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Effect of the type of the external carbon source on denitrification kinetics of wastewater

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ABSTRACT

The effective denitrification in conventional wastewater treatment plants (WWTPs) requires maintaining the concentration of organic carbon compounds on the sufficient level in the biological reactor. There are many so-called external carbon sources used for this purpose. In this paper, the effect of addition of various external carbon sources on denitrification kinetics was described. The tests were performed with the use of real municipal wastewater from the full-scale WWTP in Zgierz (Poland). Three external carbon sources were selected, that is, acetic acid and two yet untested commercial compounds being the mixtures of various organic and some inorganic substances. One of the commercial carbon sources was glycerine-based, while the second one alcohol-based. Apart from organic carbon both commercial carbon sources contained nitrogen and phosphorus at relatively high amounts and in the case of one of them it exceeded 1,000 mg NH_4^+–N L^{-1}. This compound had the lowest potential of denitrification equal to 0.102 mg NO_3^-–N mg COD^{-1}. Although the ratios of nitrogen to chemical oxygen demand (COD) of both commercial compounds did not exceed 2.5%, which indicated that the denitrification potential of these carbon sources should not be decreased, lower rates of denitrification were observed in the tests with the use of them. When acetic acid was the external carbon source, denitrification was the fastest and its rate increased from two- to three-fold in comparison with the commercial carbon sources tested. These experiments indicated that heretofore assumed critical values of nitrogen to COD ratio for external carbon sources should be verified.

Keywords: Activated sludge; Denitrification; External carbon sources; Kinetics; Wastewater

1. Introduction

Biological denitrification has been extensively used in full-scale wastewater treatment plants (WWTPs) to remove nitrogen from wastewater. In this process, heterotrophic bacteria utilize nitrate anions as the final acceptor of electrons and, as a result, they are reduced to nitrite anions and subsequently to gaseous nitrogen. This process is usually limited by available organic carbon present in wastewater, particularly it concerns municipal wastewater. Thus, external carbon sources are added in order to improve the ratio of chemical oxygen demand (COD) to nitrogen and consequently to increase the efficiency of denitrification processes.

External carbon sources can be divided into three groups: (1) conventional, (2) commercial and (3) alternative compounds [1]. The first group comprises substances being a simple source of organic carbon such as acetic acid, methanol, ethanol or glucose [1,2]. The commercial external carbon sources are the preparations containing such chemical compounds as acetic acid, glycerine, soda or black treacle. Their composition is defined and invariable [1]. Alternative

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external carbon sources comprise waste or wastewater coming from food, dairy and beet sugar factories [3–7]. The advantages of these sources are low costs and the fact that their utilization follows the idea of sustainable development. The research on the applications of various organic carbon compounds for the enhancement of denitrification process has been conducted for over 20 years [8].

Each carbon source should be characterized and experimentally tested before its application in any full-scale wastewater treatment system. Gu and Onnis-Hayden [9] proposed the procedure to assess the efficiency and feasibility of the alternative carbon sources for enhancing nitrogen removal at full-scale wastewater treatment facilities. This procedure for prescreening carbon alternatives is presented as an evaluation matrix comprising such criteria as costs, availability, COD, composition, quality and environmental aspects [9]. Other authors mentioned that more detailed characteristics of carbonaceous compounds used for denitrification should include biodegradability, content of unfavorable/toxic substances, kinetics of denitrification and the potential for the complete denitrification without the need to adapt the microflora [10–13]. Many works characterizing the external carbon sources aimed at the determination of denitrification rate and estimation of the stoichiometric coefficients of denitrification [2,8,11,13]. It indicated that the kinetics of denitrification processes is one of the most important criteria in the evaluation of the denitrification potential of carbonaceous compounds.

The main aim of the work was to compare the kinetics of denitrification of municipal wastewater depending on the various types of external carbon source used. It allowed for the recommendation of the appropriate external carbon source in the full-scale WWTP in Zgierz (Poland).

Three carbon compounds of different chemical composition were studied. These were two commercial carbon sources (ECS1 and ECS2) and one conventional carbon source, that is, acetic acid (ECS_AA). Kinetic parameters of denitrification were determined within the batch tests on the basis of the measurement of nitrate uptake rate (NUR).

2. Materials and methods

2.1. Description of the full-scale WWTP

The object of this study was the full-scale WWTP Zgierz (Poland) that generally treats municipal wastewater from the city of Zgierz and the Industrial Park "Boruta". The contribution of the industrial wastewater usually varies from 7% to 15%. The average pollutant load to the plant corresponds to approximately 94,000 population equivalent (PE). The biological stage consists of one five-zone bioreactor and secondary clarifier run in the Phoredox process configuration. The total volume of the bioreactor is 24,000 m^3. The scheme of the wastewater treatment installation, particularly its biological part, working in the WWTP Zgierz is depicted in Fig. 1(a). The sampling points are shown in Fig. 1(a).

