, the combination of conditions favourable to the selection of slow-growing microorganisms (such as nitrifiers), and low DO concentrations has led to dense aerobic granules.[19] Aerobic granules were grown at an air upflow velocity of 1.0 cm s^{-1} with DO concentration in the range of 1.8–4.2 mg l^{-1},[10] at superficial upflow air velocity of 0.58 cm s^{-1} and 0.14 cm s^{-1}, where the DO concentration was 5.5–6.6 mg l^{-1} and 4.8 mg l^{-1}, respectively,[16] superficial upflow air velocity 0.8 cm s^{-1}, 1.6 cm s^{-1}, 2.4 cm s^{-1} and 3.2 cm s^{-1}.[12] The combination of low DO concentration and higher shear forces has not shown to be successful for aerobic granule formation.[20] *Sturm and Irvine*[21] point out that the DO concentration is more important for the formation of aerobic granules than shear forces, because aerobic granulation cannot be achieved at DO < 5 mg l^{-1}, and aerobic granules disintegrate into flocs at air velocities < 1 cm s^{-1}. In contrast, *Liu and Tay*[22], at the same superficial air velocity, grew granules that had good stability over 151 days. Higher shear forces cause greater hydrophobicity of the cell surface, higher amounts of extracellular polymeric substances (EPS), and smaller, denser, and more compact granules.[20,23]

The appropriate ratio of COD/N and DO concentration are still a challenge for the aerobic granular technology for the efficiency of COD and N removal, as well as for granule stability.

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Therefore, the aim of this study was to investigate the effect of COD/N ratio and DO on COD and N removal efficiency, as well as the effect of airflow on granule size.

2 Experimental

2.1 Wastewater

The synthetic wastewater was prepared by dissolving the salts in tap water of following composition: 2 g l^{-1} $(NH_4)_2SO_4$, 1 g l^{-1} KH_2PO_4, 0.4 g l^{-1} $FeSO_4$, 0.5 g l^{-1} $MgSO_4$, 0.4 g l^{-1} NaCl, 1 g l^{-1} $MgCO_3$, 1 g l^{-1} $CaCO_3$, and the trace element solution was added (0.3 ml l^{-1}): 1.5 g l^{-1} $FeCl_3 \cdot 6H_2O$, 0.15 g l^{-1} H_3BO_3, 0.03 g l^{-1} $CuSO_4 \cdot 5H_2O$, 0.18 g l^{-1} KI, 0.12 g l^{-1} $MnCl_2 \cdot 4H_2O$, 0.06 g l^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.12 g l^{-1} $ZnSO_4 \cdot 7H_2O$, 0.15 g l^{-1} $CoCl_2 \cdot 6H_2O$, 10 g l^{-1} EDTA.[24] Sodium acetate was used as the carbon source.

2.2 Reactor set-up and aerobic granules

The experiments were performed in a lab-scale sequencing batch reactor (SBR) of 2 l working volume. Fine air bubbles for aeration were induced with compressor (HIBLOW HP 40; Hagen Model40 A-10011) over an air sparger located at the bottom of the reactor. The volumetric exchange ratio was set to 50 %. A cycle time of 4 h was maintained with 5 min of filling, aeration time of 220 min, 10 min of settling, and 5 min of discharging. The hydraulic retention time was 8 h. The experiments were conducted successively, with an increase in the COD/N ratio, and for selected COD/N ratios 1, 5, 9, 11, and 14, the initial COD was 50 ± 5 mg l^{-1}, 250 ± 10 mg COD/L, 450 ± 10 mg COD/L, 550 ± 10 mg COD/L, and 700 ± 10 mg COD/L. The SBR was equipped with probes for DO concentration, pH value and temperature monitoring, WTW Multi 3420 SET KS1, Germany. The pH value was in the range of 7–8, controlled with HCl and NaOH. The experiments were performed at room temperature. The airflow was measured by air flow meter.

The mature granules in concentration of 3.5 g l^{-1} as mixed liquor suspended solids (MLSS) were used from the parental reactor, which was operating in steady state for 2 months.

2.3 Design of experiments

The experiments were divided into two parts.

The first part of the experiment involved investigation of COD/N ratios 1, 5, 9, 11, and 14 in relation to: (i) removal efficiency of COD and N, and (ii) MLSS dynamics.

The experiments were performed in the SBR reactor at 2 mg DO l^{-1}. The initial NH_4-N concentration was set at 50 ± 5 mg NH_4-N l^{-1}. At the start of the experiments, all N in the influent was in NH_4-N form. Each COD/N ratio was maintained for 25 days.

