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# Denitrification-caused Suppression of Soluble Microbial Products (SMP) in MBRs used for Biological Nitrogen Removal

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***Significance***
*The strategies for reducing SMP-caused membrane fouling are essential for MBR sustainable operation. Comparing the bioprocesses with and without denitrification, we found that the specific yield coefficients of polysaccharides (PS) and proteins (PN) in SMP existing denitrification were significantly lower than those without denitrification. In addition, the freshly produced PS and PN from extracelluar polymeric substances (EPS) could be biodegraded and utilized by denitrifers. These findings indicated that biological denitrification was an important mechanism to reduce SMP and MBR fouling.* © 2013 American Institute of Chemical Engineers *AIChE J*, 59: 3569–3573, 2013

*Keywords: denitrification, membrane bioreactors (MBRs), membrane fouling, wastewater treatment*

Soluble microbial products (SMP) have been found to play significant roles in membrane fouling of membrane bioreactors (MBRs).[1–3] The SMP in MBRs can be controlled by the optimization of operating conditions and influent loading rates,[1] use of coagulants/flocculants[1] and inhibitors.[4,5] However, these methods are time-consuming or chemical-consuming. Theoretically, a potential approach for SMP mitigation is to optimize the electron flow in biological processes. Previously, Drews et al.[6], Jin et al.[7] and Lu et al.[8] all reported that keeping a higher level of dissolved oxygen (DO) concentration in the mixed liquor can help in eliminating SMP basing on experimental measurements and/or modeling simulation. However, the high DO means more energy input. In fact, it is ubiquitous that there exists biological denitrification with the nitrite and nitrate as the electron acceptors in the biological nitrogen removal MBR systems, which might also play the important roles in the formation of SMP under the anoxic condition. As expected, the occurrence of dentrification can change cell proliferation and extracellular polymeric substances (EPS) generation, which subsequently impact on the production of SMP. On the other hand, the nitrate is expected to be an electron accepter during the biological oxidization of SMP in the mixed liquor, as parts of the SMP fractions are of biodegradation.[9] Recently, Xie et al.[10] found that the amount of SMP was all in a low level at different C/N ratios during the denitrification process. Similarly, Rosenberger et al.[11] found that the post-denitrification MBR had a lower fouling rate than the predenitrification MBR. Winkler et al.[12] pointed out that the contributions of SMP and/or EPS to the denitrification should be considered. Indeed, Kim and Nakhla[13] found that denitrifying phosphorous accumulating organisms (DPAO) could consume the storage intracellular carbon during the endogenous denitrification, and thus result in changes in compositions and formations of SMP and EPS. Unfortunately, to date little is known about the underlying mechanisms of such a behavior. Therefore, some details will be reported in this letter for revealing the effects of denitrification on SMP formation and elimination to a certain extent.

Based on two sets of parallel batch tests (i.e., with and without the occurrence of denitrification), the main objective

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of this study is to highlight the roles of denitrification in the SMP formation under the anoxic condition, and to reveal the SMP formation can be controlled to what extent by the denitrification process. In addition, nitrate uptake rate (NUR) tests were conducted to investigate the denitrification potential using the freshly extracted EPS as electron donors, which are one major source of the SMP. Details of the experimental procedures are shown online in additional Supplementary Material.

From Figure 1A and B, it can be seen that chemical oxygen demand (COD) (i.e., acetate) was consumed much more rapidly in the denitrification occurring test, particularly in the initial 12 h (decreasing from 1160 to 241.3 mg/L). Correspondingly, there was a higher specific denitrification rate (SDNR) up to 12 mg-N/(g-VSS • h) in the initial 12 h. These results suggested that the presence of sufficient COD and electron acceptors could lead to a higher denitrification activity. As for the control test, only around 35% of COD was consumed during the whole period possibly due to the transfer of oxygen from air into the mixed liquor. However, according to the mass balance of COD, only approximately 5% of COD could be consumed by oxygen entering into the liquor in the denitrification occurring test. This difference might be due to the complex competition relationships between oxygen and nitrate for the electron acceptors. In addition, as there were enough electron acceptors in the denitrification test (the nitrate was added every 6 h), much more energy for cells growth could be supplied[10]. Hence, it was found that VSS in the denitrification occurring test increased from 1808 to 2343 mg/L, while that in the control one only reached from 1756 to 1980 mg/L. However, it was found that the SDNR decreased significantly up to 0.38 mg-N/(g-VSS·h) in the end of the denitrification occurring test, as a result of the decrease of available COD. The different conditions from the real MBR reactor and the inhibitory effects of the accumulated nitrite for the sludge in this study might be important reasons for the lower activity at the end of the tests.

