Kinetic evaluation of nitrification performance in an immobilized cell membrane bioreactor

D. Güven, E. Ubay Çokgör, S. Sözen and D. Orhon

ABSTRACT

High rate membrane bioreactor (MBR) systems operated at extremely low sludge ages (superfast membrane bioreactors (SFMBRs)) are inefficient to achieve nitrogen removal, due to insufficient retention time for nitrifiers. Moreover, frequent chemical cleaning is required due to high biomass flux. This study aims to satisfy the nitrification in SFMBRs by using sponge as carriers, leading to the extension of the residence time of microorganisms. In order to test the limits of nitrification, bioreactor was run under 52, 5 and 2 days of carrier residence time (CRT), with a hydraulic retention time of 6 h. Different degrees of nitrification were obtained for different CRTs. Sponge immobilized SFMBR operation with short CRT resulted in partial nitrification indicating selective dominance of ammonia oxidizers. At higher CRT, simultaneous nitrification–denitrification was achieved when accompanying with oxygen limitation. Process kinetics was determined through evaluation of the results by a modeling study. Nitrifier partition in the reactor was also identified by model calibration.

Key words | high rate MBR, hybrid MBR, immobilized biomass, nitrification, polyurethane sponge

INTRODUCTION

Conventional biological wastewater treatment systems target to minimize the effects of organic compounds and microbial biomass produced as pollutants through stabilization, which consumes a vast amount of energy. However, the contemporary approach for understanding organic wastes as ‘valuable energy source’ instead of ‘pollutant’ is vital for sustainable energy and environmental management. In this respect, establishment of new and emerging energy efficient technologies for wastewater treatment processes is necessary. Development of membrane bioreactor (MBR) has been a monumental step in redesigning the classical activated sludge system by eliminating gravity separation and related design constraints on sludge residence time (SRT) for good biomass settling properties. Classical MBRs were operated with long SRT and high biomass concentration that also enables nitrogen removal.

The idea of operating MBR systems at low SRT level was first explored by Ng & Hermanowicz (2005), and then studied by Harper et al. (2006) and Duan et al. (2009). As an antagonistic approach, high rate MBR operation (extremely low SRT and hydraulic retention time (HRT)), called superfast membrane bioreactor (SFMBR) was introduced by Teksoy Başaran et al. (2012) as a novel process to benefit from the energy potential of the organic matter. This study tested an SFMBR system successfully, achieving full removal of soluble readily biodegradable organic compounds at extremely low SRT and HRT levels. The significant advantages of this novel system essentially consisted of reduced reactor volume and energy requirement, elimination of sedimentation tank and separation and concentration of organic solids on membrane which could be digested, incinerated or gasified for energy generation thereafter (Sözen et al. 2014, 2015). Although high chemical oxygen demand (COD) removal was achieved in the operation of SFMBR, the reported results were limited to the assessment of system performance based on COD removal. One of the major drawbacks of the SFMBR, appears to be the inability of nitrification due to very low SRT (<2 days) operation. Such low SRTs are much below the minimum cell residence time that is required for suspended nitrifier growth for temperatures lower than 25 C (Güven & Schmidt 2009). Nitrifiers, ammonia and nitrite oxidizers are slow growers and have different levels of sensitivities to environmental factors. Immobilization techniques help overcome SRT dependent growth limitations to a large extent (Seo et al. 2001). Maintenance of a high cell density of viable culture of nitrifying...
bacteria in the active growth phase on the carriers will provide an effective nitrification (Manju et al. 2009). In such systems, enhanced SRTs could be achieved as carrier residence time (CRT). Membrane fouling is the other operational problem for the SFMBRs. Biomass characteristics, including the mixed liquor suspended solids (MLSS) content, particle and floc size distribution and extracellular polymeric substances, as well as operating conditions, membrane properties are among the major factors that directly affect the fouling phenomena (Le-Clech et al. 2006; Yang et al. 2009). Although there is not that much study reported on the effect of low SRTs on membrane fouling rate, most of the studies indicated that degree of fouling is less under higher SRTs (above 20 days) (Jinsong et al. 2006; Ahmed et al. 2007). Hybrid application of immobilized cell growth and MBR is successfully studied by several authors to overcome problems related with membrane fouling and nitrification (Fujita et al. 2000; Leiknes & Ødegaard 2007; Chu & Wang 2011; Dizge et al. 2011; Phattaranawik and Leiknes 2011; Rahimi et al. 2011b; Yang et al. 2012; Deng et al. 2014).