The second part of the experiment involved investigation of DO concentration (airflow) in relation to: (i) removal efficiency of COD and N, and (ii) size of granules.

This series of experiments was conducted at 280 mg COD l^{-1} and 20 mg NH_4-N l^{-1}, at COD/N ratio 14 in four identical SBR reactors at airflow rate of 0.4 l min^{-1}, 0.8 l min^{-1}, 1.7 l min^{-1} and 2.9 l min^{-1}, which corresponded to the DO concentration 1 mg l^{-1}, 2 mg l^{-1}, 4 mg l^{-1}, and 7 mg l^{-1}, respectively. After 25 days of the experiments at each air flow (DO concentration), the removal efficiency of COD and N was determined, as well as the diameter of the granules.

2.4 Analytics

Before analysis, the mixed liquor (ML) samples were filtrated through filter paper of 0.45 µm. COD, total N, NH_4-N, NO_3-N and NO_2-N were determined with Merck cuvette kits (Merck, Germany), analogous to the Standard Methods.[25] Spectrophotometric measurements were performed on spectrophotometer Spectroquant VEGA 400 Merck, Germany. The MLSS was determined according to Standard Methods.[25] The particle size was determined via light microscope (Carl Zeiss Jena) and stage micrometer. For each sampling, 30 ± 2 granules were used and the average value was calculated.

3 Results and discussion

3.1 COD/N ratio and removal efficiency of COD and N

The effects of COD/N ratio on COD and N removal efficiency are summarised in Fig. 1. With the increase in COD/N ratio from COD/N 1 to COD/N 14, the average COD removal efficiency increased from 57 to 91 %, and the average COD removal ≥ 90 % was achieved at the ratio of COD/N ≥ 11, and at the same time, the average N removal efficiency increased from 40 to 69 %. The effluent COD concentration was lower than 100 mg COD l^{-1} regardless of the COD/N ratio (Fig. 1a) – the requirements for the effluent quality of the discharge into surface waters had been met.[26] The microbial activity for COD removal was attributed to the activity and dominance of the heterotrophic bacteria. These results (Fig. 1a) are in agreement with *Kim et al.*,[7] *Kim and Ahn*,[9] and *Kocaturk and Erguder*.[6]

In a study conducted under anoxic-aerobic regime with acetic acid as the carbon source, *Kocaturk and Erguder*[6] reported COD removal efficiency of 63–79 % at ratios COD/N 1–7.5, efficiency of 76–90 % at COD/N 10, and at COD/N 20 and 30 the efficiency of COD removal of 94 % and 93 %, respectively. The higher efficiency of COD removal with increasing COD/N ratio is connected to the activity and dominance of heterotrophic bacteria. However, towards the end of the experiment, at ratio COD/N 30, the decrease in COD removal efficiency from 95 to 87 % was recorded, due to the excessive filament growth, which deteriorated the structural integrity of the granules.[6] A similar trend of increasing COD removal efficiency with increasing COD/N ratio was reported by *Kim and Ahn*[9] in their experiments under aerobic regime with acetate as the carbon source. At COD/N ratios of 10, 7.5, 5, and 2.5, they achieved COD removal efficiencies of 93.7, 90.8, 82.4, and 70.7 %, respectively. COD removal efficiencies of 78.9, 86.7, 92.7, and 95.9 % were recorded in exper-

