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# A kinetic study on drinking water denitrification using a membrane bioreactor

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**Abstract:** This study determines the basic parameters of Monod kinetics for microbial growth within a membrane bioreactor using the Zenon ZeeWeed 10 MBR system. The influent nitrate concentration was kept at 70 ± 2 mg $L^{-1}$ $NO_3^-$. During the experiments a constant concentration of activated sludge was maintained at approximately 0.76 g $L^{-1}$ under anoxic conditions. Sucrose was added to the activated sludge as a carbon source. The Monod kinetic parameters were calculated by numerical interpolation, by considering experimental data. The maximum specific growth rate of the biomass was determined to be 0.31 $h^{-1}$, half-saturation constant 5.4 mg $L^{-1}$, and yield coefficient 0.35 mg biomass $mg^{-1}$ COD. Afterwards, a dynamic simulation was performed within the calculated parameters. The dynamic concentration profiles for substrate and biomass were determined at different dilution rates within the range of 0.8 to 5 $d^{-1}$.

**Keywords:** kinetics, denitrification, drinking water, membrane bioreactor, sucrose

## 1 Introduction

Nitrate and nitrite removal from water have attracted significant attention over recent years, due to their risks to human health. The harmful effects of nitrates on human health are reported as methaemoglobinemia or Blue-baby syndrome [1-3], and nitrosamines, nitrosamides [4], which can cause the formation of carcinogens in the stomach.

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During the biological process of denitrification, nitrate is first microbiologically reduced to nitrate and nitrite, then nitric oxide (NO) and nitrous oxide ($N_2O$), and finally to molecular nitrogen ($N_2$) [1,5]. The biological removal of nitrate can be affected by various factors: different types of external C sources [6,7], various types of micro-organisms [6,8], various operational parameters such as C/N ratios [2,9,10], temperature [3,11-13], pH [3,11], dissolved oxygen [12,14,15], hydraulic retention time [16-18], nitrate and nitrite concentratios [11,19] and mixed liquor suspended solids [10,20]. In addition, nitrate removal highly depends on the substrate amount that influences the denitrification rate, denitrification yield, and the composition of the microflora [6,21]. The residual carbon sources can cause several problems during drinking water treatment despite the advantages of hetetrotrophic denitrification due to the high denitrifying rates [3]. Therefore, many researchers have focused on certain carbon sources like methanol [1,2,6,22], ethanol [2,10,23], acetate [7,11], glucose [7,9,22], glycerol [22], and acetic acid [22]. On the other hand, sucrose is relatively rare and has only been mentioned in a few studies. Gómez et al. [2] compared three carbon sources (sucrose, ethanol, and methanol) on submerged filters for the removal of nitrate from contaminated groundwater (100 mg $L^{-1}$ $NO_3^-$). Greater biomass production was observed with sucrose, when compared with ethanol and methanol. Fernández-Nava et al. [4] examined the properties of sacharose-rich residue during the denitrification process. Crude syrup as a C source was used in another research performed by Lee and Welander [6]. Sisson et al. [24] used sucrose in the denitrification by biological granular-activated carbon. The influent $NO_3$-N concentration was 80 mg $L^{-1}$ (carbon to nitrogen ratio C/N = 1.88/1), and the average denitrification efficiency achieved 84 to 89%. During the research when the C/N ratio increased from 1.5 up to 2.5, the removal efficiency increased up to 95% [25].

However, the MBR system is, in general, less commonly used for drinking water treatment. Nitrate removal from contaminated groundwater, drinking water, and surface water has been examined by using extractive MBRs [26], ion-exchange and gas-transfer MBRs [20],

pressure-driven MBRs [10,16,20] and other known hybrid systems. The Zenon ZW 10 membrane bioreactor was used in the denitrification of drinking water sources by Buttiglieri et al. [27].

Miscellaneous models for describing the process kinetics have been studied so far. Although, the most commonly used relation describing microbial growth is Monod kinetics [28-30] and the heterotrophic denitrification is usually assumed as being described by Monod expressions [19]. There are several factors next to the pH, temperature, and dissolved oxygen [12,31], that can affect microbial growth and kinetics: type of substrate [2,9], microbial population [13,32,33], type of water source [32]. Microbial growth, the kinetics, and the influences of different physico-chemical factors have been extensively investigated over recent papers [12,29,30,34].