In this context, application of immobilized cell growth for SFMBR operation at low SRTs, would enable to achieve a sustainable level of nitrification while minimizing fouling problems. The development of design principles and practical application of such systems still requires substantial efforts on laboratory- and pilot-scale applications. Accordingly, this study focused on reshaping the configuration and operation strategy of existing conventional activated sludge systems with the operational properties in conjunction with the immobilized systems offered by MBR technology. This approach ultimately aimed to achieve an enhanced SFMBR capable of sustaining nitrification and minimizing the membrane fouling problems by using carriers. In this context, the main objective of the study was to evaluate the efficiency of carbon removal and nitrification and to identify the limits for nitrification, as well as nitrification kinetics with respect to different residence times in sponge-submerged SFMBRs.

**METHODS**

**Experimental design rationale**

To achieve the objective of the study, the experimental studies were basically designed to evaluate the nitrification performance of a submerged MBR equipped with immobilized cell carriers operated at low CRTs. The most effective cell carrier was selected as polyurethane sponge among different alternatives (zeolite, polyvinyl alcohol (PVA), diatomic earth and polyurethane sponge) by running a detailed experimental study (Sözen & Güven 2013) and the immobilization of the cells on the effective carrier was carried out in fill and draw reactors. Fill and draw operations were essentially designed to interpret the dissimilarity of growth characteristics related to nitrification efficiency under different CRT. To better evaluate the adopted experimental design, it should be noted that the sludge age or the SRT is the essential operation and design parameter for biological treatment systems, selected independently from influent conditions and/or HRT for the appropriate treatment scheme (Orhon et al. 2009). Therefore, influent COD concentration and flow regime does not impose an SRT to the system. Obviously, it is impossible to change influent characteristics; however, a wastewater stream with specific characteristics can be treated using an SRT 2 days, 8 days or 15 days, using a high rate, conventional or nutrient removal schemes. The birth of high rate systems before 1950 was accomplished simply by changing the SRT values to low levels in existing treatment systems (Orhon 2014). Selected SRT simply changes the level of biomass, MX and the biomass concentration (Equation (1)), XH in the reactor (Equation (2)):

\[
MX = Y_{NH} \cdot CS \cdot SRT \quad (1)
\]

\[
XH = Y_{NH} \cdot CS \cdot \frac{SRT}{HRT} \quad (2)
\]

where, Q is the flow rate, CS is the biodegradable COD, and YNH is the net yield determined by the selected SRT. This way, the selected SRT determines the unit organic load level of the biomass. This sets the basis of the way the experimental system in the study was operated at three different SRT levels which corresponds to CRT. The laboratory-scale submerged MBR unit was operated at steady state at three different CRTs; starting from 52 days descending to 5 days and 2 days for representing high rate operation to identify the limits for efficient nitrification performance under steady-state conditions. The CRT was controlled by removing the relevant amount of carriers and replacing them with the same amount of new carriers each day. The system had an average HRT of 6.0 h in all experiments. Each MBR operation was started with the immobilized cells taken from the parallel operated fill and draw reactors sustained at the corresponding CRTs at steady state.

A synthetic mixture characterizing the readily biodegradable COD composition of domestic wastewaters was
prepared to have a final concentration of 200 mg COD/L and 60 mg NH$_4$-N/L by diluting the substrate, macro and micronutrients stock solutions in tap water (Henze 1992; Cokgor et al. 1998). For each 1,000 mg COD substrate fed, 20 mL of macro- and micro-nutrients were added to the reactor. The MBR unit and the fill and draw reactor for the immobilization were fed with the same synthetic mixture.

**Cell immobilization experiments**

The fill and draw reactors with a working volume of 2 L were operated for the immobilization of the cells on polyurethane sponge at 20°C. The polyurethane sponges were cut in 1×1×1 cm size in cubic shape. The reactors were run with immobilized cells at three different CRTs (2, 5 and 52 days) for 3 months at a HRT of 24 h. A suspended growth fill and draw reactor with a volatile suspended solids (VSS) concentration of 2,500 mg/L was parallel operated as a reference for the comparison to the attached growth systems under same operational conditions. Reactors were monitored to evaluate the nitrification performance regarding the different CRTs.