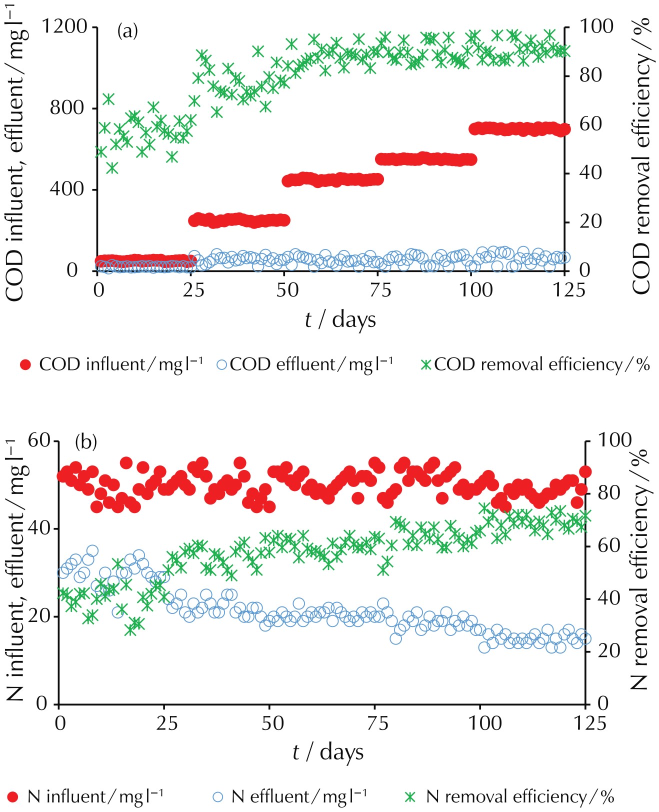

Fig. 1 – Dynamics of: (a) COD, and (b) N concentration, in influent, effluent, and removal efficiency at ratios COD/N 1–14

Slika 1 – Dinamika koncentracije: (a) KPK i (b) N, u influentu, efluentu i učinkovitost uklanjanja pri omjerima KPK/N 1–14

iments under aerobic regime with acetate as the carbon source, at ratios of COD/N 5, 10, 15, and 20.[7] However, in experiments with high MLSS concentration, such as 8 g MLSS l^{-1}, the high COD removal efficiency of 89.4–95.6 % was recorded at C/N ratio lower than 2.[27]

Kim et al.[7] showed that the microorganisms' diversity decreased with the increase in the COD/N ratio from 5 to 20, and the increase in COD/N ratio had a direct effect on the stability of aerobic granules. *Zhao et al.*[28] reported that the gradual dominance of heterotrophic bacteria occurred with the increase in COD/N ratio, thus, decreasing the number and diversity of microorganisms. *Kim et al.*[7] determined by pyrosequencing that the share of genus *Thauera* in the community of aerobic granular sludge increased from an initial 0.01 % at COD/N ratio of 5 to 63.7 % at COD/N ratio of 20. These authors suggest that, due to the high share of *Thauera* in the aerobic granular sludge, the increase in COD removal efficiency occurred and denitrification efficiency was improved. Also EPS content increase as COD/N ratio raise from 5 to 20. The genus *Thauera* contributes to the EPS production, formation and stability of the aerobic granular sludge[29], and to the aerobic denitrification.[30]

The results of N removal (Fig. 1b) show that the average N removal efficiency increased from 40 to 69 % with the increase in COD/N ratio from 1 to 14.

The average effluent N concentrations were satisfactory for discharge into the public sewage system at all investigated COD/N ratios, while the satisfactory values for effluent discharge into surface waters of < 15 mg N l^{-1} [26] were almost achieved with COD/N ratio of 14, with average effluent N concentration of 15 mg N l^{-1}, in the range of 13 and 17 mg N l^{-1} (Fig. 1b). The results of N removal (Fig. 1b) are in agreement with *Kim et al.*[7] who achieved N removals of 57.5, 61.6, 69.6, and 79.1 % (aerobic regime) at COD/N ratios of 5, 10, 15 and 20, respectively, as well as with *Kim and Ahn*[9] who recorded N removals of 72.3, 65.3, 61.7, and 52.3 % (aerobic regime) at COD/N ratios of 10, 7.5, 5, and 2.5, respectively.

The three possible reasons for the increases in N removal efficiencies with increasing COD/N ratio are: (i) denitrification during reactor fill due to sufficient organic matter, (ii) simultaneous nitrification and denitrification, and (iii) the dominance of aerobic or facultative anaerobes, as stated by *Kim et al.*[7]

Similar to our results, *Luo et al.*[8] recorded a decrease in the rate and efficiency of nitrification when the COD/N ratio decreased from 4 to 1, attributing it to the significant shift in the microbial community and the decrease in the EPS, which led to the decrease in the sedimentation, size, and physical strength of the aerobic granules. The reason being the higher amount of ammonia-oxidising bacteria (*Nitrosomonas*), while the amount of nitrite-oxidising bacteria (*Nitrospira* and *Nitrobacter*) decreased at COD/N ratio of 1 due to the growth inhibition by free ammonia (FA), and due to the decreased sludge retention time (SRT).[8] The toxic effect of FA as well as unfavourable pH for nitrification and denitrification have been reported by *Kocaturk and Erguder*.[6] They point out that the increase in COD/N ratio from 7.5 to 30 had a detrimental effect on the ammonia removal, and explain that the cause was: (i) the pH increase above 8.6, which is suitable neither for nitrification nor for denitrification, and (ii) the toxic effect of FA. Due to the long-term high pH and inhibitory effect of FA, the nitrifiers were washed out at high COD/N ratios.[6] Different results of N removal efficiency compared to the results achieved in this study are probably due to conducting experiments under anoxic-aerobic regime. They achieved 75 % ammonia removal at COD/N ratio 1, at COD/N ratios 2–7.5 they achieved > 90 % ammonia removal, and with further increase in COD/N ratio, the ammonia removal efficiency decreased to 37 % at COD/N ratio of 30. The N removal efficiency at COD/N ratios 10–30 was 17–18 %, at COD/N ratio of 1 it was 26 %, and at COD/N ratios 2–7.5 it was in the range 35–54 %.[6]