In the period of study, that is, from September 2014 to December 2014, the average inflow of wastewater was 7,700 $m^3 d^{-1}$. COD of the influent was on average 1,550 ± 606 mg $O_2 L^{-1}$. The concentration of total nitrogen in the influent was in the range from 63.3 to 97.2 mg L^{-1}, while total phosphorus varied from 8.9 to 22.4 mg L^{-1}.

Analysis of bacterial composition of activated sludge from the WWTP Zgierz made with the use of molecular biology techniques revealed that the most abundant genera were *Dechloromonas* sp., *Vogesella* sp., *Planctomyces* sp., *Thauera* sp., *Sulfuritalea* sp., *Candidatus* sp. and *Blochmannia* sp., whereas at the level of species these were *Vogesella perlucida, Candidatus Blochmannia rufipes, Dechloromonas hortensis, Runella limosa* and *Dechloromonas denitrificans* [14]. It confirmed that bacteria of strong denitrifying ability like *Thauera* sp. [15] were present in the activated sludge biomass used in the batch tests performed in this work.

2.2. Organization of the experimental study

The experiments were divided into four stages: (1) characteristics of the composition of the external carbon sources tested; (2) determination of nitrate-nitrogen concentration profile in the activated sludge chamber; (3) batch tests of ammonium uptake rate (AUR) and NUR; and (4) determination of kinetic parameters of denitrification.

Regarding stage 2, the concentration of nitrate-nitrogen in activated sludge chamber was measured in nine points (in triplicate) as it is shown in Fig. 1(a). Changes of nitrate-nitrogen concentration along the activated sludge bioreactor were determined in order to select the appropriate sampling point of activated sludge. It was assumed that at point in which the concentration of nitrate-nitrogen (NO_3^-–N) was the highest the activated sludge biomass had the highest ability to transform ammonium-nitrogen into nitrite- and nitrate-nitrogen. This biomass was used for AUR/NUR tests, which were described in the following section. Taking into account that denitrification is preceded by nitrification of wastewater in the tested WWTP, it was decided that NUR tests will be conducted after AUR tests.

2.3. Batch tests of ammonium uptake rate and nitrate uptake rate

The AUR/NUR tests were carried out in a batch bioreactor of 15 L working volume equipped with control and measurement devices. The setup for AUR/NUR tests was constructed at the WWTP Zgierz. All experiments were performed at constant temperature (20°C). The rotary speed of the stirrer was kept constant at 400 rpm.

The substrate for AUR/NUR tests was raw wastewater taken from the object 3.1 (Fig. 1(a)). The activated sludge biomass coming from the aerated part of the activated sludge chamber (point no. 5 in Fig. 1(a)) was used as the inoculum. Wastewater and activated sludge were introduced into the bioreactor at the proportion reflecting that in the activated sludge system at the WWTP Zgierz, that is, 1:1. The concentration of activated sludge biomass at the beginning of AUR test was from 3.68 to 3.83 g L^{-1}, whereas COD was on average equal to 198 ± 11.4 mg $O_2 L^{-1}$. More data characterizing substrate and biomass in AUR/NUR tests are included in Table 1.

AUR tests were not the ultimate goal of this study and were performed mainly in order to obtain nitrified substrate. Each AUR test was conducted until the concentration of ammonium- and nitrite-nitrogen in wastewater decreased to 1 and 0.1 mg L^{-1}, respectively, which indicated the end of nitrification. In that moment (usually after 12 h), aeration (oxygen supply) was stopped and the external carbon source was added when the concentration of dissolved oxygen decreased to 0 mg L^{-1}. The amount of external carbon source added was 10 mg COD mg NO_3^-–N^{-1}, which resulted in the increase of COD, on average, by 195 ± 18 mg O_2 L^{-1}. The duration of denitrification tests (NUR tests) was from 120 to 330 min depending on the external carbon source tested. The samples were taken every 15 min.

During both tests (AUR and NUR tests) COD, N–NH_4^+, N–NO_3^-, N–NO_2^-, pH and alkalinity of wastewater were measured. Additionally, the concentration of activated sludge biomass expressed as volatile suspended solids (VSS) was determined. In Table 1, the values of the aforementioned indicators at the beginning and at the end of AUR and NUR tests are shown. Moreover, the dissolved oxygen concentration was measured with the optical dissolved oxygen probe ProODO™ (YSI Environmental, USA). During AUR tests the concentration of dissolved oxygen was from 2.5 to 4 mg O_2 L^{-1}, while during NUR tests it was less than 0.4 mg L^{-1}.