Figure 1C shows the SMP formation versus COD uptake in the two sets of batch tests. At the beginning of batch tests, as a result of the high uptake rate of COD, the SMP yield was dominated by the mechanism of production rather than biodegradation, e.g., the fraction of substrate utilization-associated products (UAP).[14] So, the proteins (PN) and polysaccharides (PS) both increased in the two batch tests. According to the linear fitting of increasing curves of PN and PS vs. COD uptake, the specific yield coefficients of SMP in the denitrification occurring test were calculated to be 4.5 PS-mg/g-COD and 10.2 PN-mg/g-COD, which were much lower than those produced in the control one (8.4 PS-mg/g-COD and 27.6 PN-mg/g-COD, respectively). It suggested that the SMP yield coefficients could be reduced by about 50% by the denitrification process, regardless of PS or PN. In addition, it was noted that the yield rate coefficient of PN was always much higher than that of PS in both two sets of batch tests, which cloud be due to the higher PN

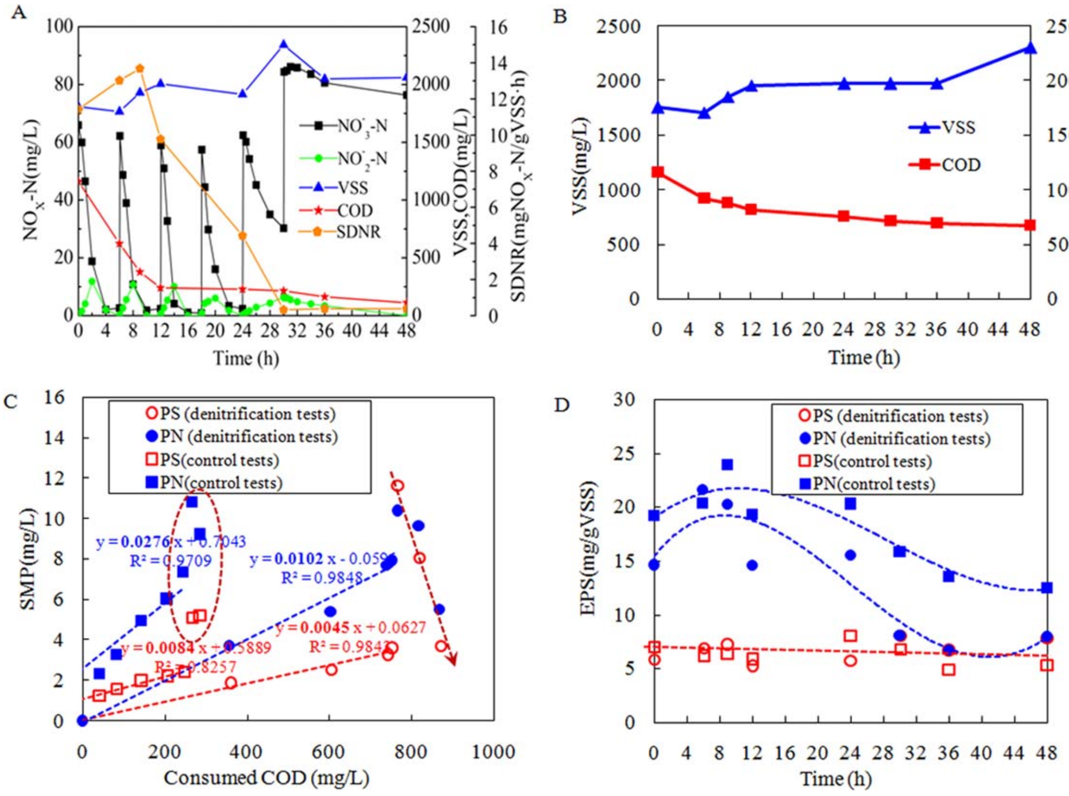

**Figure 1. (A) Variations of NO$_x$-N, COD, VSS, SDNR in the denitrification test, (B) Variations of COD, VSS in the control test, (C) SMP formation versus COD uptake in the two sets of the batch tests, and (D) EPS production in the two sets of the batch tests.**

