**In-situ fixed bed denitrification in sequential biofiltration: laboratory testing of solid substrates**

Josefine Filter, Christin Bosinsky, Sefine Oksal Kilinc, Aki Sebastian Ruhl and Martin Jekel

**ABSTRACT**

High nitrate concentrations in wastewater treatment plant effluents and aquifers can challenge sequential biofiltration systems in preventing nitrite and gas formation in the sand bed, as well as to achieve the regulated limit value for nitrate in potable water reuse applications. This study investigates the introduction of electron donors in the form of organic fixed bed materials as an in-situ anoxic zone into sequential biofiltration systems. Laboratory batch and column tests with straw, soft wood, peat, polylactic acid (PLA), and polycaprolacton (PCL) revealed incomplete denitrification with a hydraulic retention time below 10 h, high organic carbon leaching, especially during the first three months, and gas accumulation within the filter bed. Therefore, ex-situ denitrification prior to oxic biofilters or in a defined side-stream treatment is recommended. No enhanced transformation of trace organic chemicals was observed under nitrate reducing conditions. Peat revealed a sorption potential for 5-methyl-benzotriazole, carbamazepine, benzotriazole, and metoprolol.

**Key words** | DOM leaching, filling cost, fixed bed denitrification, gas clogging, sequential biofiltration, trace organic chemicals

**INTRODUCTION**

Surface water used for potable water reclamation can be influenced by upstream discharges of waste water treatment plant (WWTP) effluents (Karakurt et al. 2019). Besides this de facto potable reuse, (semi-)closed water cycles can lead to the accumulation of poorly degradable or even persistent trace organic chemicals (TOrCs) in the aquatic environment. Managed aquifer recharge (MAR) systems allow a brine-free removal of pathogens and dissolved organic compounds like various TOrCs with low operational costs. Sequential biofiltration (SBF) systems or sequential managed aquifer recharge technologies (SMART) represent an advancement of conventional biofilters and MAR systems.

They are characterized by two infiltration steps with an intermediate aeration or introduction of oxygen. While in the first filtration step, easily biodegradable DOC is removed associated with the consumption of oxygen, beneficial carbon limiting and oxic conditions are established in the second filtration step after aeration for enhanced TOrC removal. Those SBF systems can be realized in different forms. A pilot scale vertical column set up was tested and described by Müller et al. (2017). A further technical pilot application consists of a horizontal plug-flow reactor filled with technical sand and an in-situ introduction of oxygen (Karakurt-Fischer et al. 2020). Since these systems are promoting oxic conditions, contaminants such as nitrate, carbamazepine, or iodinated x-ray contrast agents which are predominantly removed or dehalogenated under anoxic and anaerobic conditions (König et al. 2016; Redeker
et al. 2018), might not be removed. This can be critical in potable reuse with a lack of dilution by natural waters.

The EU water framework directive seeks a good status of all waters by 2027 (The Council of the European Union 2008). Therefore, the limit of discharged total nitrogen into waterbodies sensitive to eutrophication is set below 15 mg/L for WWTPs exceeding 10,000 population equivalents (European Council 1991). The parametric value for drinking water is currently set at 50 mg/L as nitrate (11 mg NO₃⁻/L) (The Council of the European Union 2005). Especially in groundwater, critical nitrate concentrations resulting from excessive agriculture or decentralized wastewater treatment are becoming more and more challenging for drinking water supply. Meeting the required threshold values is essential to ensure a sufficient water quality in terms of nitrate concentrations for further indirect potable reuse options. A population-based cohort study of Schullehner et al. (2018) found statistically significant increased risks of colorectal cancer already at nitrate drinking water levels above 3.87 mg/L, which questions the adequacy of the current drinking water standard in regards to chronic effects.

In order to prevent the release of nitrate into aquifers, this study investigates possible options to integrate an anoxic zone as in-situ treatment as for a horizontal flow-through reactor (Figure S1, supporting information). Such a technical SBF system is characterized by a short HRT and limited filling volume. It is designed to treat WWTP effluents prior to aquifer recharge but also an application with surface water might be considerable. There are several possibilities to introduce electron donors. Continuous dosage of easily degradable organic carbon is a well-known technique to induce denitrification. However, it promotes biofilm growth and gas clogging in sand beds that cannot be backwashed. Another option is the application of H₂ as inorganic electron donor, but due to its low water solubility of only 1.7 mg/L (at 20 °C and normal pressure of 1.013 bar) it seems inapplicable for removing nitrate concentrations exceeding 16 mg/L in a non-pressurized system (assuming a demand of 0.106 mg H₂ per mg NO₃⁻). Furthermore, the safe handling of gaseous hydrogen would require additional technical efforts, e.g. membrane contactors or exhaust air treatment.