2.4. Analytical methods

The analysis of COD, VSS, NH_4^+–N, NO_3^-–N, NO_2^-–N, total nitrogen (N_{tot}), organic nitrogen (N_{org}), phosphorus and alkalinity was performed in agreement with the standard methods [16].

COD was measured according to the dichromate method with the use of the Dr. Lange spectrophotometer DR 5000.

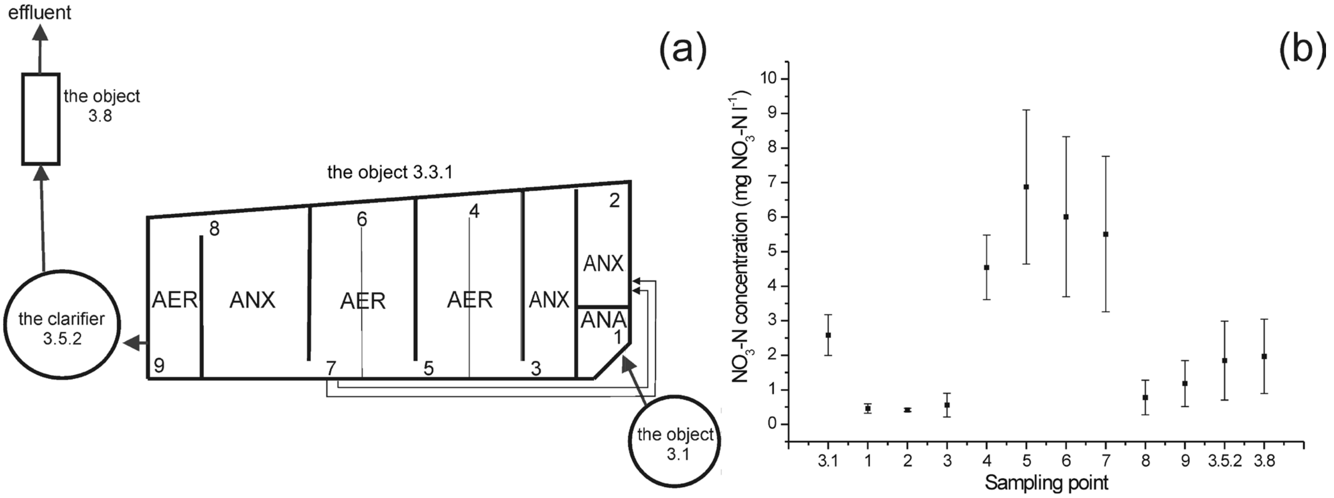

Fig. 1. (a) Main objects of the biological part of the wastewater treatment plant in Zgierz. The object 3.1 – the tank of raw wastewater after mechanical treatment; the object 3.3.1 – the activated sludge chamber; the object 3.5.2 – the secondary settler; the object 3.8 – the tank of treated wastewater; the numbers from 1 to 9 depict the sampling points, in which nitrate concentrations were determined; point 1 – anaerobic conditions; points 2 and 8 – anoxic conditions; points 3, 4, 5, 6, 7 and 9 – aerobic conditions. (b) Distribution of NO_3^-–N concentrations in the biological part of wastewater treatment plant in Zgierz.

Table 1
Characteristics of substrate composition and biomass concentration during AUR/NUR tests

ECS	Test	COD (mg O_2 L^{-1})	VSS (g L^{-1})	NO_3^-–N (mg N L^{-1})	NO_2^-–N (mg N L^{-1})	NH_4^+–N (mg N L^{-1})	pH	Alkalinity (mmol L^{-1})
ECS_AA	AUR *t* = 0 h	185 ± 23	3.68 ± 0.18	3.18 ± 1.7	0.37 ± 0.18	30.68 ± 9.74	7.66 ± 0.09	7.33 ± 0.37
	AUR *t* = 12 h	48 ± 5	3.82 ± 0.14	18.55 ± 5.7	0.074 ± 0.03	0.82 ± 0.19	7.64 ± 0.09	4.75 ± 0.72
	NUR *t* = 0 min	251 ± 26	3.83 ± 0.23	17.69 ± 6.6	0.11 ± 0.08	0.76 ± 0.17	7.66 ± 0.19	5.33 ± 0.81
	NUR *t* = 120 min	102 ± 26	3.91 ± 0.26	0.39 ± 0.16	0.06 ± 0.025	0.19 ± 0.095	7.96 ± 0.19	7.02 ± 0.66
ECS1	AUR *t* = 0 h	206 ± 17	3.83 ± 0.25	3.12 ± 0.56	2.59 ± 1.48	22.3 ± 9.14	7.73 ± 0.06	6.83 ± 0.06
	AUR *t* = 12 h	42 ± 4	4 ± 0.2	19.15 ± 5.01	0.08 ± 0.017	0.66 ± 0.05	7.67 ± 0.06	4.74 ± 0.04
	NUR *t* = 0 min	228 ± 29	3.96 ± 0.15	17.57 ± 5.23	0.59 ± 0.06	0.66 ± 0.03	7.89 ± 0.02	4.83 ± 0.03
	NUR *t* = 225 min	100 ± 17	4.13 ± 0.21	0.29 ± 0.07	0.23 ± 0.13	0.21 ± 0.084	8.08 ± 0.07	6.09 ± 0.06
ECS2	AUR *t* = 0 h	203 ± 23	3.48 ± 0.1	2.91 ± 1.23	1.72 ± 1.06	37.59 ± 6.64	7.73 ± 0.09	7.29 ± 0.82
	AUR *t* = 12 h	45 ± 7	3.78 ± 0.09	19.64 ± 6.45	0.06 ± 0.01	0.82 ± 0.12	7.73 ± 0.09	4.8 ± 0.1
	NUR *t* = 0 min	263 ± 62	3.8 ± 0.08	18.85 ± 5.9	0.07 ± 0.02	0.81 ± 0.12	7.73 ± 0.1	4.77 ± 0.07
	NUR *t* = 330 min	84 ± 35	3.93 ± 0.12	0.56 ± 0.23	0.056 ± 0.02	0.54 ± 0.11	8.05 ± 0.13	5.93 ± 0.15