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

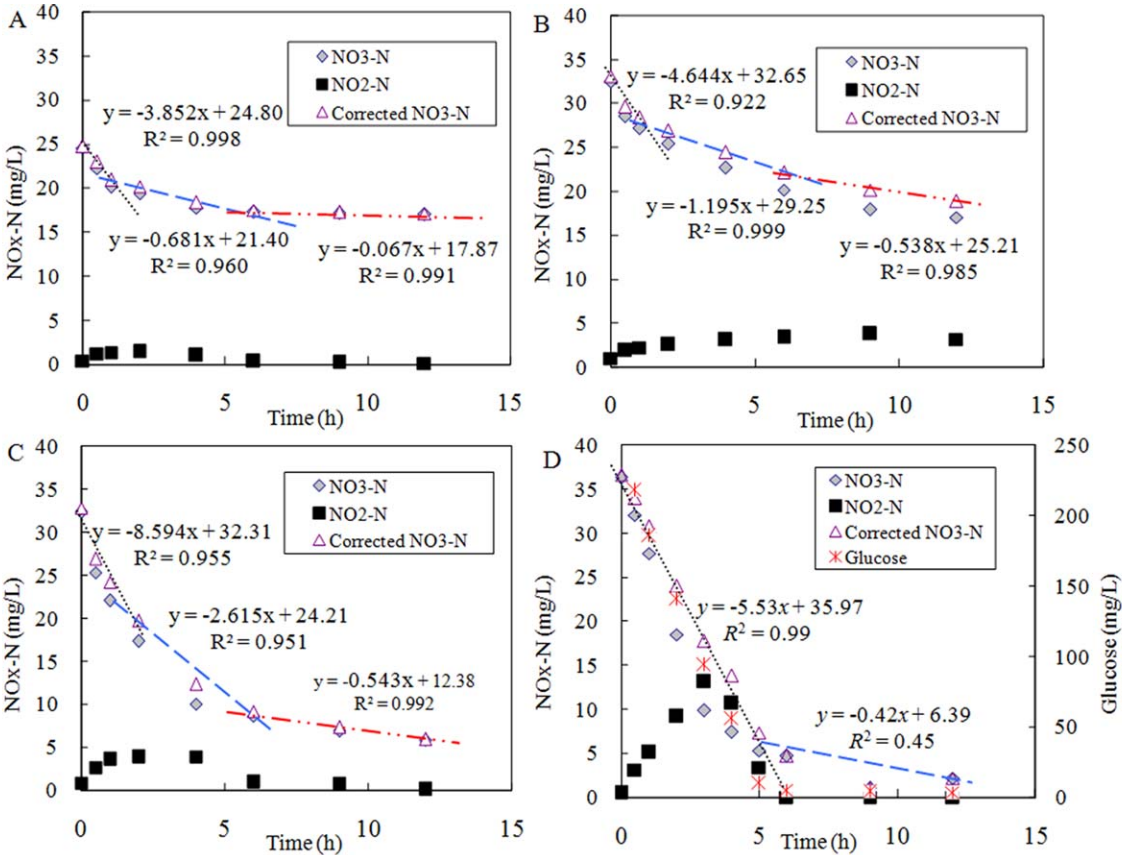

**Figure 2. The denitrification potential tests under different EPS loads (A) 150 mg/L COD, (B) 300 mg/L COD, (C) 450 mg/L COD and using the glucose at a COD of 300 mg/L as carbon source (D).**

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abundance in the sludge or EPS and then resulting in a higher release potential of PN.[15,16] In fact, the SMP production is always accompanied by their biodegradation, which can be consumed by their own producers and other microbes.[9] Hence, after a long-term operation, SMP biodegradation became more and more important, especially under the condition of COD insufficient, which finally resulted in the scattering behavior of the plotted curve in Figure 1C. For example, at the end of the denitrification occurring test, the SMP were found to significantly be utilized as carbon sources during the endogenous denitrification process. Similarly, it was noted that the EPS could also be used as another important carbon source for denitrifaication (Figure 1D). The PN of EPS reduced more rapidly in the last period of the denitrification occurring test while the PS in the EPS always kept at a low and steady level. Possibly this is a major reason that the yield rate of PN was always higher than that of PS in the SMP. The detailed descriptions on the change of EPS during the two batch tests can be found in the Additional Supplementary Material file.

To provide more detailed information with respect to the utilization of EPS during the dentrification process, the NUR tests with extracted EPS solution and glucose as carbon source were performed to estimate and compare their denitrification potential. As the hydrolysis of EPS is generally considered to be a main source of SMP formation,[16] in present study, the extracted EPS from the fresh sludge are considered as the freshly formed SMP. In Figure 2, the evolution profile of nitrate and nitrite under the anoxic condition is presented. As the reduction of 1 g nitrite to $N_2$ needs the same amount of electrons to the reduction of 0.6 g nitrate to $N_2$, the equivalent nitrate was thus corrected by $NO_3^-$-N+0.6$^*$$NO_2^-$-N.[17] As shown in Figure 2A-C,