Organic fixed bed materials appear promising for promoting denitrification in sequential biofiltration systems, since the release of electron-rich organic compounds, biomass growth, and gas formation could be limited to a defined zone. Furthermore, this approach might decrease operational effort and costs compared to a nitrate removal with membrane technologies or ion exchangers. Solid phase denitrification is broadly reviewed by Wang & Chu (2016), who identified woodchips as the most popular and competitive natural plant-like used for denitrification. Further plant-based materials for denitrification have already been evaluated in laboratory as well as in pilot studies (Gibert et al. 2008; Warneke et al. 2011). Schaffer et al. (2015) tested compost in a laboratory set up as the denitrifying layer in SAT applications. Besides natural substrates, synthetic but biodegradable polymers such as polycaprolacton (PCL), polyactic acid (PLA), polyhydroxy-alkanoate (PHA), and polybutylene succinate (PBS) were investigated as electron and carbon sources for fixed bed denitrification (Boley et al. 2000; Shen & Wang 2011; Chu & Wang 2013).

In this study, natural as well as synthetic materials were tested as electron donors for in-situ denitrification in a SBF system. Batch experiments were conducted for a first evaluation of the different materials within a month, as reported by Gibert et al. (2008). A parallel set of laboratory columns was installed to simulate an anoxic filtration zone in a SBF system. Although there are plenty of studies available on TOrC degradation under anoxic conditions, to the best of our knowledge, there is hardly any information available on TOrC removal during fixed bed denitrification. Therefore, besides monitoring nitrate removal and dissolved organic carbon (DOC) release, this study also investigates the removal of selected TOrCs.

MATERIALS AND METHODS

Materials

Straw, pine woodchips (soft wood), peat, PCL, and PLA were applied as fixed bed materials. Sand from a MAR site located at Lake Tegel in Berlin (Germany) was used as a reference filling material. Detailed specifications of the substrates are given in Table S1 in the supporting information.
Batch experiments

Batch tests were carried out according to the standard DIN EN ISO 7827. A prepared solution was purged with nitrogen in order to reduce the dissolved oxygen concentration below 1 mg/L. Amounts of 0.25, 0.50, and 1.00 g of the respective fixed bed materials were mixed with 100 mL of the solution. To provide sufficient microorganisms, 15 mg TSS/L of washed activated sludge from a municipal WWTP (with biological phosphor removal, denitrification and nitrification described by Streicher et al. (2016)) was added. In addition to the fixed bed materials, H2 was also tested as electron donor. Here, the prepared batches were purged with H2 for 10 minutes at the beginning and additional H2 was introduced during each sampling. The bottles were shaken at 120 rpm in the dark for 30 days. Samples were taken at day 5, 10, 20, and 30 under anoxic conditions by purging nitrogen or respectively hydrogen with hollow needles into the closed batches. After 30 days of incubation under anoxic conditions, batches were unsealed and shaken for another 24 h to investigate if backwards transformation of TOrCs into the parent compound occurs under oxic conditions.

Long-term column experiments

Prior to the column experiments, all materials were exposed to WWTP effluent for 48 h for inoculation with microorganisms. All materials were packed into glass columns (35 mm inner diameter, 200 mm length) which were operated in the dark and upstream with a mean flow rate of 19.0 ± 0.6 mL/h (396 individual measurements \( n = 396 \)) leading to hydraulic retention times of less than 10 hours (Table 1).

