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Carbon Removal by Activated Sludge under Fully Aerobic Conditions at Different COD/N Ratio

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Abstract

The aim of the experiment was to analyze the particular processes (biomass synthesis, oxidation, denitrification and accumulation) during carbon removal from wastewater. The experiment was carried out in a sequencing batch reactor (SBR) in two series with different organic carbon compounds to nitrogen compounds ratio (COD/N). Organic carbon loading of activated sludge was 0.07 g COD/g·d.

Organic carbon removal efficiency was higher at COD/N ratio 13 (98%), at COD/N ratio 3 (85%). Depending on COD/N ratio in waste, the processes of biomass synthesis, denitrification and intracellular accumulation changed. At low COD/N ratio biomass synthesis decreased; however, there was an increase in carbon used for denitrification and accumulation. It was observed that under fully aerobic conditions the activated sludge was able to store reserved materials expressed as poly(-β-hydroxybutyrate) PHB. When the lack of carbon source in waste was observed, the activated sludge used endogenous carbon resources as poly(-β-hydroxybutyrate) as electrons donor. Depending on activated sludge nitrogen loading, different times of endogenous denitrification were observed. In the first series the denitrification with endogenous carbon source was from the 8th hour in the second series during only 2 initial hours of the experiment. Biomass cellular respiration was on the level of 16% in both series.

Keywords: municipal wastewater, poly(-β-hydroxybutyrate), accumulation, biomass synthesis, readily biodegradable organic compounds

Introduction

Biomass synthesis, oxidation, denitrification and accumulation are the elementary processes that take place in activated sludge during organic compounds (COD) removal. These particular mechanisms depend on environmental conditions. DIONISI et al. [1] confirmed this information for the strain *Thiosphaera pantotropha*. The authors indicated that if there were a large amount of organic compounds in the environment (e.g. in the form of acetate) the microorganisms would be able to oxidize about 29% of COD, accumulate 57% in the form of poly(-β-hydroxybutyrate) (PHB) and 14% would be used for biomass synthesis. Thus, under unsteady state conditions oxidation and synthesis are not the primary processes in organic compounds removal.

Dissolved and colloidal pollutants adsorbed on the surface of zoogleal agglomeration are directly oxidized by microorganisms to CO_2 and H_2O. These compounds are then used for biomass synthesis. The rate of organic compounds removal by activated sludge results mainly from the intensity of new cell synthesis.

Barak et al. [2] showed that under aerobic conditions the activated sludge was capable of denitrifying. The source of organic compounds depending on the phase of denitrification can be organic compounds delivered with wastewater or accumulated intracellularly. Under famine conditions, when lack of organic compounds delivered

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with wastewater is observed, microorganisms can use the reserved carbon substrate as poly(-β-hydroxybutyrate) as the endogenous carbon and energy source [3, 4].

The amount of stored polymer in the form of poly(-β-hydroxybutyrate) depends on both individual microorganism features and culture conditions. According to Sun et al. [5] the carbon source and the C/N ratio in the medium mainly influence poly(-β-hydroxybutyrate) synthesis efficiency of bacteria. The authors observed that with the increase in the C/N ratio in the environment from 6 to 20 the quantity of synthesized PHB at *Azospirillum brasilense* Sp7 increased.

The growth of microorganisms that are able to accumulate the reserved substances (like poly(-β-hydroxybutyrate)), takes place under unsteady nutrient conditions. It is generally observed when one of the major factors, needed for the growth (nitrogen, phosphorus, sulphur, magnesium, oxygen), is limited in the environment by simultaneous presence of the excess carbon source [4, 6, 7, 8]. Carta et al. [9] have noticed that the substantial concentration of acetate in the medium caused an increase in the poly(-β-hydroxybutyrate) synthesis rate.

Most research has documented the ability of activated sludge to poly(-β-hydroxybutyrate) synthesis under anoxic conditions. However, Beun et al. [10] claimed that under aerobic and anoxic conditions, at sludge age of 8 days, about 70% of acetate was used for poly(-β-hydroxybutyrate) synthesis and the rest for biomass yield.

Literature findings have widely reported that research connected with the possibility of poly(-β-hydroxybutyrate) used as a potential carbon source for denitrification are still in the initial phase. By the time investigations concerned the technological conditions improvement for this polymer production. The authors of the presented study tried to determine the influence of poly(-β-hydroxybutyrate) accumulation on the N-oxidized reduction.

