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Storage phenomena in relation to carbon sources for denitrification

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ABSTRACT

This study presents the effect of substrate composition on the observed respirometric responses in denitrification process. For this purpose different mixtures of organic substrates have been investigated for both enrichment of different cultures and respirometric batch tests. The results of the study provide examples and data on the experimental assessment of storage yield for different substrates and heterotrophic growth yield based on NUR tests. In the tests conducted with biomass acclimatized to a 4-compound substrate mixture of acetate, propionate, ethanol and glucose, the observed anoxic storage yields were assessed as 0.70 gCOD(gCOD)$^{-1}$ when fed with the same mixture and 0.71 gCOD(gCOD)$^{-1}$ when fed only with acetate and propionate. However, for the culture enriched with a 2-compound mixture of acetate–propionate, the observed storage yields were estimated as 0.61 gCOD(gCOD)$^{-1}$ when fed with the 4-compound mixture and 0.71 gCOD(gCOD)$^{-1}$ when fed with acetate and propionate. This result has been evaluated as a possible consequence of culture adaptation. The anoxic growth yields in the tests were calculated to be equivalent to an average of 0.64 gCOD(gCOD)$^{-1}$. The study could serve as a new perspective for the experimental determination of model parameters for the design of activated sludge systems for different substrate compositions.

Keywords: Activated Sludge Model No. 3 (ASM3); Readily biodegradable substrate; Respirometry; Storage; Nitrate utilization rate (NUR)

1. Introduction

Strict standards for nitrogen content in effluents of biological wastewater treatment plants impose precise analysis of the nitrogen removal system and relevant operation conditions particularly for denitrification. Reliable estimation of the electron acceptor demand is one of the key issues in determining the resulting nitrogen removal efficiency [1–3]. The nitrogen removal efficiency is strongly influenced by the carbon availability and C/N ratio in the denitrification tank should be high enough to denitrify all nitrate [4–7] otherwise an external carbon source should be supplied.

One of the external carbon sources used for denitrification in recent studies is the fermentation products of primary settled domestic sewage, which embodies a high content of volatile fatty acids (VFAs), in the nature of readily biodegradable substrate, participating in the process at the highest rate. Readily biodegradable carbon sources for denitrification alternative to methanol were examined so far [6,8–13]. Since the behavior of

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Table 1
Simplified ASM3 matrix for anoxic processes

Component i→ process	S_S COD	S_{NO} N	X_P COD	X_H COD	X_{STO} COD	Process rate equation, ρ_j, all $\rho_j \geq 0$
Anoxic storage of COD	−1	$-\frac{1 - Y_{STOD}}{2.86}$			Y_{STOD}	$k_{STO}\eta_D \frac{S_S}{K_S + S_S} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic growth		$-\frac{1 - Y_{HD}}{2.86 Y_{HD}}$		1	$-\frac{1}{Y_{HD}}$	$\hat{\mu}_H \eta_D \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic endogenous respiration		$-\frac{1 - f_{EX}}{2.86}$	f_{EX}	−1		$b_{HD} X_H \frac{S_{NO}}{K_{NO} + S_{NO}}$
Anoxic respiration of X_{STO}		$-\frac{1}{2.86}$			−1	$b_{STOD} X_{STO} \frac{S_{NO}}{K_{NO} + S_{NO}}$

the activated sludge differs with the substrate type, studies are concentrated on understanding of the performances by using the respirometric methods.

Recent experimental evidence has directed the modeling studies in activated sludge systems to the introduction of biochemical storage [14]. The process is regarded as an important biochemical mechanism in substrate removal especially under transient conditions [15–17]. The composition of the raw wastewater usually fluctuates during the course of the day and results in ''feast'' and ''famine'' periods with respect to the organic compounds, which is necessary for denitrification. In this regime, microorganisms rapidly remove organic substrate and store as polymers in the feast period. When sufficient amount of external substrate is not available during famine period the stored polymers are used for microbial activity [15,18–21].

The new model, ASM3 [14], involves entire conversion of readily biodegradable substrate into storage products prior to growth on stored polymers. The model describes storage, microbial growth and endogenous decay as energy consuming processes with different rates of electron acceptor utilization.

This study was undertaken with the main intention of defining the storage stoichiometry and kinetics by using the electron acceptor utilization rates on different carbon sources under anoxic conditions (nitrate utilization rate, NUR). The second and equally important objective of this study was to demonstrate the effect of biomass acclimation to different organic substrates. For this purpose two mixtures of readily biodegradable substrates were used to outline the differences observed in the respirometric response of different cultures under anoxic conditions.