The experiment was performed for almost 200 days at a mean temperature of \( 22 ± 1.4 \, ^\circ \text{C} \) \( (n = 64) \). The influent consisted of Berlin tap water containing a non-biodegradable DOC of \( 5.5 ± 0.4 \, \text{mg/L} \) \( (n = 55) \) which was spiked with sodium nitrate to a mean nitrate concentration of \( 46.7 ± 9.9 \, \text{mg/L} \) \( (n = 26) \). Accordingly, the applied test water revealed a low ratio of bio-degradable DOC (BDOC) to nitrogen (N). Oxygen concentrations were adjusted below 2 mg/L by aeration with nitrogen. Furthermore, a defined volume of a TOrC stock solution containing 10 mg/L of acesulfame (artificial sweetener), benzotriazole (corrosion inhibitor and antifreeze) and 5-methyl-benzotriazole (transformation product of benzotriazole), diclofenac (analgesic), carbamazepine and gabapentin (antiepileptic), iopromide and iomeprol (x-ray contrast agents), formylaminoantipyrine (metabolite of analgetic metamizol), and sulfamethoxazole (antibiotic) was added to the influent to adjust TOrC concentrations of 1 \( \mu \text{g/L} \) each. Along the column, sampling ports were installed at 7.8 and 15.6 cm height. The effluent passed a gas capture bottle to quantify released nitrogen. A scheme of the experimental setup is presented in Figure 1.

Analyses

Samples were taken under anoxic conditions and filtrated with 0.45 μm membranes prior to further analyses. Oxygen was quantified with an OXY-4 mini fiber-optic oxygen meter (PreSens, Precision Sensing GmbH, Germany). The DOC was measured with a TOC Cube analyzer (Elementar, Germany) and further characterized with liquid chromatography and continuous organic carbon detection (LC-OCD) (Huber et al. 2011). The selected TOrCs were quantified by high performance liquid chromatography coupled with

### Table 1 | Operational details of the packed columns

<table>
<thead>
<tr>
<th>Material</th>
<th>Reference (Sand)</th>
<th>Rye straw</th>
<th>Soft wood</th>
<th>Peat</th>
<th>PCL</th>
<th>PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied dry matter (g)</td>
<td>323</td>
<td>30</td>
<td>20</td>
<td>49</td>
<td>153</td>
<td>177</td>
</tr>
<tr>
<td>Packing density (kg/L)</td>
<td>1.68</td>
<td>0.16</td>
<td>0.16</td>
<td>0.26</td>
<td>0.79</td>
<td>0.92</td>
</tr>
<tr>
<td>HRT (h)</td>
<td>9.0</td>
<td>7.8</td>
<td>8.8</td>
<td>9.0</td>
<td>2.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

![Figure 1](http://iwaponline.com/jwrd/article-pdf/10/4/394/831447/jwrd0100394.pdf)
tandem mass spectrometry (HPLC-MS/MS), as described by Hellauer et al. (2017) in detail.

Data processing

The mean nitrate removal rate $R_N$ (mg N/(L·d)) was calculated according to Equation (1) and the theoretical gas formation rate $R_{N2}$ (mL/d) was calculated according to Equation (2) assuming a complete reduction of nitrate to molecular nitrogen:

$$R_N = \frac{Q}{BV} \cdot \Delta N$$

$$R_{N2} = \frac{\Delta NO_3}{\delta_{N2}} \cdot \frac{Q}{BV}$$

with: $\Delta N = c_{NO_3-N,\text{in}} - c_{NO_3-N,\text{out}} - c_{NO_2-N,\text{out}}$

$Q$ volume flow (L/d)

$BV$ bed volume of the column (L)

$c_{NO_3-N,\text{in}}$ nitrate concentration in the column influent (mg/L)

$c_{NO_3-N,\text{out}}$ nitrate concentration in the column effluent (mg/L)

$c_{NO_2-N,\text{out}}$ nitrite concentration in the column effluent (mg/L)

$\delta_{N2}$ density of molecular nitrogen at 20 °C (1.16 mg/mL).

The Student’s t-test was carried out with unpaired samples with differing variance. The probability for a similar behavior of two samples is expressed by the p-value. A p-value smaller than 0.05 is considered as significant difference between the samples. To examine if the observed removal in batch tests is significant, the dataset of relative removals was tested against 0 (= no removal).

RESULTS AND DISCUSSION

Nitrate removal

The batch tests showed a nitrate removal within 30 days for PCL, straw, soft wood, peat, and H2. The nitrate removal rate constant $k$ correlates with the DOC released from the materials (Figure S2, supporting information).