The aim of the study was to analyze the use of organic compounds by activated sludge for the particular processes under constant oxygen supplying level of 2 mg/dm^3 in the reactor. The contribution of the endogenous carbon source for denitrification under fully aerobic conditions was estimated in dependence on the COD/N ratio in wastewater.

Experimental Procedures

Organisation of Experiment

Research was carried out in a sequencing batch reactor Bioflo 3000 at a volume of 5 l. The activated sludge concentration was 3000 mg/dm^3. A stable (50%) volumetric exchange rate was carried out. The reactor cycle lasted 24 hours (Fig. 1). The operation phases were the following: filling phase, when municipal wastewater with additional carbon (CH_3COOH) and nitrogen (NH_4Cl) sources was supplied to the reactor. The reaction time phase that controlled oxygen supply (DO in the reactor was at 2 mg/dm^3) was used to investigate reactor performance. The last two phases were: sedimentation and decantation.

The experiment was carried out in two series. The acetate addition to municipal wastewater caused an increase in activated sludge loading by 200 mg COD/dm^3. The wastewater contained different amounts of ammonium. Organic carbon and nitrogen compound content of wastewater is presented in Table 1.

In both series the activated sludge was adapted to the technological conditions until the stabilization of the chemical parameters in the effluent was obtained. Kinetic research was conducted at the end of each technological series.

Analytical Methods

The influent and the effluent were assayed for the concentration of the following: organic compounds, expressed as COD (PN-74/C-04578), ammonium by the Nesslerization method after distillation and by colorimetric method (PN-73/C-04576/01, 02), nitrite and nitrate by colorimetric method (PN-73/C-04576/06, 08), total Kjel-

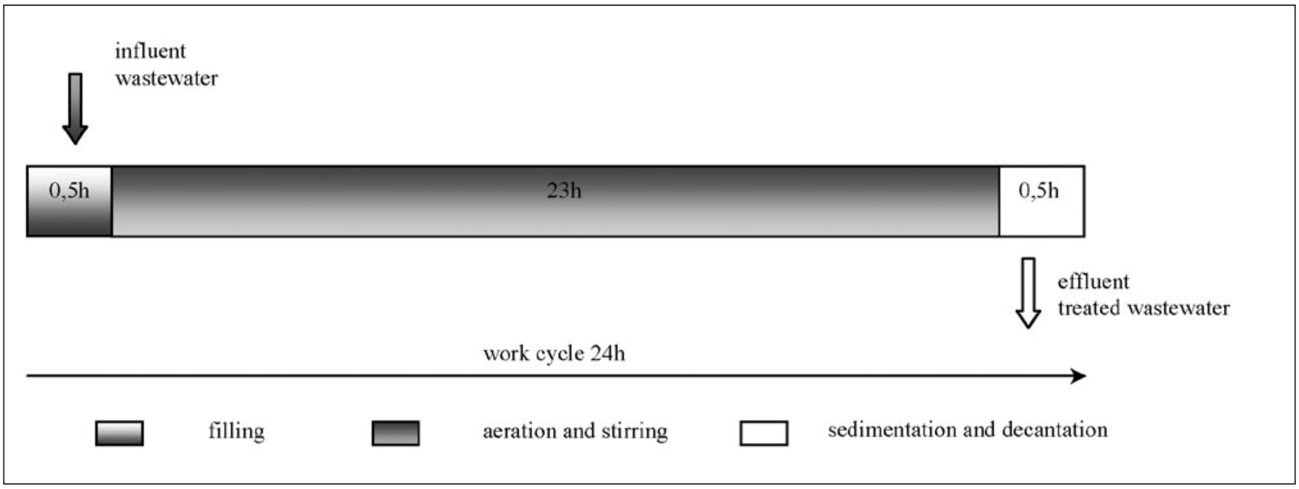

Fig. 1. Scheme of the reactor cycle.

Table 1. The amount of nitrogen and carbon compound concentrations in wastes.

Series	COD [mg/dm^3]	TKN [mg/dm^3]	N-NH$_4$ [mg/dm^3]	COD/N
I	400	30	25	13
II	400	110	100	3

dahl nitrogen (PN-73/C-04576/12) and suspended solids by drying at 103-105°C (PN-73/C-04559).

Organic substances oxidizing activity of activated sludge was calculated using respirometric method in OxiTop.