Bioreactors were aerated constantly and stirred with a magnetic stirrer to provide aeration and mixing, respectively. Desired residence time of carriers was obtained by daily wasting and replacing a certain amount of carriers in the reactor. The degree of immobilization was determined by analyzing the amount of non-attached VSS in the reactor at the end of each daily operation. It was observed that almost 99% of suspended solids were attached on sponge after 30 days of operation. In immobilized systems it is not possible and meaningful to determine the VSS concentration in sponges to a desired level of accuracy for a performance evaluation and process modeling. Instead, for such systems, the assessment of active biomass levels is much more meaningful and this parameter can only be determined by modeling, as in this study.

**Sponge-submerged MBR**

The laboratory-scale submerged MBR (Figure 1) consisted of a cylindrical Plexiglas reactor with an operating volume of 3 L, and equipped with a hollow fiber PVDF Zee Weed-1 (GE) membrane module with a total membrane surface area of 0.1 m$^2$ and a nominal pore size of 0.04 μm was continuously operated with a transmembrane pressure (TMP) range of 0.1–0.5 bar at a flux of 3.75 L/m$^2$h. The synthetic wastewater was pumped (P1) into the bioreactor from a feed tank and permeate was withdrawn at a constant flow by a permeate pump (P2). The bioreactor was aerated constantly and stirred with a magnetic stirrer to provide aeration and mixing. A level sensor was used to control the wastewater volume in the reactor. Pressure gauge was used to measure the TMP. A certain amount of carriers were wasted manually from the system to attain the desired residence time. Data acquisition and control of the system was maintained via processing signals from a pH/temperature probe and a dissolved oxygen (DO) probe connected to a multimeter (Hach-Lange sc1000, Germany). Membrane module contains an air diffuser at the bottom to supply pressurized air flow for backwash. Every 19-min filtration,
the membrane was backwashed through the air supply for 1 min. The membrane module was cleaned once every day by employing 15 min of contact with a pH = 12 NaOH solution followed by 15 min of contact with a pH = 2.5 H₂SO₄ solution. Reactor was filled with immobilized polyurethane sponge at a volume fraction of 40%.

**Respirometric analysis**

Respirometric tests were conducted in batch reactors with varying volumes of 0.7–2 L for the determination of oxygen uptake rates (OURs) and nitrogen utilization rate at 20 °C. Same representative conditions, in terms of biomass composition, were applied to batch experiments as the original MBR bioreactor at the selected CRT and HRT. Tests were started with the biomass seeding alone to observe the initial endogenous respiration level. Substrate was added to the batch after this stage at desired S₁/X₁ with an initial oxygen concentration of 6–7 mg/L.

**Model structure**

The model used was an adopted model of ASM1 for two sequential steps of nitrification together with carbon removal. For the carbon removal process, SS₁ and SS₂ represent two different readily biodegradable COD components, whereas X₇ is for the active heterotrophic biomass and SO for DO. Nitrification process was evaluated in two steps; ammonia oxidation and nitrite oxidation, where X₇ and X₉ represent ammonia oxidizers and nitrite oxidizers, S₇, S₉ and S₉₉ represent ammonia, nitrite and nitrate concentrations, respectively (Katipoglu-Yazan et al. 2013, 2015). Soluble (Sp) and particulate (Xp) microbial products were also accounted for as a part of endogenous respiration (Cokgor et al. 2011). Matrix representation of the selected model structure and process rate expressions is presented in Tables 1 and 2, respectively.

**Methods of analysis**

All samples were filtered through 0.45 μm PVDF syringe filters and directly analyzed for COD according to ISO 6060 methodology (ISO 6060, 1986). MLSS, VSS, and NH₄⁺-N were determined according to Standard Methods (AWWA 2005). NO₃⁻-N and NO₂⁻-N were measured by DIONEX ICS-1500. OUR measurements were conducted with a WTW OXI Level 2 oxygen meter. Model parameters were estimated using the AQUASIM software program (Reichert et al. 1998).
RESULTS AND DISCUSSION