In the column experiment, a mean nitrogen removal $R_N$ of 4–21 mg N/(L·d) was calculated according to Equation (1) for the tested substrates, except for the reference sand and PLA columns, which showed no nitrate removal (Figure S3). As presented in Figure 2, the highest removals were achieved with rye straw (30 g filter material) and PCL (153 g), which was already indicated by the batch tests.

The relative effluent concentrations of nitrate and nitrite reveal an incomplete denitrification to nitrite with PCL during the first four months of operation. The residual oxygen in the influent water as well as the short hydraulic retention time of only 3 h might have provoked incomplete denitrification and nitrite formation. Furthermore, biofilm seems to develop, since the total nitrogen removal increases with time. After day 125, an almost complete nitrogen removal is achieved resulting in a removal rate of $23 \pm 5$ mg N/(L·d) ($n = 9$). In contrast, Chu & Wang (2015) achieved a high removal rate of 182 mg N/(L·d) in a PCL reactor at 20 °C and 6–9 h HRT with spiked groundwater after 300 days of operation. They recommend high temperature and HRT during the initial phase. However, this could be difficult to implement in an in-situ application. Furthermore, they conducted microbial analyses of the biofilm and found denitrifying processes in deeper layers of the biofilm, requiring a certain biofilm thickness.

In the column experiments, straw achieved the highest average nitrate removal rate of 21 mg N/(L·d) during the first four months of operation. The rates were calculated only from measurements up to day 128, since air was accidently pumped into the column due to a broken pumping hose. However, Cameron & Schipper (2010) reported comparable values for wheat straw during the first ten months after start up but a significantly lower average nitrate removal of 7.8 mg N/(L·d) between 10 and 23 months, indicating a decline in nitrate removal over time. This decline was also observed with straw in this study. During the first month nitrate removal rates reached 29 mg N/(L·d) and steadily decreased to 14 mg N/(L·d). Reasons might be the decreasing pore volume and HRT due to gas entrapment as well as the washout of readily degradable BDOC during the first month.

The observed average nitrate removal for soft wood of 5 mg N/(L·d) during six months of operation corresponds to the reported value of 4.9 g N/m3·d by Cameron & Schipper (2010). The high removal rates reported by Ghane et al. (2015) can be explained by the carbon turn-over of
wastewater DOC. Therefore, soft wood seems to be a suitable fixed bed material for treating carbon-rich influents even under short HRT (Christianson et al. 2016). The results of Halaburka et al. (2017) suggest that denitrification in an aged woodchip bioreactor at constant temperature can effectively be modeled using even zero-order kinetics when nitrate concentrations are exceeding 2 mg N/L.

A direct comparison of the observed nitrate removal rates with literature data is not possible and always needs to be seen within the context of operational conditions, since HRTs are usually longer, temperatures differ, or the content of BDOC in the influent water is much higher.

Gas formation

The gas formation in the column experiments was quantified by measuring the weight loss of the replaced water in column and gas capture bottles. To transfer mass of replaced water, into a nitrogen equivalent volume, the mass was divided by 0.998 g/mL reflecting the density of water. The obtained gas formation rates correlate with the theoretical gas formation rates $R_{N_2}$ calculated from nitrogen removal, indicating that molecular nitrogen was the main gas formed during the experiment (Figure 3). Only a minor gas formation was observed for sand and PLA. In contrast, the gas retention capacity of the porous media was exceeded
for straw, PCL, wood, and peat, as more than 70% of the formed gas was released from those columns into the gas capture bottles (Figure S4).

In a biofiltration system with horizontal or downstream flow conditions the gas retention capacity can be higher, since gas entrapments would not be ‘flushed’ out with the water stream as in the up-stream operated column tests. This might lead to a further decrease of the hydraulic conductivity, as reported by Cameron & Schipper (2010), especially for coarser grain size media.

**DOC release**

All materials that induced denitrification in the experiments also released DOC. Batch tests were able to indicate the amount of released DOC from the different substrates, especially for straw and peat (Figure 4(a)). A higher DOC release was observed for PCL and soft wood in the column tests compared to batch experiments.