In the experiments of this study, conducted under aerobic conditions at pH value maintained in the range of 7–8, which is beneficial for nitrification and denitrification, the average N removal efficiency did not exceed 69–% (Fig. 1b). In addition, FA concentration was maintained below the inhibition threshold for nitrifiers due to the favourable pH value.

Yuan et al.[10] emphasised the importance of the anaerobic phase (60 min) during the reactor fill phase as it significantly contributed to the nitrogen removal (91.7 %), to the clear spherical granular shape, as well as to the compact granule structure compared to the short anaerobic phase (5 min) during reactor filling, when 58.8 % removal of N was achieved, and the granules were irregular, fluffy, and loose in structure.

Simultaneous nitrification and denitrification was probably the reason for the N removal (Figs. 1a and 1b), as suggested by *Yuan et al*.[10] and *Kim et al*.[7] Oxidation of ammonia to nitrite and/or nitrate occurred on the surface of the granule, and denitrification in the inner layers of the granule.[10,11] Lower COD/N ratios are more favourable for nitrifier proliferation, so the nitrification took place; however, at lower COD/N ratios, the amount of organics was insufficient for denitrification, therefore, lower net N removal was achieved (Figs. 1a and 1b). Higher COD/N ratios are more beneficial for heterotroph proliferation. Therefore, although sufficient organics for denitrification were ensured at higher COD/N ratios, lower N removal values were achieved (Figs. 1a and 1b), because the heterotrophs were more competitive for DO.[5−7] Also, the genus *Thauera* was probably present in aerobic granules, which contributed to the removal of nitrogen by aerobic denitrification.[30]

3.2 MLSS change in the dependence of the COD/N ratio

The biomass concentration in the bioreactor depends, among others, on the COD/N ratio and the type of carbon source.[6−10,31] The disintegration of the granules, and thus the wash-out of the biomass from the bioreactor, has been observed at low COD/N ratios, and the authors *Kim et al*.[7] and *Kocaturk and Erguder*[6] reported COD/N ratio 5, *Kim and Ahn*[9] the COD/N ratio 2.5, and *Luo et al*.[8] COD/N ratio 1.

The dynamics of the MLSS concentration variation during the change of COD/N ratio over 125 days is shown in Fig. 2. The ratio COD/N 1 was unfavourable for the maintenance of the aerobic granules, and a decrease in the MLSS concentration was recorded in this period. During the period of the experiment at ratio COD/N 5, the stagnation of the MLSS concentration was recorded, and at COD/N ratios 9 to 14, the MLSS concentration increased. After 125 days of the experiment, the biomass concentration in the bioreactor amounted to 3.1 g MLSS l^{-1}, and the net biomass concentration decreased by 0.4 g l^{-1} (Fig. 2). In addition, low COD and N removal efficiencies were observed at COD/N ratio of 1 (Figs. 1a and 1b), and the additional reason for the low efficiency can be attributed to the concentration of microbial biomass (Fig. 2). The results in this article are in agreement with *Kim et al.*,[7] *Kim and Ahn*,[9] and *Kocaturk* and *Erguder*.[6]

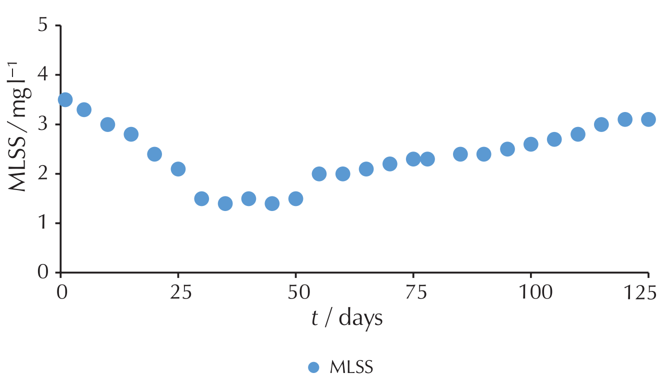

Fig. 2 – MLSS variations over 125 days related to the COD/N ratio increase
Slika 2 – Varijacije MLSS-a tijekom 125 dana povezane s porastom omjera KPK/N