Ammonium, nitrate, nitrite and total nitrogen, and total phosphorus concentrations were measured with the use of the Dr. Lange tests on the spectrophotometer mentioned above. Alkalinity was determined with the use of potentiometric titration to the end-point pH level equal to 4.5 [16]. VSS were determined gravimetrically.

2.5. Calculations and statistical elaboration of the results

The basic statistical elaboration of the obtained results comprising the calculation of mean values and standard deviations (σ) was made with the use of MS Excel. The values of the volumetric NUR were determined as the slope of the changes of nitrate-nitrogen concentration in time during NUR tests. It was made for the first and second phase of denitrification. The example of the determination of the volumetric denitrification rate is shown in Fig. 2. The zero-order kinetics model for denitrification can be definitely applied for the first phase as the changes of nitrate concentration are linear. In this way, the determined value of volumetric NUR for this phase is the maximum one in the process and thus independent of substrates concentration. After the first phase the concentration of nitrate-nitrogen initially non-linearly and later linearly decreased (the second phase) but the value of volumetric NUR was then very low. Here, the kinetics cannot be unequivocally determined as substrate limitation may have occurred. In the literature, either zero-order or first-order kinetics for denitrification was reported [17]. Rout et al. [17] found that in most cases both models fitted the experimental results well (high R^2).

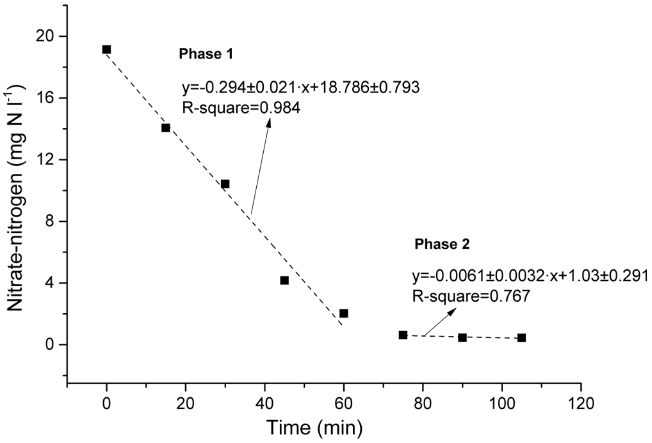

Fig. 2. Example of determination of the volumetric nitrate uptake rate in NUR test with acetic acid as the external carbon source.

Moreover, the specific NUR in NUR tests was calculated regarding the mean concentration of biomass (VSS). Yield coefficient of activated sludge biomass during denitrification tests was calculated as the ratio of biomass formed (expressed as VSS) to COD removal (mg VSS mg COD^{-1}).

Potential of denitrification was calculated in agreement with the formula proposed by Sage et al. [18] and Zhang et al. [8]:

$$P_{\mathrm{DN}} = \frac{\mathrm{N}_{\mathrm{IN}} - \mathrm{N}_{\mathrm{OUT}}}{\mathrm{COD}_{\mathrm{IN}} - \mathrm{COD}_{\mathrm{OUT}}} \qquad ()$$

where N_{IN}, $\mathrm{N}_{\mathrm{OUT}}$ – the concentration of nitrate-nitrogen at the beginning and at the end of NUR test, respectively; $\mathrm{COD}_{\mathrm{IN}}$, $\mathrm{COD}_{\mathrm{OUT}}$ – the values of COD at the beginning and at the end of NUR test, respectively.