Calculation Methods

The theoretical amount of N-oxidized forms in the reactor (N-NO$_x$) was calculated using the following formula:

$$N - NO_x = TKN_i - TKN_e - N_s$$

where:
TKN$_i$ - total nitrogen concentration in the influent [mg/dm^3],
TKN$_e$ - total nitrogen concentration in the effluent [mg/dm^3],
N$_s$ - nitrogen concentration used for biomass synthesis as a product of biomass yield, organic compounds removed [mg/dm^3] and nitrogen contents in the biomass (0.05 g N/g).

Organic compounds accumulated (COD$_a$) in activated sludge were calculated using the following formula:

$$COD_a = COD_r - (COD_o + COD_s + COD_d)$$

where:
COD$_r$ - concentration of organic compounds removed [mg/dm^3],
COD$_o$ - concentration of oxidized organic carbon compounds estimated on the basis of respirometric measurement [mg/dm^3],
COD$_s$ - organic compounds concentration used for biomass synthesis as a product of biomass yield and organic compounds removed [mg/dm^3],
COD$_d$ - organic compound concentrations used for denitrification were estimated on the assumption that for the reduction of 1 mg nitrate (N-NO$_3$) microorganisms use 4.8 mg organic compounds, expressed as COD [mg/dm^3].

Organic carbon left (COD$_l$) in the reactor was calculated using the following formula:

$$COD_l = COD_r - (COD_o + COD_s)$$

Two main COD (C$_{COD}$) fractions in wastewater: soluble and particulate were defined according to Barnard et al. [11].

Concentration of the particulate fraction (C$_{p,COD}$) was calculated using the following formula:

$$C_{p,COD} = C_{COD} - C_{s,COD}$$

C$_{COD}$ - organic compounds concentration [mg/dm^3],
C$_{s,COD}$ - soluble fraction of organic compounds concentration after filtration [mg/dm^3].

Concentration of slowly or non-biodegradable particulate fraction (C$_{ps,COD}$) was calculated using the following formula:

$$C_{ps,COD} = C_{COD} \cdot 0.05$$

0.05 - coefficient estimated by Barnard et al. [11].

Concentration of biodegradable particulate fraction (C$_{pb,COD}$) was calculated using the following formula:

$$C_{pb,COD} = C_{COD} - C_{ps,COD}$$

Concentration of slowly or non-biodegradable soluble fraction (C$_{ss,COD}$) was calculated using the following formula:

$$C_{ss,COD} = C_{COD} \cdot 0.03$$

0.03 - coefficient estimated by Barnard et al. [11].

Concentration of very readily biodegradable soluble fraction (C$_{svrb,COD}$) was calculated using the following formula:

$$C_{svrb,COD} = \frac{VFA \cdot 645}{1000}$$

VFA - volatile fatty acids concentration in wastewater (CH$_3$COOH) [mg/dm^3],
645 - 1 g/dm^3 of acetic acid corresponds to 645 mg COD/dm^3.

Concentration of readily biodegradable soluble fraction (C$_{srb,COD}$) was calculated using the following formula:

$$C_{srb,COD} = C_{s,COD} - C_{srv,COD} - C_{ss,COD}$$

Poly(-β-hydroxybutyrate) (PHB) was determined with black sudan [12].

Results and Discussion

Acetic acid was added to the municipal wastewater to increase the very readily biodegradable organic compound concentrations. This addition caused a 30% increase in those compound concentrations.

Soluble fraction that made up about 81% of total organic compounds predominated in wastewater, whereas particulate fraction was approximately 19% (Table 2).

Table 2. Organic carbon fractions in wastes.

Organic fractions (C_{COD})		Contribution in percentage terms
Soluble ($C_{s,COD}$)	very readily biodegradable ($C_{svrb,COD}$)	30 %
	readily biodegradable ($C_{srb,COD}$)	50 %
	slowly or non-biodegradable ($C_{ss,COD}$)	1 %
Particulate ($C_{p,COD}$)	biodegradable ($C_{pb,COD}$)	14 %
	slowly or non-biodegradable ($C_{ps,COD}$)	5 %

Table 3. Technological parameters of the series.

Series	Sludge age [d]	COD/N in the reactor	r_C [g/g•d]	r_N [g/g•d]	Y [g/g]
I	15	10	0.06	0.006	0.75
II	15	2.5	0.07	0.27	0.30

It is assumed that all readily biodegradable organic substrates are first taken up and stored by the heterotrophic biomass [9]. These bioassimilable substances enhance the poly(-β-hydroxybutyrate) storage.