The basic purpose of this research was to develop a kinetic model to describe the microbial growth of drinking water denitrification using MBR. A kinetic analysis was conducted by assuming Monod kinetics to be appropriate for substrate consumption, and a constant biomass concentration. First, the kinetic parameters on the basis of experimental data were determined: specific growth rate of biomass, substrate half-saturation constant, and the yield coefficient. Further, a dynamic simulation was performed dependent on the calculated kinetic parameters.

## 2 Methodology

The mode of MBR operation was due to the biomass characteristics close to the model of mixed flow bioreactor under a steady-state. The increase of biomass was neglected because of it being very low, and the influent and effluent were free biomass. The balance of biomass was obtained by using the equations for a continuous stirred-tank reactor with recycle first and the reactor without recycle second. During the second approach it was assumed that the biomass concentration in the circulation would be equal to that of the reactor, and thus produce the same result in both cases, namely the specific growth rate of micro-organisms would be equal to the dilution rate ($D = \mu$). The dilution rate (in other words the reciprocal of the residence time) is defined as the quotient of the influent flow-rate and the bioreactor volume, D = q/v ($h^{-1}$). In regard to the substrate, the equations for the continuously stirred-tank reactor with recycle were used.

Two constants were used to express the specific growth rate of biomass ($\mu$) by Monod kinetics [30,31,34]: $\mu_{max}$ ($h^{-1}$) is the maximum growth rate constant, $K_S$ represents the half-saturation constant (g $L^{-1}$) and $y_S$ is the substrate concentration (g $L^{-1}$) (Eq. 1):

$$\mu = (\mu_{max}\, \gamma_S)/(K_S + \gamma_S) \quad (1)$$

A zero-order kinetic model is usually used at high substrate concentrations, and first-order dependence can be applied and at low substrate concentrations [11,34].

The specific growth rate of active biomass is reduced due to the endogenous decay of active biomass, where $k_d$ ($h^{-1}$) represents the endogenous decay-rate [31]. Thus dynamic changes in the biomass concentration over time can be written as follows [$y_0$ and $y_X$ are the mass concentrations of biomass in the influent and effluent (g $L^{-1}$)] (Eq. 2):

$$(d\gamma_X)/dt = D(\gamma_0 - \gamma_X) + ((\mu_{max}\, \gamma_S)/(K_S + \gamma_S) - k_d)\gamma_X \quad (2)$$

The yield coefficient [$Y_{X/S}$ – amount of biomass produced in regard to the amount of food consumed (g $g^{-1}$)] can be determined according to the following equation, which describes the mass balance of substrate in the steady-state (Eq. 3):

$$D(\gamma_{S,0} - \gamma_S) = (1/Y_{X/S})\mu\gamma_X \quad (3)$$

Where $y_{S,0}$ and $y_S$ are the mass concentrations of substrate in the inflow and outflow (g $L^{-1}$). Since we assumed that during the consumption of the substrate and thus in the production of biomass only active biomass would be involved, the variable $w_X$ was therefore inserted into Eq. 3, representing the percentage of active biomass (%) (Eq. 4):

$$D(\gamma_{S,0} - \gamma_S) = (1/Y_{X/S})\mu w_X\gamma_X \quad (4)$$

Literature provides extensive information regarding the proportion of active biomass, depending on a number of factors [8,33,35]. The viability of biological sludges can be expressed as the active bacterial concentration per unit mass of volatile suspended solids [35]. Dynamic changes in the substrate concentration over time can be displayed as follows (Eq. 5):

$$(d\gamma_S)/dt = D(\gamma_{S,0} - \gamma_S) - (1/Y_{X/S})((\mu_{max}\gamma_S)/(K_S + \gamma_S))\gamma_X \quad (5)$$

Determination of the Monod kinetic parameters was based on the experimental values for the mass concentration of substrate at the outflow ($y_S$), and the calculated dilution rates ($D$). For this reason, numerical interpolation (method of least squares) was performed using a Matlab software program.