3. Results and discussion

3.1. Characteristics of the external carbon sources tested

Two commercial external carbon sources denoted as ECS1 and ECS2 were tested. Additionally, acetic acid, a commonly used external carbon substrate in denitrification of wastewater, was tested as the reference substrate to be compared with the commercial carbon sources studied. ECS1 mainly contains glycerine (about 60% w/w), while the ECS2 is a mixture of alcohols, sugars and proteins. Thus, ECS1 and ECS2 can be, respectively, denoted as glycerine-based and alcohol-based external carbon sources.

COD of both commercial carbon sources was more than twice higher compared with that of acetic acid (Table 2). High COD values were recorded for all the tested external carbon sources. Therefore, according to the guidelines formulated by Gu and Onnis-Hayden [9] they exhibited great potential

Table 2
Composition of external carbon sources tested in this work (own study)

External carbon sources	ECS_AA	ECS1	ECS2
Trade names	–	Kem-Carbo GCM 95	Brentaplus VP-1
Main components (data from manufacturers)	Acetic acid and water 50% v/v	Glycerine, water, soda, methanol	Alcohols, sugars and proteins
COD mg O_2 L^{-1}	480,000	1,158,000	1,330,000
NH_4^+–N (mg N_{NH4} $L^{-1)}$	N/A	252	1,080
NO_3^-–N (mg N_{NO3} $L^{-1)}$	N/A	134	605
NO_2^-–N (mg N_{NO2} L^{-1})	N/A	0.4	0.52
N_{org}	N/A	67.6	209.5
N_{tot} (mg N L^{-1})	N/A	454	1,895
N_{tot}/COD (%)	N/A	0.039	0.142
P_{tot} mg P L^{-1}	N/A	313	39.3
P_{tot}/COD (%)	N/A	0.027	0.0029

to be applied as the carbon source for wastewater denitrification. It concerned particularly two commercial carbon sources (ECS1 and ECS2), whose COD was of 10^6 magnitude (Table 2). Both ECS1 and ECS2 contained a certain quantity of nitrogen and phosphorus (Table 2). Although the ratio of total nitrogen to carbon compounds expressed as N_{tot}/COD was less than 2.5% [9], the addition of the carbon substrate rich in nutrients increased their concentration in wastewater and, as a result, might have influenced on efficiency of nitrogen and phosphorus removal from wastewater.

3.2. Concentration profile of nitrate-nitrogen in activated sludge chamber

The variations of nitrate-nitrogen concentration in the activated sludge system had the same character in each run (Fig. 1(b)). In the influent (object 3.1), the concentration of nitrate-nitrogen was from 1.95 to 3.18 mg N L^{-1}. In the activated sludge chamber, it initially decreased to less than 1 mg N L^{-1} (sampling points 1, 2 and 3) and then increased along the aerated part of the activated sludge chamber reaching the highest values in sampling point 5 (from 5.24 to 9.43 mg L^{-1}). However, in the further sampling points of the aerated part of the activated sludge chamber it remained approximately at the same high level (Fig. 1(b)). Then, in the second anoxic chamber of the bioreactor the concentration of nitrate-nitrogen significantly decreased (on average by 78%) in comparison with sampling point 5. In the second aerated chamber (the last part of bioreactor) and further sampling points (secondary clarifier and effluent) it slightly increased, achieving the values from 1.07 to 3.23 mg N L^{-1} depending on the measurement campaign (Fig. 1(b)).

The analysis of nitrate-nitrogen concentrations in the studied WWTP allowed for a more thorough analysis of their variations particularly in the activated sludge chamber. It exceeded the standard analytical protocol comprising the determination of nitrate-nitrogen in the influent and effluent only. What is more important, the determination of nitrate-nitrogen concentration profile in the activated sludge chamber enabled for the selection of the point, from which activated sludge biomass for AUR/NUR tests was taken. Assuming that the biomass of the highest ability to oxidize ammonium-nitrogen was in the point of activated sludge chamber at which the concentration of nitrates was the highest, the sampling point number 5 was selected to take the biomass for AUR/NUR tests.

3.3. AUR/NUR tests

The typical run of the AUR test is presented in Fig. 3. The concentration of ammonium-nitrogen decreased rapidly during the first 4 h of the test and the values reached less than 0.1 mg L^{-1} in the last hours of experiments (between 8 and 12 h). Simultaneously, the concentration of nitrite-nitrogen gradually increased to its maximum value after about 4 h of the test and then decreased (Fig. 3). At the end of the AUR tests (between 8 and 12 h) it was usually less than 0.1 mg L^{-1}, which indicated that nitrification was finished (Fig. 3). The concentration of nitrate-nitrogen in wastewater increased and after about 8 h of the AUR test it remained at the same high level until the end of experiment (Fig. 3).

