

DENITRIFICATION IN THE ACTIVATED SLUDGE SYSTEMS: STUDY OF THE KINETICS

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Received: 01.03.2012; Revised: 15.03.2012; Accepted: 22.03.2012

Abstract

The number of kinetic studies on denitrification in the activated sludge systems is limited. Moreover, most of them concern the estimation of volumetric and specific nitrate uptake rates. At the same time the experimental determination of Monod kinetic parameters for anoxic growth of heterotrophic biomass has been hardly ever made. The aim of this work is to study the kinetics of denitrification including the determination of Monod kinetic parameters. In order to do it the appropriate procedure based upon the measurement of nitrate uptake rate (NUR) was elaborated.

The proposed procedure proved reliable and gives the chance for the direct, experimental determination of kinetic parameters for denitrifying biomass to replace the commonly used correction factors. The mean values of specific NUR calculated in this work ranged from 1.33 to 1.77 mg NO₃-N g VSS⁻¹ h⁻¹ and were close to those achieved in the systems with acetate as the carbon source. The determined values of the maximum specific growth rate for denitrifying biomass ($\mu_{\max,D}$) varied from 0.28 to 0.78 h⁻¹, while the values of half-saturation constant K_{NO₃} were in the range from 0.81 to 2.05 mg NO₃-N l⁻¹.

Streszczenie

Niewiele opublikowanych dotychczas prac poświęconych denitryfikacji dotyczy kinetyki tego procesu. Co więcej ograniczają się one głównie do wyznaczenia objętościowej i właściwej szybkości zużycia azotanów (V). Niezwykle rzadko można spotkać eksperymentalnie wyznaczone wartości parametrów kinetycznych równania Monoda dla wzrostu heterotrofów w warunkach anoksydacyjnych. Celem tej pracy jest rozpoznanie kinetyki denitryfikacji wraz z wyznaczeniem wartości parametrów kinetycznych równania Monoda. Aby to osiągnąć, opracowano stosowną procedurę eksperymentalną opartą na pomiarze szybkości zużycia azotanów (V) (NUR).

Zaproponowana procedura okazała się być wiarygodna. Umożliwia ona bezpośrednie, eksperymentalne wyznaczenie kinetycznych parametrów dla biomasy denitryfikującej i zastąpienie nimi stosowanych dotychczas współczynników korekcyjnych. Średnia wartość właściwej szybkości zużycia azotanów (V) wynosi od 1.33 do 1.77 mg N-NO₃⁻ g⁻¹ Z_{org}⁻¹h⁻¹ i jest bliska wartościom otrzymanym w układach, w których źródłem węgla był octan. Z kolei wyznaczone w pracy wartości maksymalnej właściwej szybkości wzrostu biomasy denitryfikującej ($\mu_{\max,D}$) zmieniają się od 0.28 do 0.78 h⁻¹, podczas gdy wartości stałej nasycenia dla azotanów (V) (K_{NO₃}) mieszczą się w zakresie od 0.81 do 2.05 mg N-NO₃⁻ l⁻¹.

Keywords: Activated sludge; Denitrification; Kinetics; Modelling; Parameters.

1. INTRODUCTION

Nitrogen compounds are removed from wastewater by the combination of nitrification and denitrification processes. Although both processes are commonly used in the existing wastewater treatment plants (WWTPs), the efficient nitrogen removal is still a problem in some of them. This work focuses on the denitrification, particularly on the kinetics of denitrification.

Basically, denitrification is defined as the reduction of nitrate nitrogen to N_2 by heterotrophic bacteria under anoxic conditions [1]. Then heterotrophs utilise nitrates as the terminal electron acceptor instead of oxygen. Thermodynamically, oxygen is the best electron acceptor ($\Delta G^0 = -219.07 \text{ kJ}/2e^-$) to oxidise NADH, while nitrates are on the second place ($\Delta G^0 = -206.12 \text{ kJ}/2e^-$). It may contribute to slower transformation of organic compounds and/or less efficient biomass synthesis under anoxic conditions. It was found that the maximum rate of organic substrate removal under anoxic conditions was often lower than under aerobic conditions because either maximum specific growth rate is lower under anoxic conditions or only a fraction of the heterotrophic biomass is able to metabolise nitrate as the terminal electron acceptor [2].