Organic compounds sludge loading was similar in both series – on average 0.07 g COD/g·d. The addition of ammonium to the wastewater changed the COD/N ratio from 13 in series I to 3 in series II. Research was carried out in two series that differed in the nitrogen loading of activated sludge. At the activated sludge loading of 0.006 g N/g·d in series I the value of the biomass yield (Y) was 0.75 g/g. With the increase in the activated sludge loading of 0.027 g N/g·d the decrease in the biomass yield (Y) to the value of 0.3 g/g was observed. The reactor was operating at a constant sludge age of 15 days. The other parameters of both series are shown in Table 3.

The organic compounds concentration, expressed as COD was about 10 mg/dm^3 in the effluent of series I, whereas series II was on the level of 60 mg/dm^3. In series II a decrease in carbon removal efficiency from 98% to 85% was observed.

In the presented experiment it was shown that at a different COD/N ratio in wastewater, diversified contributions of elementary processes concerning organic compounds removal was observed. The increase in nitrogen loading of activated sludge and the decrease in COD/N ratio caused a decrease in the organic compounds used for biomass synthesis. In the consequence of biomass synthesis higher carbon removal efficiency was achieved in series I (52%) in contrary to the second series (21%). The decrease in sludge biomass production during the complex organic removal could result from the monovalent cations of NH_4^+ presence in supplied wastewater. Novak [13] has indicated that lower biomass production was caused by the increase in ammonium ions concentration in wastewater. These studies showed that an increase in

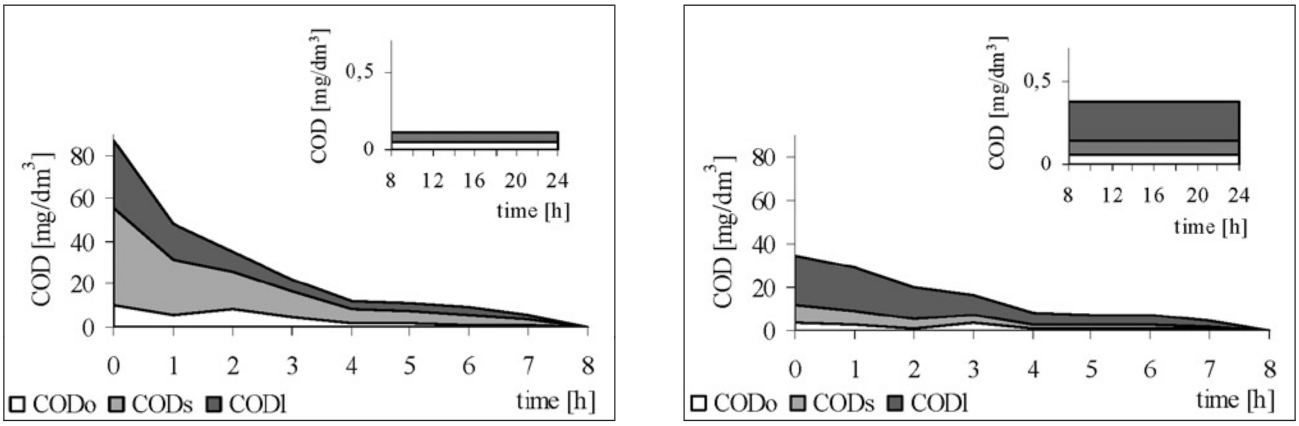

Fig. 2. The amount of organic carbon concentration that was left (COD_l) and was used for oxidation and biomass synthesis a) series I, b) series II.

the ammonium concentration would lead settling properties of activated sludge to deteriorate. When the monovalent-to-divalent cation ratio on a charge–equivalent basis was increased from 2.4 to 4.7 by ammonium, the interfacial settling velocity decreased.

Organic compounds oxidation was on the same level of approximately 16% in both series. From Fig. 2 it may be concluded that the amount of organic compounds used by activated sludge for biomass synthesis and oxidation depend on the COD/N ratio in wastes. Additionally, Fig. 2 shows the amount of organic carbon concentration left (COD_l) in the reactor during reaction time that was not used for biomass synthesis and oxidation. From the 7th hour of the reaction in series I the lack of the organic compounds left in the reactor that could be used for accumulation or for nitrate reduction was observed. In the second series organic carbon left in the reactor was still present during reaction period and it could be used for carbon storage and denitrification (Fig. 2).