The intensive oxidation of ammonium-nitrogen was accompanied by the high values of oxygen uptake rates (OUR). During the first 4 h of the tests the mean value of OUR was at the level of 24.45 mg O_2 L^{-1} min^{-1}. Then, it decreased to 2.53 mg O_2 L^{-1} min^{-1}, on average. At the end of the AUR test, that is, between 10 and 12 h, it was less than 0.5 mg O_2 L^{-1} min^{-1}, which confirmed that nitrification was finished.

The transformation of various forms of nitrogen during the AUR tests well corresponded with the changes of NH_4^+–N, NO_2^-–N and NO_3^-–N concentrations in nitrification process of wastewater in activated sludge chamber. Two stages of nitrification could be distinguished in the AUR tests [11]. Alkalinity was utilized and its value decreased, on average, from 7.15 to 4.77 mmol L^{-1} in the AUR tests (Table 1). The value of pH was at the level from 7.66 to 7.73 at the beginning of AUR test and then slightly decreased to 7.64 or 7.67 (Table 1). These values were close to the optimum pH found for nitrification, that is, pH between 8 and 9 [19].

Summing up the run of the AUR tests, ammonium-nitrogen present in wastewater was oxidized within no longer than 10 h of the test. The same observation was made for nitrite-nitrogen. Thus, the changes of ammonium-, nitrite-, nitrate-nitrogen and alkalinity observed during the AUR tests indicated that they were performed properly.

The concentration of nitrate-nitrogen decreased rapidly in each NUR test irrespective of the external carbon source used (Fig. 4). It was accompanied by the decrease of COD. The curves of COD had the same character as those of nitrate-nitrogen concentration. According to Isaacs and Henze [20] the demand for carbon compounds in denitrification processes was from 5 to 10 mg COD mg NO_3^-–N^{-1}, while Peng et al. [2] estimated its value between 4 and 15 mg COD mg NO_3–N^{-1}. In the NUR tests performed in this study it was, on average, 7.89 mg COD mg NO_3–N^{-1} and it was in agreement with the values reported by Isaacs and Henze [20] and Peng et al. [2].

Upon the changes of nitrate-nitrogen concentration during each NUR test two stages of denitrification can be distinguished. These were the intensive denitrification (phase I), in which the concentration of nitrate-nitrogen decreased rapidly,

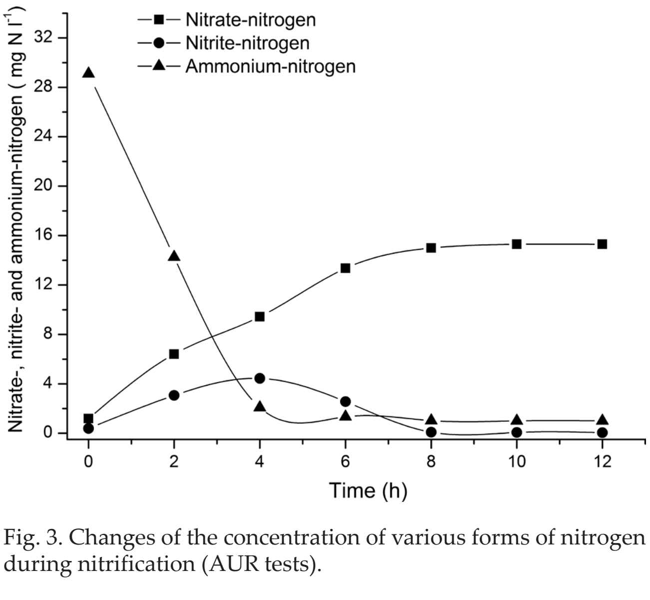

Fig. 3. Changes of the concentration of various forms of nitrogen during nitrification (AUR tests).

and the slow denitrification (phase II), in which only small changes of the concentration of nitrates were observed.

As opposed to the changes of nitrate-nitrogen concentration (NO_3^-–N), the changes of nitrite-nitrogen concentration (NO_2^-–N) were affected by the type of external carbon source used in the denitrification tests. In the case of acetic acid, the concentration of nitrite-nitrogen was at the same low level and usually did not exceed 1 mg N L^{-1}, whereas in the case of ECS1 it was higher and varied from 0.04 to 2.67 mg L^{-1}. The third carbon source tested, that is, ECS2, contained about four times higher amount of total nitrogen than ECS1. Most probably due to this reason the concentration of nitrite-nitrogen in the NUR tests, in which ECS2 was used, was the highest and in one trial it even exceeded 12 mg L^{-1}. On average, the concentration of nitrite-nitrogen in the NUR tests with ECS2 varied from 0.06 to 5.36 mg L^{-1}. These results indicated that if the external carbon source contains more nitrogen, the increase of nitrite-nitrogen concentration in the denitrification process is more probable (Fig. 4).