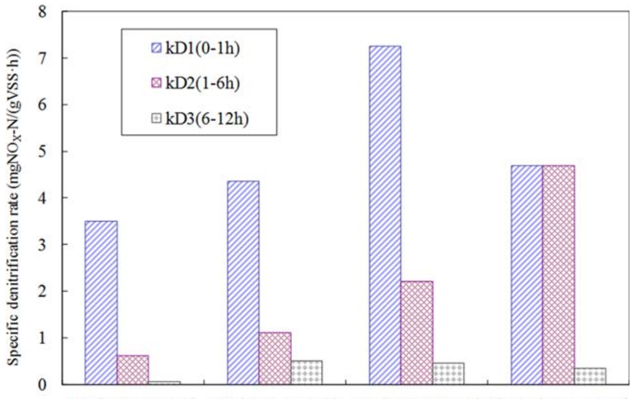

**Figure 3. The SDNR (i.e., $k_{D1}$, $k_{D2}$, $k_{D3}$) calculated according to the evolution profile of the corrected nitrate ($NO_3^-$-N+0.6$^*$$NO_2^-$-N).**

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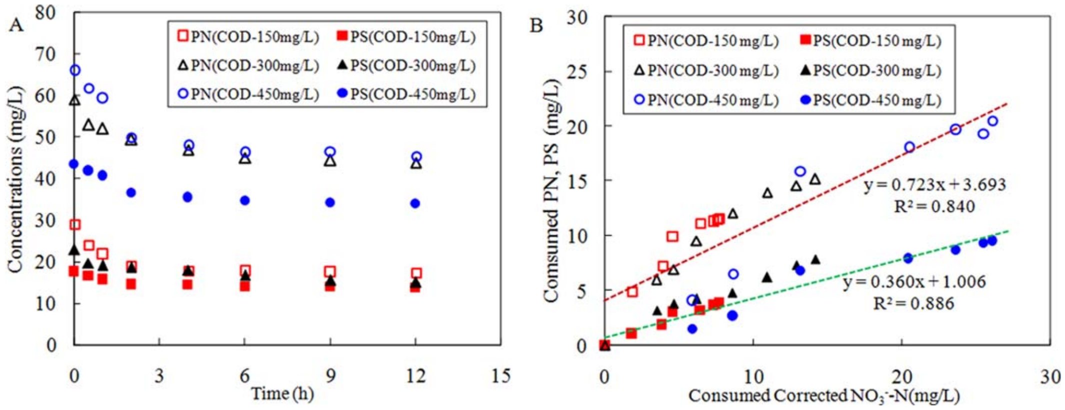

**Figure 4. (A) The variations of PN and PS in the different EPS loads solutions during the denitrification potential tests, and (B) the correlations between the consumed PN/PS and the consumed corrected $NO_3^-$-N with each EPS loads.**

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the evolution profile of the corrected nitrate can be apparently divided into three phases, and thus three SDNR (i.e., $k_{D1}$, $k_{D2}$, $k_{D3}$) could be obtained. As shown in Figure 3, it can be seen that an increase of the EPS loads could result in a great increase of $k_{D1}$ (i.e., from 3.55 to 4.36 and 7.26 mg-$NO_x$-N/(g-VSS·h)). In addition, the $k_{D2}$ and $k_{D3}$ were also impacted by the EPS loads. In fact, the EPS solution with high organic loads could provide more available electron acceptors. Interestingly, we noted that the glucose with COD = 300 mg/L had a comparable $k_{D1}$ to the EPS with COD = 300 mg/L, implying that the readily bioutilizable matter in the EPS had a similar denitrification potential to the glucose. Our finding also is generally in good agreement with a previous study that the supernatant of solubilized excess sludge could act as electron donors for denitrifaction.[18] This phenomenon further confirms that a great portion of the freshly produced SMP from EPS in the mixed sludge liquor could be readily biodegraded by the denitrifiers as carbon source.

Corresponding with the three different SDNR, the biodegradation trends of PN and PS at different EPS loads could be also divided into three different periods (Figure 4A), implying that the PN and PS were composed of readily biodegradable, slowly biodegradable, and nonbiodegradable compounds. In the first 2 h, the EPS were consumed rapidly due to the consumption of the readily biodegradable PN and PS. However, subsequently, their degradation rates slowed down. Till the end, the PN and PS maintained at a stable level as the residual compounds were much more difficult to be degraded. Interestingly, it was noted that the decrease rate of PN was always higher than that of PS under each EPS loading rate. It suggested that the PN in EPS was more readily unitized by denitrifiers than the PS. In addition, there was a significantly positive correlation between the consumed PN/PS and the consumed corrected $NO_3^-$-N (Figure 4B), with $R^2$ of 0.84 and 0.89 for PN and PS, respectively.

According to the results mentioned previously, the main conclusions of this study can be drawn as follows (1) the occurrence of denitrification under anoxic conditions can help in suppressing and consuming the PN and PS of SMP, and (2) the readily biodegradable fraction in the extracted EPS (referred to the freshly formed SMP) had a as high SDNR as that of glucose. Besides, the PN in the extracted EPS had a higher denitrification potential than the PS. Overall, denitrification could be an important mechanism to reduce SMP and EPS, and thus to mitigate membrane fouling in MBRs.

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