In Activated Sludge Models (ASMs) elaborated by the *International Water Association* (IWA) task groups, the rate expression for anoxic growth of heterotrophs is analogous to the one for aerobic growth. Both expressions for aerobic growth (eq. 1) and anoxic growth (eq. 2) are presented below. They both come from Activated Sludge Model no 1. (ASM1) [2].

$$\rho_1 = \mu_{\max H} \cdot \frac{S_s}{K_s + S_s} \cdot \frac{S_o}{K_o + S_o} \cdot X_{BH} \quad (1)$$

$$\rho_2 = \eta_g \cdot \mu_{\max H} \cdot \frac{K_o}{K_o + S_o} \cdot \frac{S_s}{K_s + S_s} \cdot \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot X_{BH} \quad (2)$$

The explanation of the symbols used in the equations (1) and (2) can be found elsewhere [2].

The main difference between the equations (1) and (2) concerns the incorporation of oxygen inhibition term in the kinetic expression describing anoxic growth. Moreover, in order to regard that maximum rate of organic substrate removal under anoxic conditions often lower than under aerobic conditions, the correction factor (η_g) was introduced. It was estimated on the basis of a few measurements that the value of η_g ranges

from 0.6 to 1.0. In ASMs the value of η_g equal to 0.8 at 20°C was assumed. At the same time in the activated sludge model elaborated by *Pai et al.* [3] the default values of reduction factors for denitrification varied from 0.5 to 0.6, while in the model by *Kaelin et al.* [4] the values between 0.15 and 0.25 were suggested on the basis of the model calibration process.

Denitrification is less sensitive to environmental factors, particularly temperature, than nitrification. The rate of denitrification is independent of nitrate concentration but depends on the concentrations of biomass and electron donor in wastewater.

Although the biochemical mechanisms as well as the kinetics of denitrification in the activated sludge systems have been investigated for last two decades, the values of such basic kinetic parameters for anoxic growth as maximum specific growth rate and half-saturation constant have not been determined experimentally yet. The aim of this work is to study the kinetics of denitrification including the determination of Monod kinetic parameters. For this purpose the appropriate procedure based upon the measurement of nitrate uptake rate (NUR) was proposed.

2. MATERIALS AND METHODS

2.1. Biomass cultivation system

Biomass used in NUR tests was taken from the laboratory activated sludge system. It consisted of three parallel aeration chambers of 5.2 litres working volume. Each chamber was coupled with a clarifier of 1.6 litres working volume. Activated sludge used for the inoculation came from the Wastewater Treatment Plant (WWTP) in Zgierz (Poland). Then, the influent, i.e. synthetic municipal wastewater, was delivered continuously to the aeration chamber at the constant volumetric flow rate equal to 0.15 l h^{-1} . The effluent was continuously removed from the system by means of the overflow mounted in the clarifier. The activated sludge was cultured at ambient temperature ($22 \pm 1^\circ\text{C}$) and constant aeration rate (0.321 vvm). More details about the laboratory activated system used in this work were presented elsewhere [5].

Synthetic municipal wastewater (MW) contained 300 mg peptone, 100 mg sodium acetate (CH_3COONa), 50 mg dipotassium hydrogenphosphate (K_2HPO_4), 50 mg sodium bicarbonate (NaHCO_3), 50 mg diammonium hydrogenphosphate ($(\text{NH}_4)_2\text{HPO}_4$), 5 mg magnesium sulphate (MgSO_4) and 5 mg sodium chloride (NaCl) per litre.

The activated sludge was acclimated in the laboratory conditions for six days. The acclimation time corresponded to at least three residence times in the aeration chamber. After that time the NUR tests with the use of the acclimated sludge was started. The laboratory activated sludge system was continuously maintained for the following two weeks. Two series of activated sludge biomass cultivation were carried out in this mode.

The activated sludge from the laboratory system had typical properties as the sludges from the aeration chamber of WWTPs. The content of total suspended solids (TSS) for this laboratory sludge varied from 3.95 to 4.5 g l⁻¹, while the content of volatile suspended solids (VSS) was in the range from 3.1 to 3.8 g l⁻¹. The sludge had also very good settling properties. The sludge volume index (SVI) was in the range from 45 to 56 ml g TSS⁻¹.