Alkalinity consumed during nitrification (AUR tests) regained in the NUR tests. The increase of alkalinity was, on average, from 4.97 to 6.34 mmol L^{-1} (Table 1). More than one-half of the alkalinity utilized in the AUR test was restored in the NUR test. The changes of pH level well corresponded with the changes of alkalinity. The value of pH increased in each NUR test (Table 1).

The duration of denitrification tests depended on the type of the external carbon source used. It was assumed that the decrease of the concentration of nitrate-nitrogen less than 0.5 mg L^{-1} indicated the end of denitrification processes. The NUR tests lasted from 120 min (ECS_AA) to 330 min (ECS2). The concentration of dissolved oxygen was less than 0.4 mg L^{-1} in each denitrification test.

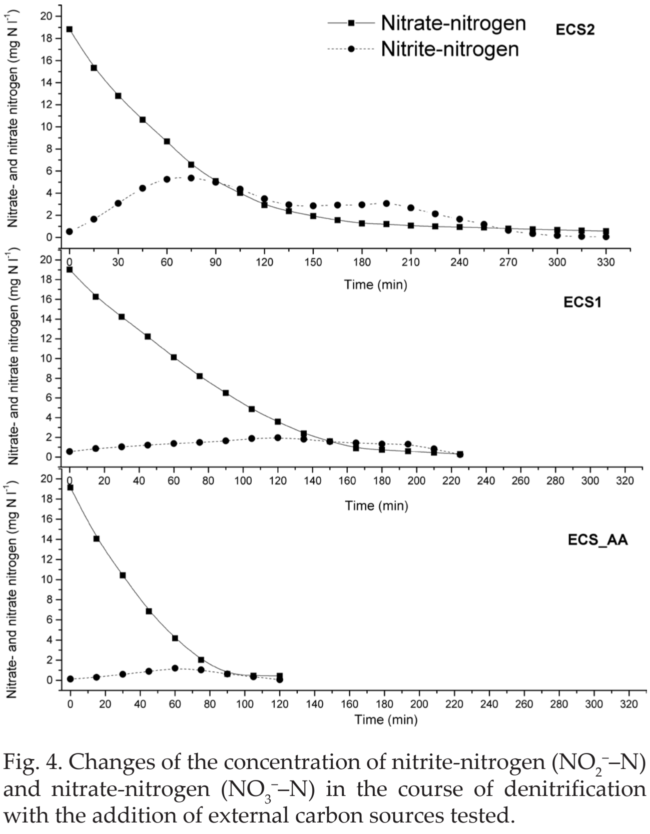

Fig. 4. Changes of the concentration of nitrite-nitrogen (NO_2^-–N) and nitrate-nitrogen (NO_3^-–N) in the course of denitrification with the addition of external carbon sources tested.

The increase of biomass concentration expressed as VSS was observed during the AUR/NUR tests (Table 1). It was, on average, 393 mg L^{-1} in the AUR tests, while in the NUR tests it was 119 mg L^{-1}.

3.4. Kinetics of denitrification

The kinetic parameters of denitrification were obtained on the basis of the results of NUR tests. The rate of denitrification was expressed as the rate of nitrate-nitrogen (NO_3^-–N) uptake in these tests. The volumetric and specific rates of denitrification were determined. Due to the fact that two stages of the changes of nitrate concentration were observed (Fig. 4), two rates of denitrification were calculated. These were the rate of the intensive denitrification (phase I) and rate of the slow denitrification (phase II). The values of volumetric and specific denitrification rates for both phases are collected in Table 3.

The values of the volumetric NUR determined for two commercial carbon sources studied (ECS1 and ECS2) were more than twice lower in comparison with the volumetric NUR calculated for acetic acid (phase I). In the second phase of denitrification the highest rate was determined for ECS1, while the lowest for ECS2 (Table 3). The specific NUR was above twice higher in the tests with acetic acid than in the tests with ECS1 and ECS2 (Table 3). The values of kinetic parameters of denitrification confirmed the previous experimental observations that denitrification ended earlier in the NUR tests with acetic acid than with ECS1 and ECS2 (Fig. 4). The simple organic compounds as acetic acid occurred to be easily metabolized by denitrifying bacteria than the other more complex organic compounds. Taking kinetic criteria into account, acetic acid was significantly better than the other two carbon sources studied in this work. However, the advantage of ECS1 and ECS2 was that they were manufactured with the use of by-products or waste materials.