The decrease in biomass concentration as well as the disintegration of the granules at C/N 5 due to the insufficient concentration of organics, and the sludge wash out from the reactor was pointed out by *Kim et al*.[7] in their experiments with the mature granules, with acetate as the carbon source at C/N ratios 5, 10, 15, and 20. They suggest that the C/N ratio greater than 5 is necessary for granule maintenance.[7] *Kocaturk* and *Erguder*[6] suggest that granule fragmentation occurs due to the lack of organics (low COD/N ratio) leading to the reduced concentration gradient between the outer and inner part of the granule, and due to the diffuse COD transport to the inner part of the granule containing denitrifiers. Degradation of the denitrifiers after the starvation period causes fragmentation by forming voids in the granule structure. As the denitrifiers are removed from the inside of the granule, the size of the granule decreases, and smaller and denser granules are formed. The reduction in granule size also occurs due to the decrease in the amount of aerobic heterotrophs, due to the lower amount of available organics (lower COD/N ratio).[6]

Kim and Ahn[9] recorded the increase in biomass concentration at higher C/N ratios (ratios C/N 10 and 7.5), and biomass loss at lower ratios (C/N 5 and 2.5) in experiments with acetate as the carbon source when conducting experiments with mature granules at ratio C/N 10, 7.5, 5, and 2.5, the experiments started with 2.87 g MLSS l^{-1}. A similar trend was recorded by *Kocaturk* and *Erguder*[6], who conducted experiments with acetic acid as the carbon source and mature granules at ratios COD/N 7.5, 10, 20, and 30, and at COD/N ratios 7.5, 5, 3.5, 2, and 1. At COD/N ratios 7.5 and higher, the biomass concentration generally increased, and at COD/N ratios of 5 or lower, granule disintegration and biomass wash-out from the bioreactor occurred. However, at the COD/N ratio 10, the formation of fluffy granules and disturbance of the granule shape occurred due to ammonia toxicity and high pH values. At the COD/N ratio 20, the granules became even fluffier, and at COD/N ratio 30, proliferation of filamentous organisms was observed.[6] *Yuan et al.*[10] recorded an increase in biomass concentration with an increase in COD/N ratio.

3.3 Effect of dissolved oxygen concentration on the organics and nitrogen removal efficiency from wastewater

Hydrodynamic shear forces induced by aeration have a significant effect on the removal efficiency in the nitrification and denitrification processes. Adequate aeration ensures high ammonia oxidation efficiency, and reduced hydrodynamic shear forces can result in unsatisfactory nitrification efficiency and contribute to denitrification.[4] The effect of DO concentration (air flow) on mature granules and their removal efficiency of COD and N was investigated and the results are shown in Fig. 3a.