2.2. Nitrate utilisation rate (NUR) tests procedure

The NUR tests were performed in a stirred tank batch reactor of 4 litres working volume. Temperature was maintained constant 20°C and rotation speed of the impeller was 140 min⁻¹. During each test nitrate, nitrite and acetate concentrations, COD and VSS were determined every two hours during 24-hour tests.

Each NUR test was preceded by the nitrification test performed in the same bioreactor. It was made in order to obtain nitrified wastewater (NW) containing such high concentration of nitrate as to avoid limitation with this substrate in the NUR tests. In consequence, the physicochemical parameters (Table 1) of NW slightly varied in the performed denitrification tests. Thus the standard deviations for these parameters were also included in Table 1.

Table 1.
Physicochemical properties of the nitrified wastewater before the addition of acetate

Analysis	The average value	Standard deviation
Alkalinity (meqv l ⁻¹)*	2.4	0.2
COD (mg O ₂ l ⁻¹)	26	12
Ammonium (mg N l ⁻¹)	0.51	0.23
Nitrate (mg NO ₃ ⁻ -N l ⁻¹)	44.11	1.75
Nitrate (mg NO ₂ ⁻ -N l ⁻¹)	0.024	0.006

*1 meqv l⁻¹=50 mg CaCO₃ l⁻¹

The short description of the nitrification test was presented in [6]. Having completed nitrification, oxygen supply was switched off and rotation speed of the impeller was lowered from 200 to 140 min⁻¹. As a result within less than 30 minutes dissolved oxygen dropped below 0.5 mg l⁻¹. In this moment 2 g of acetate was added to the bioreactor and NUR test was started. The initial acetate concentration in the bioreactor was equal to 595±12 mg l⁻¹, which corresponded to COD in the liquid phase at the level of 414±15 mg O₂ l⁻¹. At the same time the average biomass concentration at the beginning of the NUR tests was 1.467±0.180 g VSS l⁻¹ or 992±104 mg COD l⁻¹. The initial substrate to biomass ratio was 0.44±0.06 mg of COD in the liquid phase per mg of biomass COD. The NUR tests were made in four replicates.

2.3. Physicochemical analyses

Nitrate, nitrite and acetate concentrations were measured with the help of ionic chromatograph Dionex ICS-1100. Total and liquid phase COD were measured according to the standard dichromatic method with the use of the DR LANGE spectrophotometer DR 5000. Also ammonium nitrogen concentration was determined with the help of this spectrophotometer by salicylate method. Alkalinity was determined with the use of potentiometric titration to the end-point pH equal to 4.5 [7]. The gravimetric measurements of total solids (TS), total suspended solids (TSS), volatile solids (VS) and volatile suspended solids (VSS) were performed in agreement with the standard procedures [7].

2.4. Calculation of kinetic parameters

As a result of the NUR tests the discrete changes of nitrate, nitrite, acetate, COD and VSS were achieved. In order to calculate such kinetic parameters of denitrification as maximum specific growth rate for biomass ($\mu_{\max,D}$) and half-saturation constant for nitrate (K_{NO_3}), the changes of nitrate concentration were transformed to the continuous data. Thus, NUR profiles were achieved upon the nonlinear approximation of nitrate concentration data with the help of third order spline curves and their differentiation. The changes of NUR in time were used for the determination of $\mu_{\max,D}$ and K_{NO_3} . The value of $\mu_{\max,D}$ was determined as the slope of NUR changes in time during the exponential growth phase as it was previously shown by *Kappeler* and *Gujer* [8]. Thus, in order to calculate the slope, these changes were presented in

a semi-logarithmic coordinates system, i.e. $\ln(\text{NUR})=f(t)$. This approach for $\mu_{\max,D}$ determination is based upon the fact that biomass concentration in any biological batch system is directly proportional to the volumetric substrate, including nitrate, uptake rate.

Furthermore, the NUR curve perfectly expresses the amount of nitrate utilised for denitrification and can be applied to estimate substrate affinity constant. In a batch system the value of half-saturation constant for denitrifiers (K_{NO_3}) influences nitrate uptake rate especially in the range of nitrate substrate limitation, i.e. when the decrease of NUR values appears. In this work the values of K_{NO_3} were determined by the integration of NUR curves in the range from the time, at which specific growth rate is equal to the half of maximum specific growth rate for denitrifying biomass, to the end-time of the experiment, when NUR hardly changed in time:

$$K_{\text{NO}_3} = \int_{t=0.5\mu_{\max}}^{t=t_{\text{end}}} \text{NUR}(t)dt \quad (3)$$

Additionally, the mean values of volumetric NUR and specific nitrate uptake rate (SNUR) were calculated. The values of SNUR were calculated in reference to biomass concentration expressed as VSS and biomass COD.