The obtained values of kinetic parameters of denitrification indicated that the presence of nitrogen in the external

Table 3
Determined mean values of parameters characterizing kinetics of denitrification

Parameter	ECS_AA	ECS1	ECS2
Volumetric nitrate uptake rate – phase I (mg N–NO_3 L^{-1} min^{-1})	0.294	0.115	0.139
Correlation coefficient R^2	0.984	0.987	0.978
Volumetric nitrate uptake rate – phase II (mg NO_3^-–N L^{-1} min^{-1})	0.0061	0.0092	0.0053
Correlation coefficient R^2	0.767	0.990	0.946
Specific nitrate uptake rate (mg NO_3^- g VSS^{-1} min^{-1})	0.1022	0.0396	0.0491
P_{DN} (mg NO_3^-–N mg COD^{-1})	0.116	0.134	0.102
Y_{XS} (mg VSS mg COD^{-1})	0.526	0.701	1.298

carbon source decreased the denitrification rates (Tables 2 and 3), even if the content did not exceed 2.5% [9]. Comparing the values of the denitrification potential (P_{DN}), the lowest one was found for ECS2 (Table 3), which contained the highest amount of nitrogen out of the three tested external carbon sources (Table 2). However, the value of P_{DN} was higher for ECS1 than that for acetic acid. It confirmed that the selection of the appropriate carbon source required the analysis of several criteria. The values of P_{DN} determined in this work were lower than those found by Zhang et al. [8] for the fermentation liquid from the food waste (P_{DN} = 0.174 mg NO_3^-–N mg COD^{-1}). The presence of additional nitrogen and phosphorus in the external carbon sources favored the growth of biomass that was proved by the values of Y_{XS}. The yield coefficients were higher for both commercial carbon sources than those for acetic acid (Table 3). It indicated that the biochemical processes might be performed more efficiently, particularly if not only the concentration of biomass but also its catabolic activity were at the high level. Simultaneously, the amount of the excess sludge would be higher for ECS1 and ECS2 than in the case of lower yield coefficient determined for the processes with acetic acid.

Denitrification rates determined in this work for ECS1 and ECS2 were relatively low in comparison with the values of denitrification rates found in the other studies for a variety of carbon sources (compare Table 3 with Table 4). At the same time the specific NUR calculated for ECS1 and ECS2 well corresponded with those determined for methanol [2], glucose [21], fusel oil [13] and industrial wastewater [22] (Table 4).

In this work, denitrification rate with acetic acid was twice lower than the corresponding value determined by Peng et al. [2]. It was most probably due to the fact that Peng et al. [2] used undiluted acetic acid, whereas here it was a 50% v/v solution of acetic acid. However, also other factors, such as biomass activity, composition of the influent (municipal wastewater vs. starch wastewater) might have influenced the obtained results. According to previous studies the values of denitrification rates for acetate were in the range from 0.0515 mg NO_3^-–N g VSS^{-1} min^{-1} [22] to 0.177 mg NO_3^-–N g VSS^{-1} min^{-1} [23]. The values determined in this work fitted this range (Table 3). Cherchi et al. [24] claimed that the variability of these rates was likely influenced by the sludge sources, type of reactors and environmental factors that generally affect biological processes.

Table 4
Comparison of denitrification rates achieved with various external carbon sources at 20°C

External carbon source	Mean specific nitrate uptake rate (mg N–NO$_3^{-1}$ g VSS^{-1} min^{-1})	Reference
Organic waste from sweets factory	0.693	[11]
Organic waste from soft drinks factory	0.780	[11]
Acetic acid	0.200	[2]
Ethanol	0.160	[2]
Methanol	0.0533	[2]
Glucose	0.041–0.0967	[21]
Industrial wastewater (brewery, distillery and fish-pickling process)	0.0400–0.100	[22]
Winery waste	0.113	[9]
Fusel oil	0.0333–0.100	[13]

4. Conclusions

Comparing the applicability of three external carbon sources for denitrification of wastewater it may be concluded that:

- Two main phases of denitrification can be distinguished in each NUR test. In the first phase, the rapid uptake of nitrate-nitrogen takes place, while in the second one the decrease of nitrate-nitrogen concentration is slow and minor regarding the absolute value of nitrate-nitrogen utilized. The second phase usually starts, when the concentration of nitrite-nitrogen reaches its maximum value.
- Both commercial external carbon sources contain the significant amount of nitrogen and phosphorus, even above 1,000 mg NH_4^+–N L^{-1}. The external carbon source containing the highest amount of nitrogen (ECS2) has the lowest denitrification potential (0.102 mg N–NO_3^- mg COD^{-1}).
- Although the ratios of nitrogen to COD of both commercial compounds do not reach 2.5%, which indicates that the denitrification potential of these carbon sources should not be decreased, lower rates of denitrification are observed in the tests with the use of them. It indicates that heretofore assumed critical values of nitrogen to COD ratio for external carbon sources should be verified.
- The rates of denitrification are more than twice higher for acetic acid comparing with two other commercial carbon sources used in the NUR tests. It is most probably caused by the fact that acetic acid is more easily assimilated by denitrifying bacteria. Moreover, the content of nitrogen in the commercial carbon sources studied may also lead to the decline of denitrification rate.

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