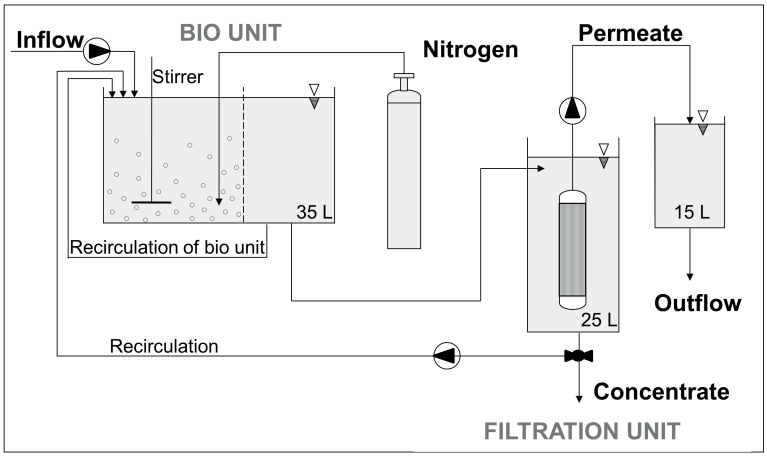


**Figure 1:** The process scheme of MBR for drinking water treatment

## 2.1 Set-up of membrane bioreactor and operating conditions

Experiments were performed using the Zenon ZeeWeed 10 membrane bioreactor having a 60 L volume. The reactor was operated under anoxic conditions at 26.3°C on average, and the pH value within the range of 7.4–8.7. Variations in operating temperatures were a result of changes in the external temperatures. The membrane module consisted of a submerged hollow-fibre membrane with a pore size of 0.04 μm and a 0.93 $m^2$ active area. The process for the drinking water treatment is shown in Fig. 1, and the UF membrane specifications are presented in Table 1.

The influent was simulated with sodium nitrate at a concentration of 70 ± 2 mg $L^{-1}$ $NO_3^-$, while the concentration of nitrite was 0.05 ± 0.02 mg $L^{-1}$ $NO_2^-$. The membrane bioreactor was inoculated with activated sludge from an existing water resource recovery facility. A constant concentration level of activated sludge was maintained during the experimental work at approximately 0.76 g $L^{-1}$ (expressed as MLSS), and the excess biomass was occasionally removed from the system. Sucrose was used as a substrate due to its low cost. The inflow mass concentration of sucrose was constant throughout the entire experiment (0.1126 g $L^{-1}$). Based on previous papers [2,24,25] and our previous investigations, the appropriate value for the C/N ratio was 3/1.

A series of experiments was performed to follow the influence of drinking water flow-rates (dilution rates) on the effluent substrate concentration. The flow-rate of the feed was increased stepwise from 10 to 170 mL $min^{-1}$. At each flow-rate (or dilution rate) sufficient time was ensured for establishing a steady-state.

**Table 1:** UF membrane specifications

| Specifications | Description |
|---|---|
| Type of membrane | Hollow fibre (HF) |
| Material | polyvinylidene difluoride (PVDF) |
| Surface properties | Neutral, hydrophilic |
| Nominal membrane area | 0.93 $m^2$ |
| Pore size | 0.04 μm |
| Max. temperature | 40°C |
| pH range | 5–9 |
| Max. trans-membrane pressure | 62 kPa |
| Max. pressure of backpulse | 55 kPa |
| Max. capacity of process pumps | 1.4 L $min^{-1}$ |

## 2.2 Analytical methods

All analyses were carried out daily except for the activated sludge concentration determination. The samples were filtered to exclude any solids that might be present in the water. Chemicals prescribed as standards were used for the analyses.