2. Conceptual framework

The new model ASM3 was chosen to describe the stoichiometric and kinetic relationships of biochemical processes under anoxic conditions. A simplified matrix representation of ASM3 is shown in Table 1. As the table shows, the storage of organic substrate is an electron acceptor consuming process, which is identified with an anoxic storage yield of Y_{STOD}. The kinetic expression of the storage mechanism is defined by a Monod type rate expression, which involves the maximum storage rate constant k_{STO}, reduced by a factor η_D, when the electron acceptor is nitrate instead of oxygen. The factor 2.86 represents the oxygen equivalent of nitrate as electron acceptor. The stoichiometry of the growth process occurred on stored polymers (X_{STO}) is defined by the anoxic growth yield Y_{HD}. The growth rate is expressed by a saturation type equation, involving the maximum specific growth rate of heterotrophs $\hat{\mu}_H$ and the half saturation constant K_{STO}. In the model two different endogenous respiration processes are defined for the decay of heterotrophic biomass (X_H) and the decay of stored products (X_{STO}), by first order process rates with respect to X_H and X_{STO} concentrations, respectively.

The processes identified in the ASM3 model can be observed in a sequence in respirometric batch tests as

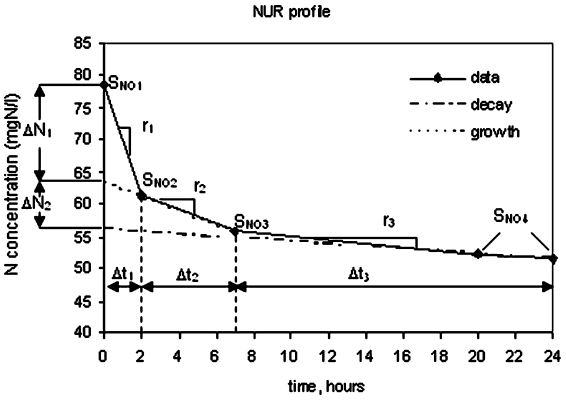

Fig. 1. Graphical representation of a typical NUR profile.

illustrated in Fig. 1. The NUR profile (S_{NO} curve) generally consists of three segments, the slopes of which represent three different rates of electron acceptor utilization, namely r_1, r_2 and r_3. In a batch anoxic reactor, the rate is affected by storage, growth and endogenous decay processes. The high level of nitrate utilization in the first stage (r_1) is representative for mainly storage besides growth and decay. The second stage (r_2) is controlled by mainly growth and the third rate is attributed to endogenous decay processes (r_3).

$$r_1 = \frac{(S_{NO1} - S_{NO2})}{\Delta t_1} \quad (1)$$

$$r_2 = \frac{(S_{NO2} - S_{NO3})}{\Delta t_2} \quad (2)$$

$$r_3 = \frac{(S_{NO3} - S_{NO4})}{\Delta t_3} \quad (3)$$

For the determination of the utilized amount of oxidized nitrogen for storage ($\triangle N_1$), the amount utilized in the first sequence is to be corrected for the interference of nitrate used for the growth and decay processes [1,2]. Depending on process stoichiometry, given in Table 1, the corresponding yields for storage and growth can be calculated with the expressions given in Eqs. (4) and (5), when the amount of readily biodegradable substrate, S_{S1}, consumed in the reactor is known.

$$Y_{STOD} = 1 - \frac{2.86 \cdot \Delta N_1}{S_{S1}} \quad (4)$$

For the calculation of the anoxic heterotrophic yield coefficient, Y_{HD}, the total amount of stored polymers, X_{STO}, has to be estimated as the fraction of initial readily biodegradable COD converted to storage products, as given below:

$$Y_{HD} = 1 - \frac{2.86 \cdot \Delta N_2}{Y_{STOD} \cdot S_{S1}} \quad (5)$$

In case of complex wastewater or substrate mixtures, the experimentally determined storage yield should be regarded as an overall observed yield, corresponding to the lumped yields of individual compounds. The amount of electron acceptor utilized for the storage of each substrate should be added in order to calculate the total nitrate consumption for storage:

$$\Delta N_1 = \frac{1}{2.86} \sum_{i=1}^{k} (1 - Y_{STODi}).S_{S1i} \quad (6)$$

The analogy is not correct for the growth yield Y_H, since the growth occurs on the stored product X_{STO} and Y_H is to be considered as the same for the similar type of stored polymers.

3. Materials and methods

Two parallel sets of experiments were conducted with two different enriched cultures. Respirometric batch tests for the measurement of NUR were conducted under anoxic conditions. Results were corrected and evaluated by considering the nitrite accumulation as described by Sözen and Orhon [3].