In the column tests, especially PCL and straw showed an excessive release of DOC. While DOC release from straw mainly occurred within the first 10 days, DOC washout from PCL was observed for several weeks, as can be seen in Figure 4(b). LC-OCD analyses revealed a release of low molecular weight compounds from PCL (Figure S5) which were found to be biodegradable in batch tests. In contrast, Boley et al. (2000) reported only a slow DOC increase in the effluent of a PCL reactor. A high DOC release from wheat straw was also observed by Aslan & Türkman (2004). Although the DOC release from the PCL column decreased with time, denitrifying activity increased. This might be another indication for an incomplete biofilm development in the PCL column. In contrast to PCL, straw revealed a full nitrate removal during the first weeks, associated with the washout of DOC. Possibly, the DOC was more diverse and more easily accessible for microorganisms than the PCL polymers. Furthermore, straw as a natural material already might have contained microorganisms, which initiated denitrification after a short runtime.

Released DOC from peat was found to be mainly hardly biodegradable humic substances.

**Removal of TOCs**

Data reported in the literature often show different observations for the removal of TOCs under nitrate reducing conditions. Due to potential micro-milieus and insufficient resolution, it can be hard to distinguish between processes under micro-aerophilic and nitrate reducing conditions in field as well as in column studies; especially, since denitrifying bacteria are commonly facultative aerobes which can switch between aerobic and anoxic respiration (Madigan & Brock 2009).

Therefore, removal data from the batch experiments were evaluated to verify biotransformation under nitrate reducing conditions. To eliminate oxic transformation with residual oxygen, data from the first five days was excluded, as well as data when sulfate was reduced. The mean relative removal under nitrate reducing conditions, which can be

![Figure 4](http://iwaponline.com/jwrd/article-pdf/10/4/394/831447/jwrd0100394.pdf)
linked to biotransformation, is listed in Table 2. Acesulfame was removed by 44% in batch tests independently of the material under denitrifying conditions. The artificial sweetener is known to be transformed under micro-aerophilic and nitrate reducing conditions with sulfamic acid being the main transformation product (Castronovo et al. 2017). Gabapentin, as well, shows a significant removal of 11% under nitrate reducing conditions, which might be attributed to biotransformation. Schaper et al. (2019) already observed a removal of gabapentin in the absence of oxygen during their field study. Even though the Student’s t-test also reveals a p-value below 0.05 for the removal of 5-methyl-benzotriazole, the biodegradability under nitrate reducing conditions found by Liu et al. (2011) cannot be clearly confirmed, due to its low mean removal of only 6%.

Column experiments also show a removal of 5-methyl-benzotriazole in the nitrate reducing columns. However, we cannot excludeoxic transformation due to residual oxygen. As for all other compounds, no significant removal is observed for denitrifying conditions. Sulfamethoxazole is removed strictly under oxygen or sulfate reducing conditions (Figure S6), which concurs with the findings in sand column investigations of Baumgarten et al. (2011).

Comparing the different electron donors, especially peat, revealed a nitrate reduction and a high removal for some TOrCs in batch and column experiments (Figures 5 and S7). As for carbamazepine, which is considered persistent under anoxic conditions, the removal by peat can be attributed to sorption (0.13 ± 0.05 μg/g in batch experiments). Benzotriazole, 5-methyl-benzotriazole and metoprolol were removed with peat in batch experiments and especially during the initial phase in column experiments, that can be linked to adsorption (Figure S8). Due to sorption effects, peat batches were not taken into account for quantifying biotransformation in Table 2. Surprisingly, acesulfame was also well removed in the peat column. In contrast to carapamazine or benzotriazole, the decreasing effluent concentrations during the column experiment indicate biotransformation as the main removal process.

Boley et al. (2006) reported a successful removal of a-endosulfan due to sorption on PCL. However, no removal of the investigated TOrCs linked to adsorption was observed with PCL in this study.

Denitrification with H₂ as electron donor did not show an enhanced removal of the investigated TOrCs in batch experiments (Figure S9).