Activated sludge concentration (MLSS-mixed liquor suspended solids) was determined according to standard methods for the examination of water and wastewater given by APHA 1995 [36]. The sample (25ml) was filtered through a filter paper labelled with black tape and dried to a constant weight (3 hours) at 105°C. Analyses for nitrate and nitrite concentrations were conducted spectrophotometrically by using an Agilent 8453 UV-Visible Spectrophotometer at 324 and 540 nm wavelengths, respectively [37,38]. The calculations for both analyses were made according to the previously

prepared calibration curve. Sucrose concentrations within the influent and effluent were determined indirectly by the method of measuring the chemical oxygen demand (COD). The COD was determined by a volumetric method using titration with $KMnO_4$ (Determination of the permanganate index) according to ISO standard 8467:1993 [39]. 25 ml of the sample was used for the COD analysis. The samples were diluted before the analysis if warranted. A solution of resorcinol was used as a control sample. In addition, the flow and circulation of the water were also monitored.

# 3 Results and discussion

## 3.1 Determination of the Monod kinetics parameters

The experimental data required for the numerical interpolation, the mass concentration of substrate at the outflow ($y_S$) and the dilution rate ($D$), are presented in Table 2. Mass concentration of substrate is expressed as chemical oxygen demand. The nitrate and nitrite concentrations at the outflow in a steady-state depending on the dilution rate, are shown in Fig. 2. Such conditions allowed nitrate removal efficiency of up to 98%.

Eq (1) was adopted since the MBR operation mode was close to the mixed-flow bioreactor under steady-state (at steady-state: $\mu = D$). The curve $D = f(y_S)$ was plotted when compiling this equation, as shown in Fig. 3. The following results for Monod kinetics were obtained by numerical interpolation: maximum specific growth rate of biomass $\mu_{max}$ = 0.31 $h^{-1}$ (7.4 $d^{-1}$) and the half-saturation constant (as COD) $K_S$ = 5.4 mg $L^{-1}$, both with $R^2$= 0.94.

Within the existing literature there is little information regarding the Monod parameters for drinking water denitrification, and it is impossible to find data relating to the value of maximum specific growth-rate and the half-saturation constant for similar denitrification processes. However, a few values are available for wastewater. Sözen et al. [15] studied the heterotrophic denitrification of domestic water. The values reported for $\mu_{max}$ were 1–6 $d^{-1}$ and for $K_S$ 2.5–20 mg $L^{-1}$. Tchobanoglous et al. [40] indicated that typical kinetic coefficients for activated sludge process regarding domestic wastewater (at 20°C) were between 15 to 70 mg $L^{-1}$ for $K_S$ and from 2 to 10 $d^{-1}$ for $\mu_{max}$. The Monod kinetic coefficients for heterotrophic growth determined by Vesilind [41] were within the range of 3–13 $d^{-1}$ for $\mu_{max}$ and 10–20 mg $L^{-1}$ for $K_S$. The results of this study aligned with the reported values. However, the half-saturation constant in this research was lower compared with the above-mentioned studies while, on the other hand, the value for the maximum specific growth-rate of biomass was quite similar. The differences between the obtained results and those values from literature are probably due to several factors that may influence microbial growth and product formation: temperature, pH, dissolved oxygen concentration, microbial population, nutrient composition, and changes in the physicochemical properties [31,32,34]. Usually, the values for $\mu_{max}$ vary particularly regarding the type of micro-organism, and the $K_S$ depending on the type of substrate [34].