3.1. Biomass and growth conditions

The biomass was originated from a denitrifying domestic wastewater treatment plant was used as seed for enrichments. Two different cultures, namely culture A and culture B, were enriched in two parallel reactors. Reactors with a 1 L volume were operated in fill and draw mode in a sequence of aerobic and anoxic conditions, with a hydraulic retention time of 1 day and a sludge age of 7 days. Culture A has been acclimatized to a substrate mixture consisting of 50% acetate, 20% propionate, 10% ethanol and 20% glucose on COD basis. Culture B has been acclimatized to a substrate mixture of only 50% acetate and 50% propionate. Synthetic wastewater was prepared with substrates mentioned above and mineral medium with trace elements and phosphate buffer (K_2HPO_4, 640 mg L^{-1}; KH_2PO_4, 320 mg L^{-1}; $MgSO_4 \cdot 7H_2O$, 30 mg L^{-1}; $FeSO_4 \cdot 7H_2O$, 1 mg L^{-1}; $ZnSO_4$ċ$7H_2O$, 1 mg L^{-1}; $MnSO_4 \cdot H_2O$, 0.6 mg L^{-1}; and $CaCl_2$, 4.0 mg L^{-1}).

3.2 Batch experiments

Batch experiments were carried out to estimate the electron acceptor utilization rates on different carbon compounds under anoxic conditions. Batch experiments were conducted in 50 mL serum bottles, each bottle inoculated with 3 mL of biomass from culture A and culture B as identified in Table 2. The mixed liquor volume was adjusted to 40 mL and N_2 was flushed through the reactor to remove oxygen. In all sets C/N ratio was supplied as 3:1 to avoid nitrate limitation with a concentrated solution of nitrate (KNO_3). The reactors were maintained in suspension with continuous mixing. Batch experiments with culture A were conducted in two parallel NUR tests as A1 and A2. In test A1, the mixture of acetate, propionate, ethanol and glucose were used, while the second test (A2) was conducted with acetate/propionate mixture.

Table 2
Experimental set-up of batch tests

Culture	Set no.	Substrate mixture	COD (mg L^{-1})	NO_3^-–N (mg L^{-1})
A	A1	50% acetate + 20% propionate + 10% ethanol + 20% glucose	160	65
	A2	50% acetate + 50% propionate	150	80
B	B1	50% acetate + 20% propionate + 10% ethanol + 20% glucose	160	75
	B2	50% acetate + 50% propionate	150	90

Analogous tests were also performed with culture B. The initial COD and nitrate contents in the reactors are also given in Table 2. COD was measured according to open reflux titrimetric method as described in ISO [22]. Ammonium (NH_4^+) [23], nitrate and nitrite (NO_2^- and NO_3^-) measurements were performed on filtered samples by colorimetric methods [24].

4. Experimental results

The results obtained were evaluated on the basis of process stoichiometry and kinetics with the emphasis on the effect of substrate composition and biomass acclimation. Respirometric batch tests carried out with two different acclimatized biomasses and two different substrate mixtures are illustrated in Figs. 2 and 3. Three phases with descending slopes could be identified in all the experiments as shown in the figures. The different NURs were used for the assessment of storage, growth and decay parameters as described in the conceptual framework.

Results obtained from the experiment on culture A indicate that the storage process has been completed in 2 h for both the sets, A1 and A2. The kinetic structure of NUR experiments has been considered in terms of volumetric rates with the understanding that a direct comparison is not justifiable as the literature data are expressed on VSS basis. The rates in the first phase (r_1), representing mainly the storage rates, were estimated as 11 and 10 mg L^{-1} h^{-1} for sets A1 and A2, respectively.

The profiles showed that the microbial growth phase lasted 5–6 h with a rate of 2 mg L^{-1} h^{-1} for both the sets. The third rate associated with endogenous decay resulted in much lower rates of 0.25 and 0.20 mg l^{-1} h^{-1} for A1 and A2, indicating that the substrate available in the reactor has been depleted. Besides the kinetic interpretation, these sets have also been used for the stoichiometric evaluation. The NURs (corrected for nitrite accumulation) were effectively used for the assessment of the storage yields by using Eq. (4). An observed storage yield of 0.70 gCOD(gCOD)$^{-1}$ was calculated for set A1, whereas 0.71 gCOD(gCOD)$^{-1}$ was found for A2. Using the results of set A2, fed only with acetate and propionate, the anoxic storage yield of propionate was estimated as 0.77 gCOD(gCOD)$^{-1}$ by Eq. (6), with the assumption of a Y_{STOD} of 0.66 gCOD(gCOD)$^{-1}$ for acetate as determined by [25]. Following this assessment, the anoxic storage yield of ethanol was calculated as 0.68 gCOD(gCOD)$^{-1}$, using the experimental data for glucose as 0.90 gCOD(gCOD)$^{-1}$ under aerobic conditions [26], reduced by an η factor of 0.85. An equally important issue is the assessment of the growth yield. The observation in the second phase of the NUR tests resulted in anoxic yields of 0.61 and 0.65 gCOD(gCOD)$^{-1}$ for the sets of A1 and