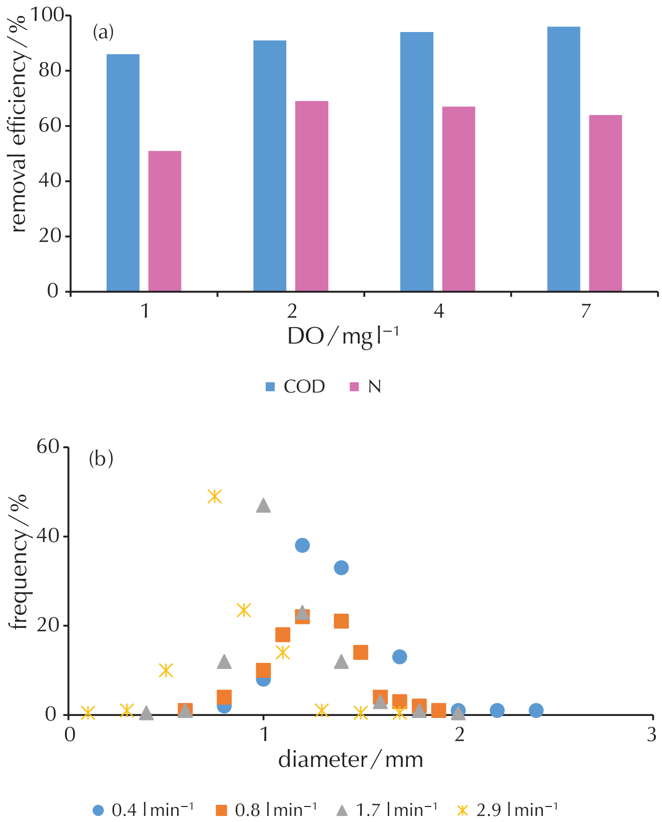

Fig. 3 – DO effect on COD and N removal efficiency (a), and granule frequency and diameter at airflows 0.4 l min^{-1}, 0.8 l min^{-1}, 1.7 l min^{-1}, and 2.9 l min^{-1} (b)

Slika 3 – Učinak DO-a na učinkovitost uklanjanja KPK i N (a) i frekvencija i dijametar granula pri protoku zraka 0,4 l min^{-1}, 0,8 l min^{-1}, 1,7 l min^{-1} i 2,9 l min^{-1} (b)

Despite the higher efficiency of COD removal at concentration of DO > 2 mg l^{-1}, there is actually very little difference in the efficiency of COD removal at DO 2 mg l^{-1} and at DO > 2 mg l^{-1} (Fig. 3a), while the increase in DO concentration disrupts N removal efficiency, and the difference in energy consumption/savings is significant,[16] since energy consumption for aeration represents 40–60 % of total energy costs at a biological wastewater treatment plant.[32] High DO concentration favours heterotrophs and high values of COD removal efficiency (> 90 %) were achieved at DO concentration ≥ 2 mg l^{-1} and airflow ≥ 0.8 l min^{-1} (Fig. 3a). High concentration of DO also favours nitrifiers, for the oxidation of ammonia to nitrate. However, due to the high concentration of DO, some of the oxygen also passed into the granules and reduced the anoxic zone. Due to the reduced anoxic zone (increased aerobic zone) of the granule, despite sufficient concentration of organics for denitrification of the accumulated nitrates, denitrification was inhibited by high concentrations of DO, resulting in reduced N removal efficiency at concentrations DO > 2 mg l^{-1}. The results in this article (Fig. 3a) are in agreement with *Yuan et al.*[10] who, at COD/N ratio 6, at DO concentrations 1.5 mg l^{-1}, 2.5 mg l^{-1}, 4.6 mg l^{-1} and 6.3 mg l^{-1} (airflows 0.5 l min^{-1}, 1 l min^{-1}, 2 l min^{-1} and 3 l min^{-1}) achieved NH_4-N removal ≥ 93.4 %, and TN removal ≥ 92.2 %, as well as with *He et al.*[33]

Higher values of COD removal efficiency (above 97 %) and NH_4-N removal efficiencies (96 and 92 %) were achieved by *Gao et al.*,[16] at COD/N ratio of about 13 with glucose as the carbon source under aerobic regime, at DO concentrations of 5.5–6.8 mg DO l^{-1}, and 4.8 mg DO l^{-1}. They point out that, in mature granules, the intensity of aeration had no effect on the removal of COD, and the higher intensity of aeration favours the removal of NH_4-N. No granule disintegration was observed at both aeration intensities (5.5–6.8 mg DO l^{-1} and 4.8 mg DO l^{-1}), and the granules had similar morphological structures, clear contours without filament outgrowths, but different sizes.[16] *He et al.*[13], at COD/N ratio 4, at aeration intensity of 1.5 l min^{-1}, 0.9 l min^{-1} and 0.6 l min^{-1} in combination of 90, 120, and 150 min, under anaerobic-aerobic-anoxic configuration, achieved reliable and stable removal of COD and NH_4-N regardless of aeration intensity and aeration time. However, the intensity and duration of aeration affected residual nitrogen. Moderate aeration intensity along with aeration time favoured the best performance of the system, as well as other granule characteristics.[13]

Higher values of N removal efficiency compared to the results achieved in this article (Fig. 3a) were achieved in the study by *He et al.*[13] since the anaerobic-aerobic-anoxic configuration is more favourable for the removal of N.