3. RESULTS AND DISCUSSION

3.1. Description of NUR tests

The substrate as well as biomass changes ran similarly in all denitrification tests. The concentration of nitrate nitrogen decreased from about 44 mg l^{-1} to less than 0.2 mg l^{-1} after 24 hours of the experiment (Fig. 1). Nitrates were metabolised very fast particularly during first fourteen hours of the test. At this time the concentration of nitrate nitrogen dropped below 19 mg l^{-1} . The time courses of the nitrite concentration were different in comparison to nitrate during denitrification tests. Initially, the concentration of nitrite increased linearly, then after about twelve-fourteen hours linearly decreased (Fig. 2). The concentration of the second denitrification substrate, i.e. readily biodegradable organic compounds, decreased during the test from about $414 \text{ mg O}_2 \text{ l}^{-1}$ to below $60 \text{ mg O}_2 \text{ l}^{-1}$ (Fig. 3). At the same time biomass concentration increased from 992 to $1179 \text{ mg COD l}^{-1}$ on average (Fig. 3).

Alkalinity plays a very important role in the nitrogen removal processes during biological wastewater

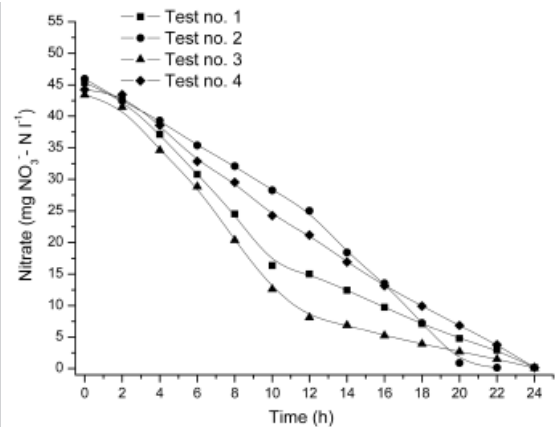


Figure 1. Changes of nitrate concentration in the performed denitrification tests

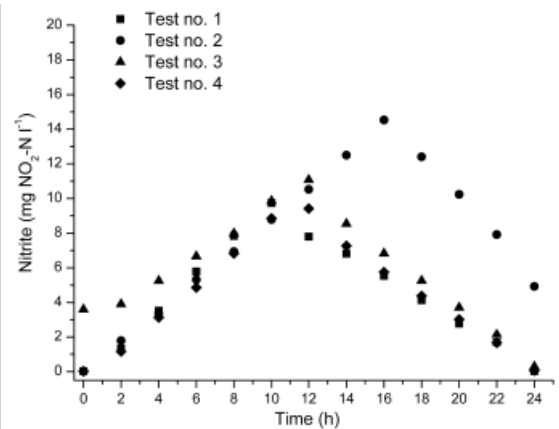


Figure 2. Changes of nitrite concentration in the performed denitrification tests

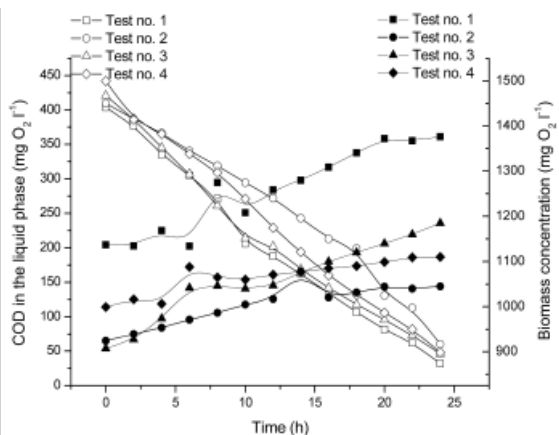


Figure 3. Changes of liquid phase COD and biomass COD in the performed denitrification tests