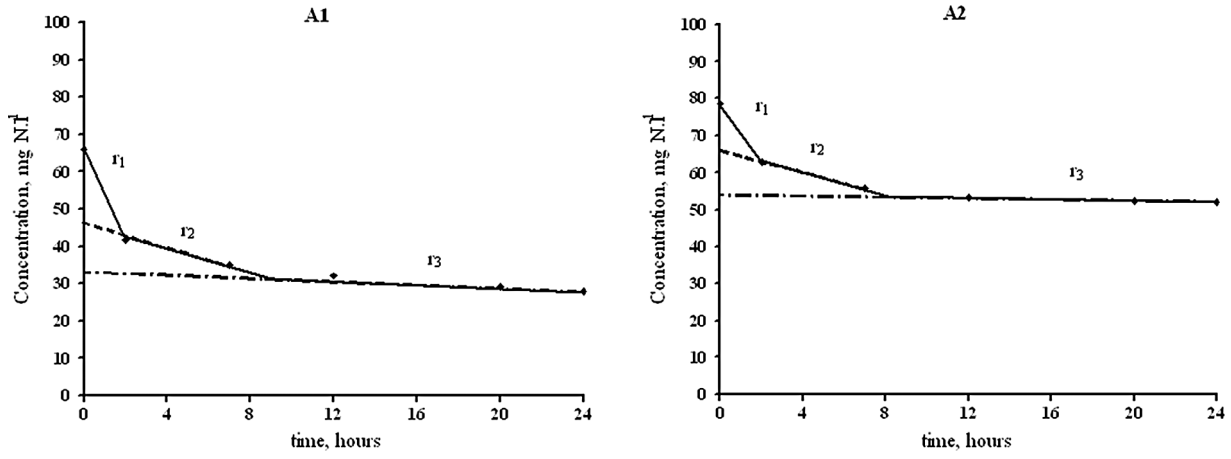

Fig. 2. Illustrations of NURs (r_i) in anoxic batch experiments with culture A, (a) batch test no. A1 and (b) batch test no. A2.

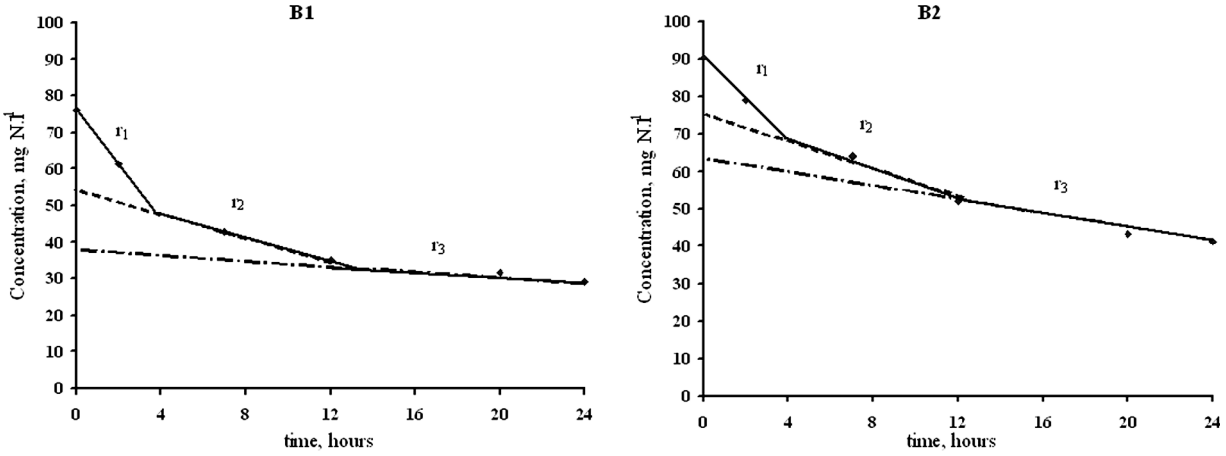

Fig. 3. Illustrations of NURs (r_i) in anoxic batch experiments with culture B, (a) batch test no. B1 and (b) batch test no. B2.