Under the conditions of constant oxygen supplying on the level of 2 mg/dm^3, N-oxidized forms in the reactor ($N\text{-}NO_2$, $N\text{-}NO_3$) was lower than it could be achieved after nitrification - $N\text{-}NO_x$ (the amount of $N\text{-}NO_x$ was estimated on the assumption that denitrification did not occur in activated sludge). The difference between N-oxidized concentration ($N\text{-}NO_x$) and nitrite and nitrate concentration ($N\text{-}NO_2$, $N\text{-}NO_3$) during reaction time in the reactor indicated that denitrification in activated sludge took place (Fig. 3). Nitrogen removal efficiency in series I was 75%. In series in which ammonium was added to municipal wastewater nitrogen removal efficiency was on the level of 30%.

The accessibility of organic compounds for denitrification in series with added acetic acid and ammonium was analyzed. The results indicated that during 7 hours of the reaction time at the COD/N ratio 13 in wastewater in series I, carbon source for denitrification was carbon left in the reactor. From the 8th hour of the experiment organic compounds that could be used as electron donors for the N-oxidized reduction were not observed. Those compounds were used up earlier.

In series II at the COD/N ratio 3, with the exception of 2 initial hours of the experiment, activated sludge was able to use organic compounds that were present in waste-

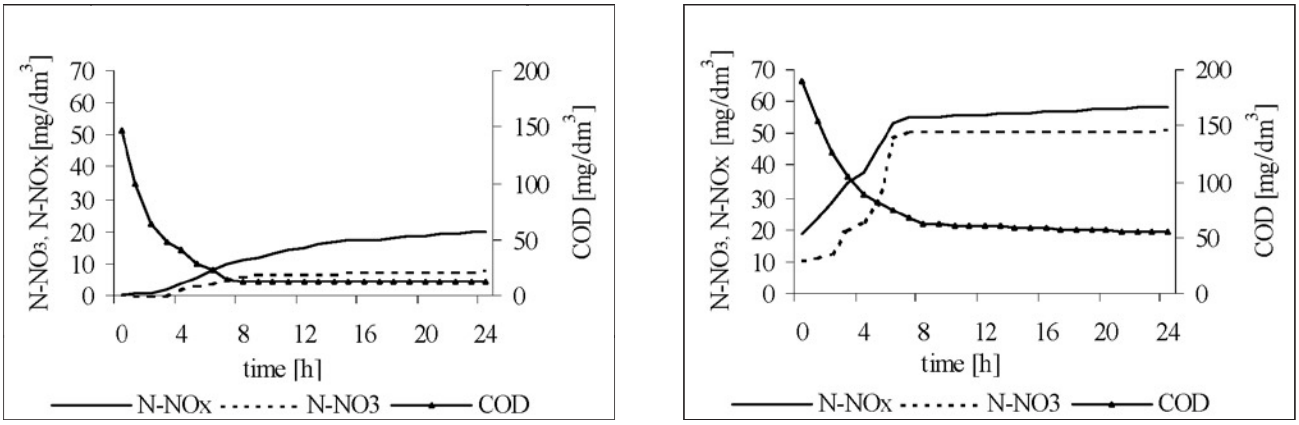

Fig. 3. Change of nitrite ($N\text{-}NO_2$) and nitrate ($N\text{-}NO_3$) concentration in the reactor, estimated N-oxidized ($N\text{-}NO_x$) concentration and organic concentration (COD) a) series I, b) series II.