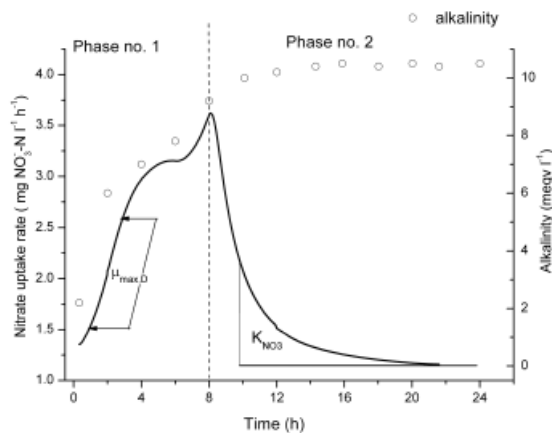


Figure 4.
Determination of the kinetic parameters upon the NUR curve and alkalinity changes in time in test no. 1

treatment. The nitrification process decreases the alkalinity in wastewater, whereas in the denitrification processes alkalinity is recovered. In the NUR tests performed in this work alkalinity increased rapidly from about 2.4 to 6.5 meqv l⁻¹ within 4 hours (Fig. 4). The changes of the measured physicochemical indicators proved that the denitrification was carried out properly in the NUR tests [1, 9]. What is more, no substrate limited the kinetics of denitrification. It concerns particularly the first hours of the NUR tests. Neither was the inhibition of denitrification by substrates or intermediates observed.

3.2. Denitrification kinetics

In this work two Monod kinetic parameters for denitrification, i.e. maximum specific growth rate for biomass ($\mu_{\max,D}$) and half-saturation constant for nitrate (K_{NO_3}), were determined. The values of both parameters were

achieved on the basis of NUR changes in time.

In Fig. 4 the typical NUR curve obtained in this study is presented. In each NUR test two phases, which can be respectively called growth and decline phase, were observed. Initially, the increase of NUR was noticed. It was associated with the exponential growth of denitrifying organisms and denoted as phase no. 1 (Fig. 4). Later on, after approximately eight to ten hours NUR started to decrease. This phase was denoted as phase no. 2 (Fig. 4). The experimental points from phase no. 1 of the NUR test were used for the determination of maximum specific growth rate for denitrifying biomass ($\mu_{\max,D}$), while the results of phase no. 2 for the determination of half-saturation constant for nitrate (K_{NO_3}). The estimated values of $\mu_{\max,D}$ and K_{NO_3} are presented in Table 2.

The values of $\mu_{\max,D}$ obtained in each performed NUR test were relatively high (Table 2). So was the mean value of $\mu_{\max,D}$ calculated upon them, which equalled 0.60 h⁻¹. To compare, the values of maximum specific growth rate for ordinary heterotrophic organisms (OHOs) $\mu_{\max,H}$ in the activated sludge systems treating municipal wastewater are usually in the range from 0.17 to 0.55 h⁻¹ [1, 8]. However, for activated sludge bacteria also higher than given above values of $\mu_{\max,H}$, even up to 0.9 h⁻¹, were found [10, 11]. The value of $\mu_{\max,H}$ strongly depends on substrate composition and the conditions of growth.

The authors of ASMs assumed the value of $\mu_{\max,H}$ for OHOs equal to 0.25 h⁻¹. It means that the value of maximum specific growth rate for denitrifying heterotrophs is lower because $\mu_{\max,D} = \eta_g \cdot \mu_{\max,H}$, where $\eta_g = 0.8$. It is worth emphasizing that the default values of correction factors for anoxic conditions as well

Table 2.
Values of kinetic parameters for denitrification

Parameter	Test no. 1 24.01.11	Test no. 2 30.01.11	Test no. 3 14.03.11	Test no. 4 21.03.11	Mean value ±SD
$\mu_{\max,D}$ (h ⁻¹)	0.28	0.60	0.78	0.74	0.60 ±0.22
R^2 for $\mu_{\max,D}$	0.997	0.983	0.986	0.993	-
K_{NO_3} (mg NO ₃ -N l ⁻¹)	1.11	0.81	2.05	1.33	1.32 ±0.52
Mean NUR (mg NO ₃ -N l ⁻¹ h ⁻¹)	2.41	2.30	2.57	1.99	2.32 ±0.25
NUR _{max} (mg NO ₃ -N l ⁻¹ h ⁻¹)	3.65	3.17	4.11	2.78	-
Mean SNUR (mg NO ₃ -N g VSS ⁻¹ h ⁻¹)	1.40	1.57	1.77	1.33	1.52 ±0.20
Mean SNUR (mg NO ₃ -N g COD ⁻¹ h ⁻¹)	2.01	2.24	2.53	1.88	2.17 ±0.28