Fill and draw operations

Nitrification is commonly expected to be achieved at sludge ages longer than 5 days in suspended growth systems depending on the environmental conditions. This was the argument to run the suspended growth system at a sludge age of only 2 days to test the possible limits of nitrification. The results of the fill and draw operations (Table 3) indicated that nitrification was not achieved at a sludge age of 2 days in the suspended growth system, as expected. On the other hand, meaningful partial nitrification efficiencies were succeeded in the reactors with immobilized cells even at 2 days’ residence time, whereas a full nitrification was detected for the CRT of 52 days. It is interesting to note that both reactors with CRTs of 2 days and 5 days reflected a similar trend in ammonia removal (about 60%) and nitrification pathways. The observed nitrite accumulation as a result of incomplete nitrification could be explained by a distinctive DO gradient along the sponge-inward depth, although oxygen limitation was avoided in the reactors. Low DO concentration due to the consumption and diffusion limitation inside the biofilm may limit the activity of nitrite oxidizing bacteria, since they are more sensitive to low DO than the aerobic ammonia oxidizers (Bernet et al. 2001; Park et al. 2010; Park et al. 2015). Thus, it could be concluded as the indication of dominancy of ammonia oxidizers over nitrite oxidizers.

Sponge-submerged MBR operations

The laboratory-scale MBR unit was first operated at a CRT of 52 days without carrier wastage and monitored on the basis of nitrogenous compounds, such as ammonia-, nitrite- and nitrate-nitrogen (Figure 2). In this operation mode, two different observations were noticed related to operational conditions: (i) 0–33th days under oxygen limitation and (ii) days 33–52 without oxygen limitation. In the first 10 and 16 days of operation only 50 mg/L of ammonia removal was obtained due to the undesired oxygen limitation (0.5–2.3 mg O₂/L) of the diffuser failure. Moreover, only 30 mg N/L of nitrate formation was observed in relation to 50 mg N/L of ammonia removal. Based on the mass balance for N compounds, this could be evaluated as a strong indication of simultaneous nitrification-denitrification (SND). The system was continued to run to the day 33 under oxygen limiting conditions to maintain SND process. At day 33, the
system was turned to fully aerobic conditions and operated for 14 more days at steady state. A complete nitrification with the formation of about 55 mg NO₃-N/L was obtained, without denitrification.

The results relating to 5 days of CRT at steady state conditions showed that only about 57% of ammonia oxidation obtained was sourced by nitrite (up to 26 mg N/L) and nitrate (10 mg N/L) production (Figure 3(a)). The nitrification pathway was similar to the one observed in fill and draw reactor where the dominant end product was nitrite. The excessive nitrite accumulation can be explained with the uncontrolled limitation of the DO along the depth of the sponge although it was kept between 4 and 6 mg/L in the mixed liquor. This could be evidence for the fact that ammonia oxidizers were the dominant species in the culture, nitrite oxidizers were partially washed out from the system or inactivated because of the oxygen limitation. Finally, MBR was tested for the assessment of the nitrification limits at CRT of 2 days. At this run, about 50% ammonia removal was noticed with 15 mg N/L of nitrite formation at steady state and a nitrate production of 10 mg N/L, the same as observed for CRT at 5 days. Obviously, not only nitrite oxidation but also ammonia oxidation was deteriorated (Figure 3(b)). This result elucidates that 2 days of CRT in sponge-submerged MBR could be the minimum residence time before washout of all ammonia oxidizers. All through the MBR operation, the optimum HRT of 6 h was sustained with an average chemical wash period of 3 days, which is three-fold higher than that observed (1 day) in the suspended growth operated SFMBR (Aysel 2012).