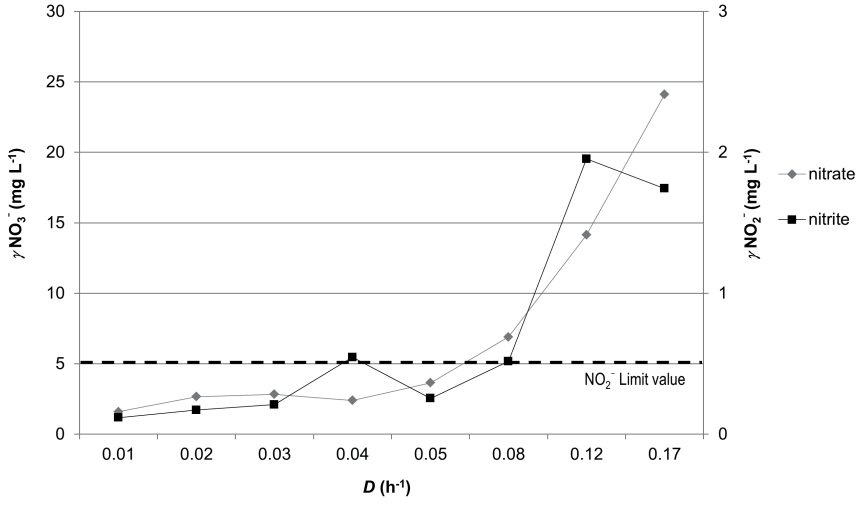


**Figure 2:** The concentrations of nitrate and nitrite at the outflow in a steady-state depending on the dilution rate

**Table 2:** Experimentally-determined mass concentration of substrate vs the dilution rate

| $\gamma_S$ (mg L$^{-1}$) | $D$ (h$^{-1}$) |
|---|---|
| 0 | 0 |
| 0.51 | 0.01 |
| 0.77 | 0.02 |
| 0.79 | 0.03 |
| 0.96 | 0.04 |
| 1.07 | 0.05 |
| 1.12 | 0.08 |
| 3.10 | 0.12 |
| 7.02 | 0.17 |

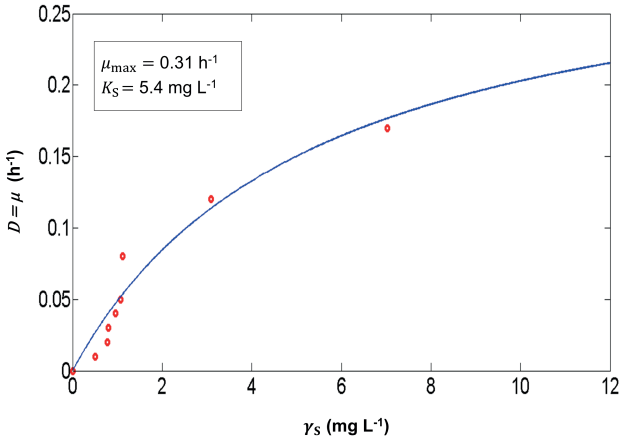


**Figure 3:** The specific growth rate of biomass as a function of substrate concentration at the outflow

During the next step of the study, the yield coefficient was determined, and dynamic simulation was performed afterwards. The value of the yield coefficient was computed according to Eq. (4). The equation considered whether in the consumption of the substrate and thus during the production of biomass, only the active part of the biomass was involved. Sears et al. [8] reported that under typical operating conditions, the microbial fraction of the activated sludge flocs represents approximately 40% by weight, while Chung and Neethling in their research discovered that only 5 to 10% of the total volatile suspended solids represented active bacterial biomass. Similar values for MBR processes have been reported, namely that an active fraction of biomass is between 4 and 7% [33]. Based on these data the active fraction of biomass ($w_x$) in this study was set at 5%. Numerical interpolation of the experimental results (Table 2) using the Matlab software program (method of least squares) was performed in order to determine the yield coefficient ($Y_{X/S}$). The calculated value of the yield coefficient was $Y_{X/S}$ = 0.35 mg biomass mg$^{-1}$ COD ($R^2$ = 0.94), which meant that approximately 35% of biomass was produced regarding the consumed substrate.

To date no data for the yield coefficient have been available for drinking water denitrification by MBR using sucrose as carbon source. However, the values for the yield coefficients of heterotrophic growth reported by Vesilind [41] were within the range of 0.46–0.69, which were higher than those in this investigation. The growth yield for saccharose-rich crude syrup obtained during another research was within the range of 0.26–0.35 g TSS g$^{-1}$ COD removed (TSS – total suspended solids in g L$^{-1}$) [6]. The yield coefficient of the aerobic organism's growth using glucose was typically between 0.4–0.6, while the anaerobic growth was less efficient and the yield coefficient was reduced substantially [31]. Thus, the results for the yield coefficient obtained during this research are quite close compared to previously mentioned values.