Table 3.
Comparison of the values of SNUR

Carbon source	SNUR (mg NO ₃ -N g VSS ⁻¹ h ⁻¹)	Literature
Acetate	1.33-1.77	this study
Different single and binary and complex substrate (lactate, lactose, proteins, fat)	1.7-6.9	[15]
Municipal wastewater (fraction S _S)	1-3	[14]
Municipal wastewater (fraction X _S)	0.6-1	[14]
Methanol	2-58	[16]
Acetate	2-4	[17]

as maximum specific growth rate for OHOs under aerobic conditions were often elevated within the calibration of ASMs. For example, *Makinia* et al. [12] increased the value of η_g from 0.8 to 0.9 in the calibration of model ASM2d for the Hanover-Gümmerwald pilot wastewater treatment plant (WWTP).

The values of K_{NO_3} determined in this study were also higher than the values of this parameter assumed in ASMs. Here, the values of K_{NO_3} were between 0.81 and 2.05 mg NO₃-N l⁻¹ (Table 2), while in ASMs the default value of K_{NO_3} is 0.5 mg NO₃-N l⁻¹. It should be noticed that the values of half-saturation constants assumed in ASMs are relatively low. It particularly concerns half-saturation constant for growth of OHOs (K_S), whose default value equals 4 mg O₂ l⁻¹ for growth on soluble biodegradable fraction of COD (S_S). This value indicates that the affinity of activated sludge biomass to municipal wastewater is very high. However, in real conditions municipal wastewater can often be less willingly metabolised by bacteria. Therefore, the need to elevate the values of K_S occurs [13].

The high values of both Monod kinetic parameters estimated in this work could have resulted from the conditions of the test, which favoured denitrifying biomass. Nevertheless, the obtained values of kinetic parameters indicate that a large fraction of OHOs is able to use nitrate as the terminal electron acceptor and/or there is a small difference between the growth rate under anoxic and aerobic conditions. It also indicates that the default values of these parameters in ASMs are relatively low and one should be careful during the calibration process of ASMs.

In Table 2 the mean values of volumetric nitrate uptake rate (NUR) and specific nitrate uptake rate (SNUR) calculated for each NUR test are collected. Additionally, maximum volumetric NUR was included. SNUR was calculated with regard to biomass

expressed in VSS and COD. The obtained values of NUR and SNUR did not differ from the values found experimentally by other authors [14, 15]. The rate of denitrification strongly depends on the carbon source. It is well illustrated in Table 3, in which the literature data for denitrification on different carbon sources were compared with the results of the present study.

4. CONCLUSIONS

The performed NUR tests ran properly because both nitrates and nitrites were depleted. Also the time courses of other components as COD, alkalinity and biomass confirmed it. The methodology of NUR test proposed in this study occurred to be reliable to study the kinetics of denitrification. What is more important, it allows not only for the determination of volumetric and specific nitrate uptake rate but also for the calculation of Monod kinetic parameters. Thus, the proposed procedure gives the chance for the direct, experimental determination of kinetic parameters for denitrifying biomass to replace the correction factors, which are commonly used in the complex activated sludge models.

The determined values of $\mu_{max,D}$ were relatively high in comparison to the values of $\mu_{max,H}$ for growth of heterotrophic biomass under aerobic conditions assumed in ASMs and varied from 0.28 to 0.78 h⁻¹. It shows that the difference between the growth rate in aerobic and anoxic conditions for mixed cultures is small and high percentage of OHOs is able to use nitrate as the terminal electron acceptor. The values of K_{NO_3} calculated in this work varied from 0.81 to 2.05 mg NO₃-N l⁻¹. At the same time the mean values of specific NUR were between 1.33 and 1.77 mg NO₃-N g VSS⁻¹ h⁻¹ and were in the range of those achieved in the systems with acetate as the carbon source.

Applying the proposed procedure a user should be aware that it may favour denitrifying biomass compared to other heterotrophs. Nevertheless, the values of kinetic parameters for denitrifiers determined in accordance with this procedure, including the ones found in this work, will support those scientists and practitioners who calibrate one of the complex activated sludge models.

ACKNOWLEDGEMENTS

This work was financed by Ministry of Science and Higher Education (Republic of Poland), project no. N N209 102 637.

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