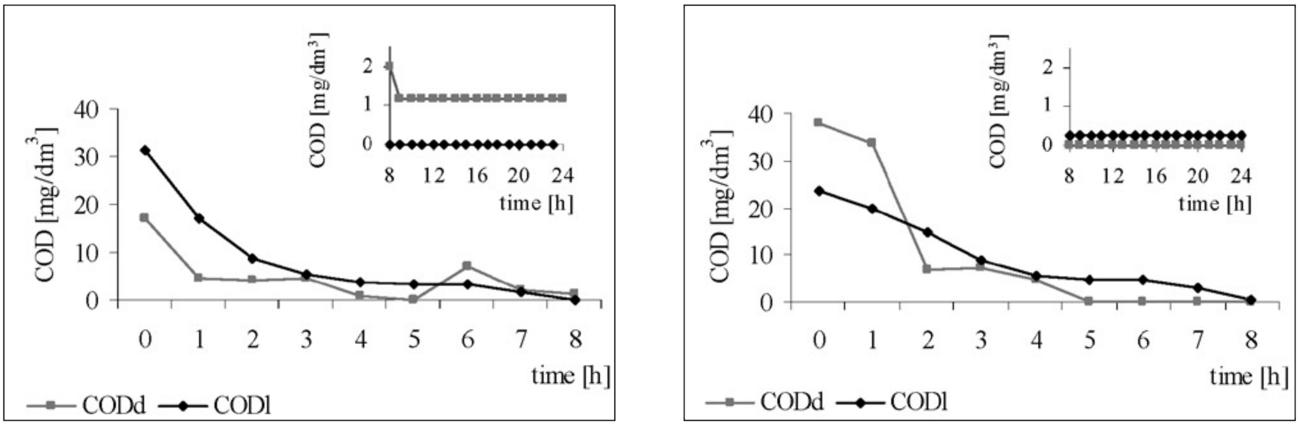

Fig. 4. Accessibility of organic compounds left (COD_l) in the reactor for denitrification a) series I, b) series II.

water for the N-oxidized reduction. The accessibility of organic compounds left in the reactor for denitrification by activated sludge is shown in Fig. 4.

It should be emphasized that the intracellular accumulation in form of poly(-β-hydroxybutyrate) in activated sludge was observed. Below is a photograph of the activated sludge with stored poly(-β-hydroxybutyrate) (Fig. 5).

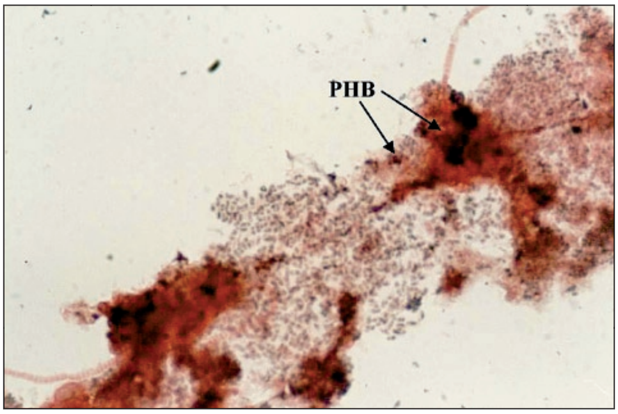

Fig. 5. Photograph of activated sludge with stored poly-β-hydroxybutyrate.

In series I at activated sludge loading of 0.006 g N/g·d poly(-β-hydroxybutyrate) was stored in microorganisms cells till the 7th hour of reaction time. In the consequence of PHB accumulation, activated sludge was able to remove about 10% of organic compounds (COD).

During the 7 hours of the reaction time in series I when the COD/N ratio in wastewater was at level 13, carbon left in the reactor (COD_l) was used by activated sludge as electron donors for denitrification. Simultaneously, intracellular accumulation of stored compounds as poly(-β-hydroxybutyrate) by activated sludge was observed (Fig. 6a_1). During that time activated sludge was able to carry out the N-oxidized reduction using exogenous carbon source from wastewater. Despite the fact of exhaustion of the accessible carbon left in wastewater (COD_l) from the 7th hour of the reaction time, denitrification was still observed. It is shown in Fig. 6a_2 that the endogenous carbon source was used for denitrification during this time. Carbon accumulated intracellularly during the initial hours of the experiment (when excess of organic compounds in the reactor was determined) was the electrons donor for this process.

The literature data [14, 15] indicate that the PHB accumulation in microorganisms' cells under steady state conditions is possible. According to Yamane et al. [16], under