## 3.2 Dynamic simulation

Dynamic simulation was performed based on the results obtained for $\mu_{max}$, $K_S$ and $Y_{X/S}$. Using dynamic simulation by means of the Scientist software program, the time required to establish steady-state was estimated and the impact of the dilution rate was studied on the concentration profiles of the substrate and biomass. The applied equations were as follows: Eq. (2), which provides the dynamic changes in the biomass concentration over time, and Eq. (5), which describes the dynamic changes of the substrate concentration over time. The dilution rates used during the dynamic simulation were chosen according to the experimental values, and varied from 0.8 to 5 d$^{-1}$ (or from 0.03 to 0.21 h$^{-1}$, Table 2). The value for the specific endogenous decay rate for the heterotrophic biomass was taken from literature [41], $k_d$ = 0.05 d$^{-1}$. All other parameters remained unchanged. Dynamic simulation was performed according to the proposed model by anticipating two different outflow substrate concentrations at the start of an operation (i.e. at the time of zero): first, the value of $y_S$ close to zero (the software allows a minimal value of 0.001 g L$^{-1}$) and second, the actual experimental value, 0.1126 g L$^{-1}$. These values allow the creation of two concentration profiles depending on which the start-up of the drinking water denitrification process may be carried out. The dynamic concentration profiles can be seen in Figs. 4 and 5, respectively.