Table 2 | Mean relative removal: 1 − Cc/C0 (9 = 9) associated with biological transformation in batch tests under nitrate reducing conditions independent of the applied material (peat was excluded due to sorption effects)

<table>
<thead>
<tr>
<th>TOrCs</th>
<th>Removal in batch tests</th>
<th>p-values (Student’s t-test)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame</td>
<td>0.44 ± 0.13</td>
<td>7.58 × 10⁻⁶</td>
<td>+</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>0.11 ± 0.11</td>
<td>0.02</td>
<td>+</td>
</tr>
<tr>
<td>5-Methyl-benzotriazole</td>
<td>0.06 ± 0.08</td>
<td>0.04</td>
<td>+</td>
</tr>
<tr>
<td>4-Formylaminoantipyrine</td>
<td>0.05 ± 0.21</td>
<td>0.53</td>
<td>+</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.05 ± 0.18</td>
<td>0.44</td>
<td>+e+dd+c</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.04 ± 0.19</td>
<td>0.62</td>
<td>+</td>
</tr>
<tr>
<td>Benzotriazole</td>
<td>0.05 ± 0.08</td>
<td>0.10</td>
<td>+</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.05 ± 0.07</td>
<td>0.10</td>
<td>+b+d</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>−0.02 ± 0.08</td>
<td>0.56</td>
<td>+c+dd</td>
</tr>
</tbody>
</table>

Literature references which consider a TOrC as removable (+) or not removable (−) under nitrate reducing conditions.

*aCastronovo et al. (2017).
*bSchaper et al. (2019).
*cLiu et al. (2011).
*dWiese et al. (2011).
*eBanzhaf et al. (2012).
*fBaumgarten et al. (2011).
*gWick et al. (2009).
*hKönig et al. (2016).
Highest TOrC removal was found in the oxic PLA and sand column. Generally, higher removal rates are found for oxic than for anoxic conditions (Greskowiak et al. 2014). The MAR sand removed most of the monitored TOrCs even under suboxic conditions below 1 mg/L. The sand might have had a more diverse microbiome, since it was used as filter material in soil aquifer treatment for years, whereas PLA was only inoculated with sewage sludge for 2 days. Furthermore, the HRT was almost double in the sand column compared to PLA, which might explain the better removal under suboxic conditions in MAR sand.

The availability of oxygen after anoxic conditions might cause a backwards transformation of already transformed TOrCs. This might be the case after re-aeration in SBF systems. This effect was simulated in the end of the batch tests. After 30 days of anoxic conditions, the batch tests were opened and shaken for another 24 h. The introduction of oxygen significantly improved the general removal of TOrCs, as shown in Figure S9 (Student’s t-test $p = 0.02$). Only a slight increase of TOrC concentrations was observed in some batches after re-aeration, indicating no severe backwards transformation into the parent compound.

**Material costs**

An overview of the estimated material costs (Table 3) for one cubic meter of a fixed bed reactor illustrates the comparatively high costs for the synthetic materials PCL and PLA. Currently, they are more than 200 times higher than for natural filling materials. However, the specific costs related to nitrate removal can be lower, assuming that synthetic materials show a better long-term performance as electron donor as indicated in the column experiment with PCL.

For wood chips and wheat straw, Saliling et al. (2007) detected weight losses of 16.2% and 37.7% over 140 days while Chu & Wang (2013) calculated consumed PCL...
amounts that ranged between 1.6 and 3.7 g PCL/g NO₃-N for their experiments.

**CONCLUSIONS**

An effective nitrate removal was achieved with PCL and straw at HRTs below 10 hours. Whereas the nitrogen removal with straw decreased over time, a complete removal was achieved with PCL after an adaptation time of 125 days. With a better adjustment of HRT, influent oxygen concentrations or even temperature, this time could be decreased and PCL might be a possible substrate for long-term fixed bed denitrification. Only a minor removal of TOrCs was observed under denitrifying conditions, except for peat, which partly removed benzotriazole, 5-methy-benzotriazole, metoprolol, carbamazepine, and acesulfame.

Especially during the initial phase, high DOC leaching from the materials and nitrite formation can occur, which would cause additional oxygen consumption in a subsequent oxic zone in an SBF system. In-situ fixed bed denitrification seems to be difficult to control, especially in a technical system with limited HRT or space. A better option might be the combination of denitrification with total suspended solids removal in the rapid sand filter prior to the technical SBF system (Figure S1). For this purpose, the rapid sand filter needs to include additional biofilm carriers for the denitrifiers and an optional dosage of easily degradable organic carbon. Treating just a part of the influent as a recirculated side-stream in a separated and controlled denitrification unit could be another possibility to achieve the required threshold values. This option allows for better control of the system conditions and the application of fixed bed materials as carbon and electron source can be reconsidered. By optimizing the HRT, the leaching of DOC and nitrite formation could be minimized.

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**SUPPLEMENTARY MATERIAL**

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