He et al.[33] conducted the experiments with acetate as the carbon source, under anaerobic-aerobic-anoxic regime, at COD/N ratio 10 at aeration rate 4 l min^{-1}, superficial gas velocity 0.59 cm s^{-1}, corresponding to the DO concentration of 7–8 mg l^{-1}, and shortened the length of the aerobic period (120, 90, and 60 min). They point out that aerobic granules can maintain integrity and stability during long-term operation at high-intensity aeration and different duration of aeration, and shorter aeration time favoured biomass retention, better sedimentation and higher EPS production. Effective removal of organics was achieved, as well as removal of N with shortened aeration time. Aeration time shaped the diversity of the bacterial community, its composition, and distribution of functional groups for the removal of organics and N. They recorded the efficiency of COD removal at all three studied aeration times > 94 % and NH_4-N > 97 %, but also different NO_3-N values in effluent. The reduction in the duration of aeration led to a reduction in NO_3-N in the effluent, and the removal efficiencies of N were 64.29, 71.81, and 86.18 %.[33]

Franca et al.[34] point out that high concentrations of DO are needed to sustain the stability of aerobic granules and to avoid oxygen restriction in aerobic granules. DO concentration and air flow intensity need to be optimised to reduce energy utilisation in full-scale aerobic granule systems, and to achieve effective N removal. Currently, there are no specific values for minimum aeration rate or DO concentration for the operation of the aerobic granule system, since the oxygen demand in the presence of high substrate concentration depends on particular factors, like the type of substrate and loading, aerobic granule size, biomass concentration, and microbial community inside the aerobic granule.[34]

3.4 Size and frequency of granules at different airflows

The COD/N ratio and the airflow rate are factors that affect granule size and stability. As the COD/N ratio increases, the granule size increases, and as airflow increases, the granule size decreases.[4,6,13,33,34] The purpose of these experiments was to investigate the effect of airflow on the size of the granules. The obtained results showed a trend of decreasing granule diameter with increasing airflow (Fig. 3b). The airflow affected granule size, and for each examined airflow in the bioreactor, granules of different diameters were found, and the share of each granule size was directly dependent on the airflow. With increasing airflow, the curve of granule frequency and granule diameter changed its shape, from a rounded central part at airflows of 0.4 $l\,min^{-1}$ and 0.8 $l\,min^{-1}$, to a pointed peak of the curve at airflows of 1.7 $l\,min^{-1}$ and 2.9 $l\,min^{-1}$. The diameter of the granules decreased from 1.2–1.4 mm (71 % granules) at airflows of 0.4 $l\,min^{-1}$ to 0.75 mm (49 % granules) at 2.9 $l\,min^{-1}$ (Fig. 3b). The results obtained in this study (Fig. 3b) are in agreement with *He et al.*[13], but in contrast with *Yuan et al.*[10] who believe that acetate as carbon source and anaerobic feeding mode are necessary to achieve compact granules (when the experiments were conducted with aerobic feeding mode, the granules were loose – mostly larger than 2 mm or less than 0.5 mm; larger granules began to disintegrate and smaller ones were washed out). Suitable hydrodynamic shear forces need to be provided to form strong and compact granules, and low shear forces result in porous and weak granules.[35] As the granule size increases, substrate removal becomes limited by transport, and organisms compete for space and substrate within the granule, so the complex internal structure of the granule and granule size have an important effect on mass transfer process and harshly affect aerobic granule stability.[36] The size of the aerobic granule is the significant link between the macroscopic properties of aerobic granular sludge and its microstructure.[31] In aerobic granules at the depth of 0.8–0.9 mm from the surface, anaerobic zones containing dead microbes have been observed, while aerobic granules of size less than 0.6 mm are completely composed of living microbes.[23,37] In the outer layers of the aerobic granule, rapid oxygen consumption occurs[36], and in granules larger than 0.5 mm, the concentration of DO in the inner layers is limited.[38] *Liu et al.*[39] point out that, in granules bigger than 0.7 mm, diffusion is the limiting circumstance for removal of substrate. However, the more compact formation of small aerobic granules suggests that large molecules could not enter their pores.[36] *Long et al.*[31] point out that 2–3 mm granules are favourable for maintaining the stability of aerobic granular sludge in the pilot plant, and emphasise the importance of maintaining optimal granule size by controlling sludge age with selective sludge discharge. In order to maintain long-term operational stability and good sedimentation during low-strength wastewater treatment, the aerobic granule size of less than 1 mm is recommended.[37,40] Since large-scale SBR reactors that operate at high organic loading rate would involve very high shear forces to maintain aerobic granules of this size, which would lead to high energy consumption, it is recommended that aerobic granules be in the range of 1–3 mm.[37,41] *Franca et al.*[34] indicate that the optimal size of the aerobic granule depends on the composition of the wastewater, and on the different operating and physical conditions of the reactor. *Chen et al.*[12] reported that shear forces of 2.4 $cm\,s^{-1}$ (airflow rate 3 $l\,min^{-1}$) and 3.2 $cm\,s^{-1}$ (airflow rate 4 $l\,min^{-1}$) resulted in robust granules with the potential for long-term operation, the granules had a clear external morphology, were compact and dense, and with good performance. Shear forces of 0.8 $cm\,s^{-1}$ (airflow rate 1 $l\,min^{-1}$) and 1.6 $cm\,s^{-1}$ (airflow rate 2 $l\,min^{-1}$) disrupted the obtained granules to large filamentous granules of irregular shape, loose structure, operational instability, and poor performance.[12] *He et al.*[13] achieved the densest granules at an aeration intensity of 0.9 $l\,min^{-1}$ for 90 min, of the investigated combinations of 1.5 $l\,min^{-1}$, 0.9 $l\,min^{-1}$ and 0.6 $l\,min^{-1}$ for 90, 120, and 150 min, and in combination 1.5 $l\,min^{-1}$ during 150 min a loss of biomass was recorded. *Gao et al.*[16] indicate that lower aeration intensity (4.8 mg DO l^{-1}) results in larger and looser granules, and higher aeration intensity (5.5–6.8 mg DO l^{-1}) results in smaller and more compact granules, and explain: (i) due to the hydraulic properties, the stronger intensity of aeration might induce stronger friction between granules and liquid/bubble, and more common collisions and attrition among granules, and limit the formation of large granules, and (ii) stronger intensity of aeration leads to greater DO concentration and prolonged starvation period, resulting in longer endogenous respiration and lower growth rate of microorganisms, which reduce the aerobic granules size.[16]