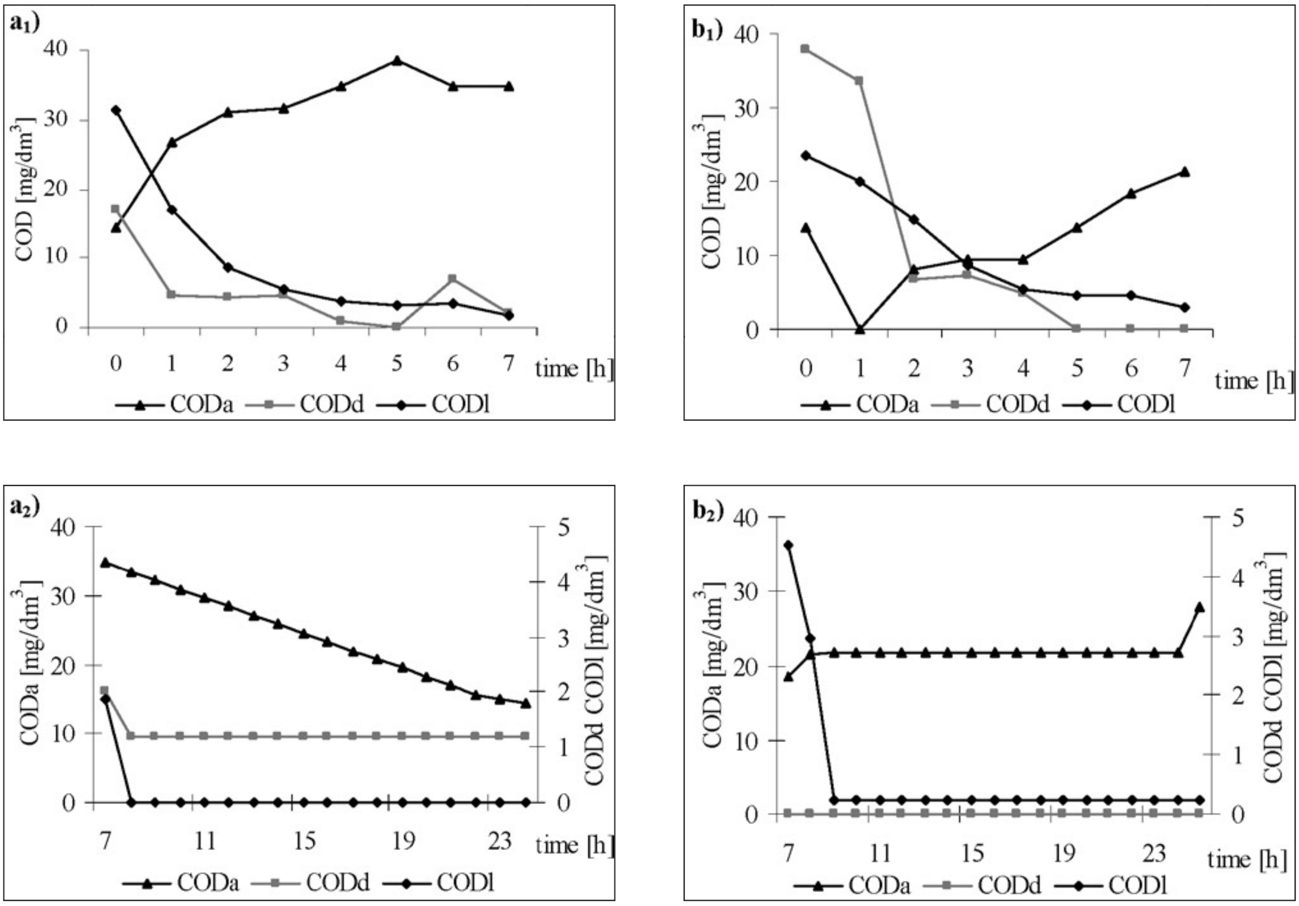

Fig. 6. Carbon left (COD_l) using for denitrification and accumulation in activated sludge in series I a_1)-a_2) t=0 h to t=7 h, in series II b_1)-b_2) t=7 h to t=24 h.

substrate balance conditions *Alcaligenes latus* is capable of storing poly(-β-hydroxybutyrate) in the amount of 80% of dry biomass mass.

With the decrease in COD/N ratio in the reactor to the value of 1.75 the decrease in poly(-β-hydroxybutyrate) storage in activated sludge cells was observed. In presented investigations, at the COD/N ratio in the reactor on this level the degradation of poly(-β-hydroxybutyrate) was observed. Barak et al. [2] have suggested that poly(-β-hydroxybutyrate) polymer can be an electron donor for N-oxidized reduction. The authors have found that the decrease in nitrate concentration was simultaneous with the decrease in poly(-β-hydroxybutyrate) in biomass cells. According to Barak et al. [2] *Pseudomonas denitrificans* is able to use stored poly(-β-hydroxybutyrate) for the nitrate reduction and does not use it for polyphosphate accumulation.