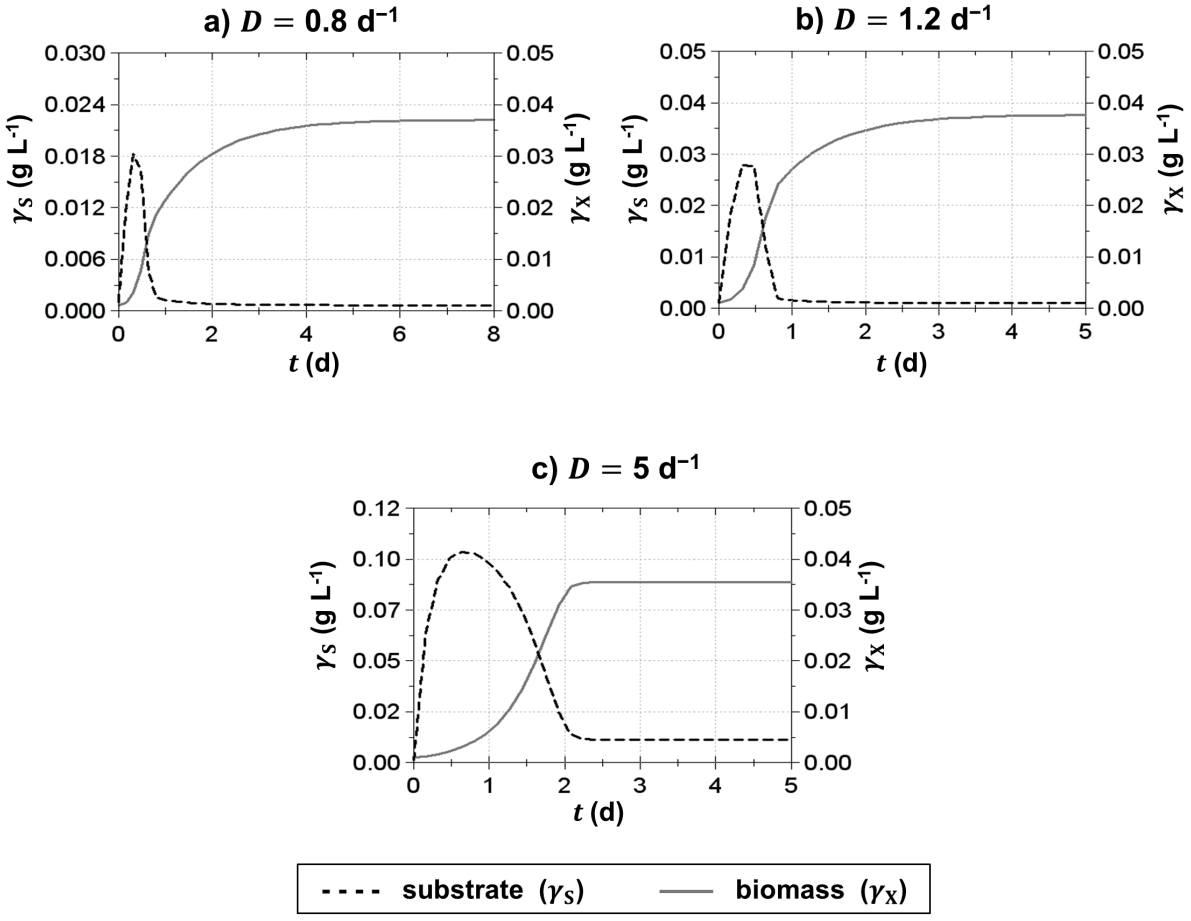


**Figure 4:** Dynamic concentration profiles ($\gamma_S$ = 0.001 g L$^{-1}$) for substrate and active biomass at three different dilution rates: a) $D$ = 0.8 d$^{-1}$, b) $D$ = 1.2 d$^{-1}$ and c) $D$ = 5 d$^{-1}$

Fig. 4 shows that the time required to establish a steady-state decreased with any increase in the dilution rate. At flow-rates lower than $D$ = 0.8 d$^{-1}$, the time needed to reach the steady-state was nearly 20 days, but decreased by 4–6 days in the cases of the dilution rates $D$ = 0.8 and 1.2 d$^{-1}$, respectively (Figs. 4a and 4b). At higher flow-rates (Fig. 4c), however, it was shorter than 2.5 days. During the adaption phase, the amount of biomass was low and the substrate concentration was high, and consequently the substrate was consumed less. After a while, the value of the substrate reduced (because of increased consumption) and the biomass increased to a value corresponding to the steady-state. Fig. 4a shows that a steady-state was achieved after approximately six days of continuous operation. The active biomass and substrate concentrations in steady-state were 37 mg L$^{-1}$ and 0.8 mg L$^{-1}$, respectively. At a dilution rate of 1.2 d$^{-1}$ (Fig. 4b) steady-state was achieved in 4 days. The concentration of substrate in the steady-state at this dilution rate increased to 1.5 mg L$^{-1}$, while the concentration of biomass was quite similar. At higher dilution rates steady-state was achieved even faster, but the substrate concentration in the steady-state increased up to 9 mg L$^{-1}$, and the biomass concentration decreased to 35 mg L$^{-1}$. Thus, the substrate concentration in the steady-state increased with the flow-rate.

During the final phase of research, a second model was developed based on an actual substrate concentration at the outflow, 0.1126 g L$^{-1}$. Figs. 4 and 5 comparisons show that the outflow substrate concentration at the start of an operation has insignificant impact on the concentration of active biomass and substrate in the steady-state. It just causes a change in the shape of the profile at the beginning of the operation. The times required to reach the steady-states (for each dilution rate) were practically the same as presented in Fig. 4. Fig. 5a shows that steady-state was achieved in approximately five to six days, which is similar or almost the same as presented in Fig. 4a. The same applied for dilution rate 5 d$^{-1}$, where the times