A2, respectively, higher than 0.50 gCOD(gCOD)$^{-1}$, theoretically calculated for domestic wastewater on the basis of energetic considerations [27].

The other group of experiments on culture B is illustrated in Fig. 3. Same procedures were applied on the experimental data for the assessment of volumetric rates reflected that r_1 and r_2 were the same for both B1 and B2. The first rate was assessed as 9 mg L^{-1} h^{-1}, whereas the rate of growth phase was calculated as 2 mg l^{-1} h^{-1}. A significant point of interest reflected by the experimental results was the observation in the endogenous decay phase exerting a utilization rate of 0.5 mg L^{-1} h^{-1}, which is twofold higher than the rate obtained with culture A. This observation can only be explained by a possible interference of the utilization of nitrate due to extended growth.

It has been observed that only 58% of the maximum storage yields could be achieved for glucose and ethanol in system B1, when maximum Y_{STOD} for glucose is 0.77 gCOD(gCOD)$^{-1}$ and maximum Y_{STOD} of ethanol is 0.68 gCOD(gCOD)$^{-1}$ as found in system A1. This is due to insufficient adaptation, since the culture was not able to adjust its metabolism for maximum storage capacity in the period of the short test. At this point, due to the lack of optimization for storage of glucose and ethanol, the electron acceptor consumption was greater and thus, the process efficiency was lower than that of the acclimatized mixed culture A. On the other hand, evaluation of the tests on enriched culture B2 showed that the calculated Y_{STOD} for propionate was 0.76 gCOD(gCOD)$^{-1}$ confirming the yield achieved in system A2. The growth yields observed for culture B have been estimated to be the same for both sets B1 and B2, the Y_{HD} values being 0.64 gCOD(gCOD)$^{-1}$. This result can be interpreted as a consequence of the assumption of ASM3 stating that growth only occurs on stored products.

5. Conclusions

Nitrate utilization profiles provide a sensitive basis for both biochemical storage and heterotrophic growth stoichiometry and kinetics. Evaluation of batch tests conducted showed that the observed storage yields for substrate mixtures were lumped yields and these experimental values should be investigated in detail to obtain individual anoxic storage yields of each compound. In this context, the anoxic storage yield of propionate was estimated as 0.77 gCOD(gCOD)$^{-1}$, with the assumption of an Y_{STOD} of 0.66 gCOD(gCOD)$^{-1}$ for acetate and that of ethanol was calculated as 0.68 gCOD(gCOD)$^{-1}$, using the experimental data for glucose as 0.90 gCOD(gCOD)$^{-1}$ under aerobic conditions, reduced by a factor of 0.85. The anoxic growth yields in the tests were calculated to be equivalent to an average Y_{HD} of 0.64 gCOD(gCOD)$^{-1}$.

The comparative evaluation of NUR profiles showed that the NURs in the three different phases of the batch experiments were in good agreement with similar electron acceptor consumption rates, except for the endogenous rate of culture B, due to possible interference of extended growth. It has been observed that insufficient adaptation which does not allow the culture to adjust its metabolism for maximum storage capacity to a new substrate introduced to the system in the period of the short test, only 58% of the maximum storage yields could be achieved for glucose and ethanol in the system seeded with culture enriched with acetate and propionate. At this point, it can be concluded that when the substrate composition is changed under dynamic conditions, the electron acceptor consumption will be greater due to the lack of optimization of the microbial population for storage and thus, the process efficiency of the culture will be lower than that of the acclimatized mixed culture.

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Symbols

η_D	Reduced factor
$\triangle N_1$	The utilized amount of oxidized nitrogen for storage
b_{HD}	Endogenous respiration coefficient for denitrifiers (day^{-1})
b_{STOD}	Anoxic decay rate of stored polymers (day^{-1})
f_{EX}	The fraction of inert metabolic products generated (gCOD/gCOD)
K_S	Half-saturation constant of substrate (mg COD/L)
K_{STO}	Half-saturation constant of stored polymers (gCOD/gCOD)
K_{NO}	Half-saturation constant for nitrate concentration (mg N/L)
k_{STOD}	Maximum rate of anoxic storage (day^{-1})
S_{NO}	Nitrate used as the electron acceptor (mg N/L)
S_S	Readily biodegradable COD (mg COD/L)
X_H	Heterotrophic biomass (mg COD/L)
X_{STO}	Storage polymers (mg COD/L)
$\hat{\mu}_{HD}$	Maximum growth rate for denitrifiers (day^{-1})
Y_{HD}	Anoxic yield coefficient for growth on stored polymers (gCOD/gCOD)
Y_{STOD}	Anoxic storage yield (gCOD/gCOD)

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