The experimental results showed that under fully aerobic conditions from the 7th hour of the reaction both the nitrate concentration and the amount of poly(-β-hydroxybutyrate) decrease in activated sludge were observed. Research by Majone et al. [17] proved that there was a possibility of poly(-β-hydroxybutyrate) being used for denitrification. The authors indicated that about 90% of aerobic heterotrophic organisms able to poly(-β-hydroxybutyrate) accumulation could use this polymer for nitrate reduction.

PHB synthesis was observed from the 2nd hour of the reaction in series II, when activated sludge loading was 0.027 g N/g·d. In the consequence of intracellular accumulation of this polymer, activated sludge was able to remove 47% of organic compounds from wastewater.

At the COD/N ratio 3 in series II, denitrification was observed for the 5 initial hours of reaction. During the 2 first hours of the experiment both exogenous and endogenous carbon were used by activated sludge as electron donors for the nitrogen oxidized forms reduction (the decrease in COD_l and COD_a were observed - Fig. $7b_1$). Carbon left (COD_l) in the reactor that was not used for oxidation and biomass synthesis were electron donors for denitrification during the next 3 hours of the reaction. From the 1st to 7th hour of the reaction carbon compounds were accumulated intracellularly by activated sludge (Fig. $6b_1$). Organic accumulation stayed at the same level throughout the experiment (Fig. $6b_2$).

The results obtained in series II indicated that at the COD/N ratio 1, activated sludge is able to biomass and reserve substance synthesis simultaneously. Organic compounds removal from the 5th hour of the reaction time was possible in the consequence of the accumulation process. The research of Dionisii et al. [1] confirmed the accumulation process in similar conditions. The authors observed that under unsteady state conditions substrate removal mechanisms could be different from those occurring under steady state conditions. Under unbalanced conditions the intracellular accumulation in biomass is the dominant process. Less physiological adaptations are required in accumulation so microorganisms are able to store substrates. Next, it can be used as endogenous carbon and an energy source for the growth.

The denitrification rate decreased when activated sludge loading increased to 0.027 g N/g·d in series II. However, nitrogen reduction was carried out during the 6 initial hours of the reaction time (from t=0 h to t=6 h). From t=7 h the nitrate concentration was at the same level. Neither the nitrate increase nor the decrease were detected. It is assumed that lack of denitrification activity of activated sludge was observed because of a shortage of electron donors in the environment. It may be concluded that the accessibility of carbon source in the reactor determines denitrification efficiency. Under such conditions ammonium oxidizing to nitrate by autotrophic organisms predominated.

The results indicated that in both series (irrespective of the COD/N ratio in wastewater) activated sludge was able to accumulate carbon compounds as the PHB polymer. When lack of carbon left in the reactor was observed, microorganisms could use this polymer for N-oxidized reduction during the endogenous phase of denitrification.

On the basis of the experiment it can also be assumed that both high and low COD/N ratios in wastewater the poly(-β-hydroxybutyrate) accumulation process and its use for the N-oxidized forms reduction are possible. Particular elementary processes in organic compounds removal from wastewater change depending on environmental conditions. Contrary to the sustainable conditions provided in series I, the presence of a considerable amount of ammonium ions in the environment in series II caused a change in the activated sludge metabolism. It has shown an inhibition of the growth rate for the advantage of the accumulation process.

Conclusion

From the investigations carried out in a sequencing batch reactor (SBR) under fully aerobic conditions at different COD/N ratio in wastewater it can be concluded:

1. There is the possibility of poly(-β-hydroxybutyrate) synthesis at low COD/N ratio (3) when wastewater contained predominantly readily biodegradable compounds on the level of 80% of C_{COD}.
2. There is more important role during carbon removal of poly(-β-hydroxybutyrate) accumulation and denitrification at low COD/N ratio 3 (77% of total carbon). Biomass synthesis and cellular oxidation predominated at COD/N 13 in wastewater (about 68% of total carbon removed).
3. When lack of organic compounds left (COD_l) in the reactor are observed, microorganisms can use reserved carbon substrate as poly(-β-hydroxybutyrate) as the endogenous carbon and energy source during endogenous denitrification. At low COD/N ratios endogenous denitrification was observed from the 8th hour of the experiment. In the case of higher COD/N ratios in wastewater this process occurred during the initial 2 hours.

4. The reserved substances in form of poly(-β-hydroxybutyrate) are significant in carbon and nitrogen removal by activated sludge under fully aerobic conditions.

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