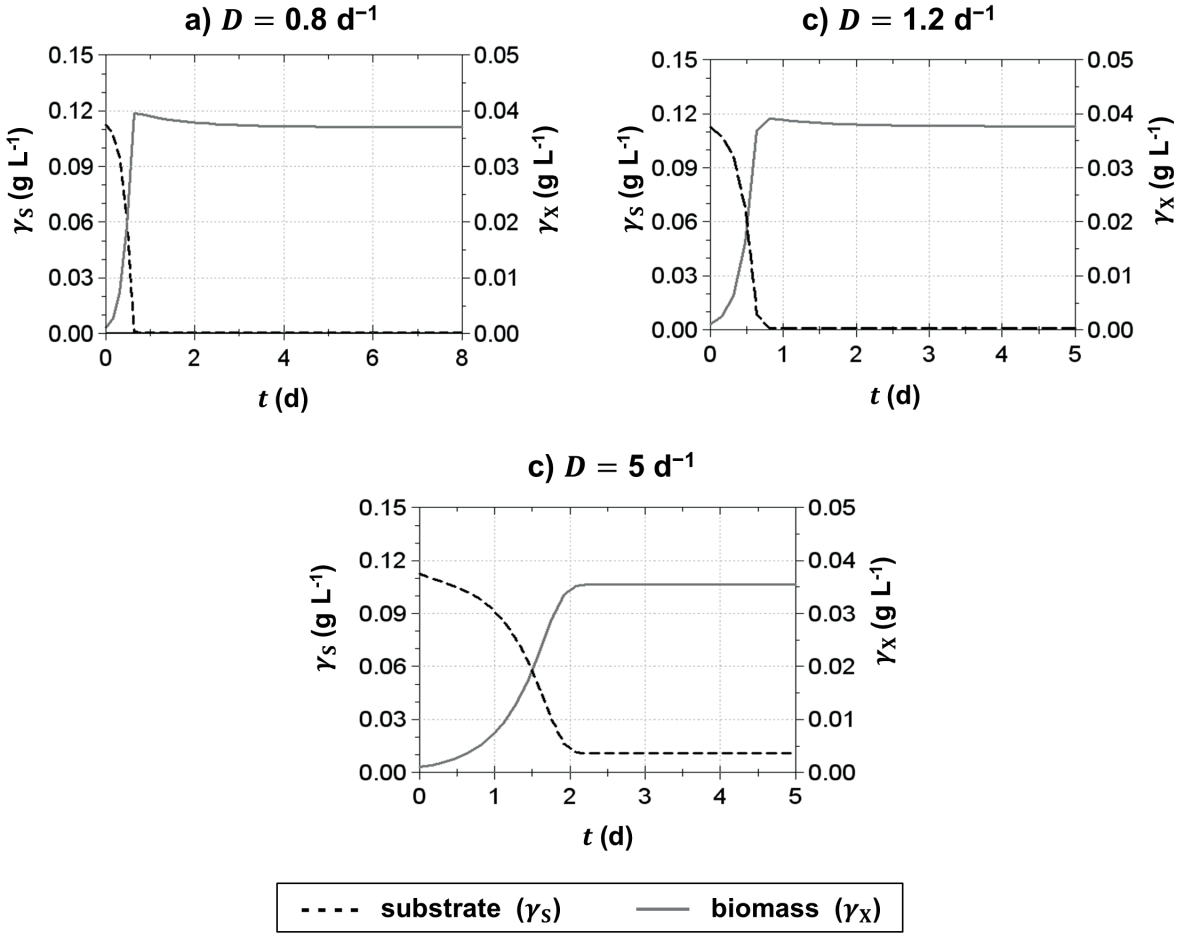


**Figure 5:** Dynamic concentration profiles ($\gamma_s$ = 0.1126 g $L^{-1}$) for substrate and active biomass at dilution rates: a) $D$ = 0.8 $d^{-1}$ b) $D$ = 1.2 $d^{-1}$ and c) $D$ = 5 $d^{-1}$

needed to reach the steady-state in both cases were shorter than 2.5 days (Figs. 4c and 5c). Therefore, it can be concluded that the outflow substrate concentration at the start of an operation has insignificant impact on the final concentration of active biomass and substrate in the steady-state.

In conclusion, an efficient operation of MBR cannot be achieved without knowing the basic operational conditions and kinetic properties of the biological system. By using such simulations or models, in which the kinetic parameters and concentration profiles are taken into account, the MBR operation system can be more easily managed, and the operating conditions may be determined to ensure the highest removal efficiency and the lowest costs. As from an economic point of view, operation of the MBR is optimal only if the steady state is achieved; this work may help to improve the operations of similar full-scale systems.

# 4 Conclusions

Drinking water denitrification using a membrane bioreactor was studied and the validity verified regarding the Monod kinetics of microbial growth. First, the Monod kinetic parameters were determined by numerical interpolation of the experimental results and then a dynamic simulation was performed. The maximum specific growth-rate was determined to be 0.31 $h^{-1}$, half-saturation constant 5.4 mg $L^{-1}$ and a yield coefficient of 35%. Dynamic simulation showed that any increase in the dilution rate decreased the time required to reach the steady-state.

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