**Modeling studies**

Evaluation of the OUR profile clearly indicated that there were different levels of oxygen utilization rates representing the growth of microorganisms on different substrates. The first level at 70 mg/L.h and the second level at 40 mg/L.h were dominantly expressing different utilization rates of the readily biodegradable five compounds together with the ammonia and nitrite oxidation (Cokgor et al. 1998). In modeling studies, ammonia oxidation was analyzed in two steps; ammonia oxidation and nitrite oxidation. Description of the process rate expressions for both autotrophic and heterotrophic activities and the basic stoichiometry in matrix format were given in Sözen & Güven (2013). Kinetic evaluation of sequential ammonia oxidation was conducted with the calibration of OUR, ammonia, nitrite and nitrate profiles obtained from batch experiments. The profiles were generated during steady state MBR operation with CRT of 52 days and 5 days. Model calibration yielded the most suited values for the model coefficients defining process stoichiometry and kinetics for each experimental set.
as given in Sözen & Güven (2013). Figure 4 illustrates the interpretation of the OUR (a) and N (b) data for CRT of 52 days by modeling, and demonstrates the close fit between the experimental profiles. Model evaluation (Table 4) first indicated that the maximum specific growth rate and half saturation constant for ammonia oxidizers (X_{NH}) were 1 day\(^{-1}\) and 1 mg NH\(_4\)-N/L, whereas these values were designated as 0.5 day\(^{-1}\) and 0.5 mg NO\(_2\)-N/L for nitrite oxidizers (X_{NO}), respectively, smaller than the values (maximum specific growth rates of 1.4 day\(^{-1}\) and 0.65 day\(^{-1}\), respectively) reported by Katipoglu-Yazan et al. (2013), but in the range suggested as a default value of 1.0 day\(^{-1}\) in some activated sludge models (Jubany et al. 2009; Munz et al. 2011). Secondly, the total nitrifier fraction of the system (the ratio of active autotrophic to total active biomass) was obtained as 8.8%, with a share of 4.8% for ammonia oxidizers and 4% for nitrite oxidizers. In conventional activated sludge systems, this fraction remains between 4 and 5% for nitrifiers, which is almost half of the fraction observed in the immobilized carrier system. Same results in terms of maximum specific growth rate and half saturation constants have been discovered in the model evaluation for 5 days of CRT. Modeling studies indicated that about 4.6% of the total active biomass was nitrifier, with a share of 4.2% of ammonia oxidizer and 0.42% of nitrite oxidizer. However, in a recent study (Sayi Ucar 2013) on a suspended growth activated sludge system operated at 4 days, no nitrifier activity was noticed under the same environmental conditions. A similar nitrifier fraction was observed in a wastewater treatment plant in Istanbul operated only at a very high sludge age (approximately 17 days) (ISKI 2008; Katipoglu-Yazan et al. 2013).

The experimental results showed that the nitrifier activity was significantly decreased with the decrease of CRT to 2 days. The simulations presented that about 3 mg/L of active ammonia oxidizers and a small amount of nitrite oxidizers were still remained in the system. Obviously, nitrite oxidizers were partially washed-out from the system by decreasing the CRT to 5 and 2 days. Model calibration provided that the system operated with sponge as carriers sustained a significant ammonia oxidation at even 2 or 5 days of CRT, which is usually not possible to achieve in a suspended growth system at the same sludge ages. The nitrification limit was

<table>
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<th>Model parameters and state variables</th>
<th>Unit</th>
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<tr>
<td>Maximum growth rate for X_{H1}</td>
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<tr>
<td>Maximum growth rate for X_{H2}</td>
<td>1/day</td>
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</tr>
<tr>
<td>Half saturation constant for growth of X_{H1}</td>
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<td>15</td>
</tr>
<tr>
<td>Half saturation constant for growth of X_{H2}</td>
<td>mgCOD/L</td>
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<tr>
<td>Endogenous decay rate for X_{H1} and X_{H2}</td>
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<td>0.1</td>
</tr>
<tr>
<td>Maximum growth rate for X_{NH} (AOB)</td>
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<td>1.0</td>
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<tr>
<td>Half saturation constant X_{NH}</td>
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</tr>
<tr>
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<td>0.08</td>
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<tr>
<td>Fraction of biomass converted to Xp</td>
<td>–</td>
<td>0.12</td>
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Figure 4 | OUR (a) and N (b) profile for CRT of 52 days.
observed as 5 days in the carrier system, with a nitrite accumulation, unavoidable with shorter sludge residence times depending on the operational parameters.

CONCLUSION

The results of the study provided experimental evidence that significant nitrification could be maintained in a hybrid MBR integrated with immobilized cell systems at low CRT levels. Even at CRT of 2 days, immobilization provided partial nitrification preventing the total wash-out of ammonia oxidizers from the system; partial nitrification resulted in nitrite accumulation in the reactor, enabling short-cut denitrification with lower organic carbon requirement, when coupled with a separate denitrification unit. This finding justified the current understanding that challenged the classical sludge retention time, by redefining cell residence time as CRT in immobilized systems, where a fraction of the vulnerable biomass remains in the reactor volume and continues its metabolic functions. The reduced frequency of chemical washing in a way of cell immobilization was another important acquisition of this hybrid system. In conclusion, this system could be a good representative model study for a super fast membrane, low energy demanding, low volume requiring, energy-efficient, novel treatment system.

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