4 Conclusion

The COD/N ratio 14 was almost satisfactory for the achievement of N removal for the discharge into surface waters, while the average COD removal of ≥ 90 % was achieved at COD/N ratio ≥ 11. With DO concentration increase, the COD removal efficiency increased; however, N removal efficiency increased with increasing DO concentration from 1 $mg\,l^{-1}$ to 2 $mg\,l^{-1}$, and with further DO concentration increase, N removal efficiency decreased. Therefore, the recommended DO concentration for mature granule, considering COD and N removal efficiency, is 2 mg DO l^{-1}. With increasing airflow, the diameter of the granules decreased from 1.2–1.4 mm (71 % granules) at airflows of 0.4 $l\,min^{-1}$ to 0.75 mm (49 % granules) at 2.9 $l\,min^{-1}$.

List of abbreviations
Popis kratica

COD – chemical oxygen demand
KPK – kemijska potrošnja kisika

N – nitrogen
– dušik

DO – dissolved oxygen
– otopljeni kisik

SBR – sequencing batch reactor
– šaržni reaktor koji radi u slijedu

SRT – sludge retention time
– vrijeme zadržavanja mulja

EPS – extracellular polymeric substances
– ekstracelularne polimerne tvari

MLSS – mixed liquor suspended solids
– suspendirane čestice miješane tekućine

ML – mixed liquor
– miješana tekućina

FA – free ammonia
– slobodni amonijak

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SAŽETAK

Uklanjanje dušika pomoću aerobnih granula – učinak otopljenog kisika i omjera ugljik/dušik

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U ovom radu istražena je učinkovitost uklanjanja dušika povezana s koncentracijom otopljenog kisika (engl. *dissolved oxygen*, DO) (DO 1 – 7 mg l^{-1}) i omjerom ugljik/dušik (KPK/N 1–14) te učinak protoka zraka (0,4 – 2,9 l min^{-1}) povezan s veličinom granula. Prosječno smanjenje vrijednosti organske tvari izražene preko kemijske potrošnje kisika (KPK) od ≥ 90 % postignuto je pri KPK/N ≥ 11, a zadovoljavajuće vrijednosti N u efluentu za ispuštanje u površinske vode gotovo su postignute pri KPK/N 14. DO od 2 mg l^{-1} preporučuje se za učinkovito uklanjanje N i organske tvari izražene preko KPK vrijednosti sa zrelim granulama. Veličina (promjer) granula smanjuje se s povećanjem protoka zraka.

Ključne riječi
Aerobne granule, uklanjanje organske tvari, N uklanjanje, natrijev acetat, sintetska otpadna voda

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