Effects of sludge settling characteristics in the BNR system

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Abstract Although the BNR system design assumes no sludge settling problems in the final settling tank, the fluctuation of daily loading and up to 40% of anaerobiosis in the BNR system would be considerable factors in determining the sludge settleability. The sludge volume index (SVI) is a classical parameter to examine the sludge settleability but it has a limited value to evaluate the overall settling characteristics. The extracellular polymer (ECP) content in sludge has long been considered as an indicator of biological flocculation in activated sludge process, but the skepticism on analytical accuracy limits the usage in settling study. This study focused on evaluating the BNR system performance related to the sludge settling characteristics under the controlled laboratory environment. A 5-stage BNR system (anaerobic-anoxic-3 stage oxic) was operated with the HRTs from 3.9 to 7.6 hours. In order to determine the sludge settling properties, both SVI and ECP content were monitored. The ECP contents in the sludge sample were measured by the slime-washing step followed by high-G centrifugation and sonication. The gel-electrophoresis was used to detect the DNA in the sonicated samples to determine the intracellular polymer contamination. It has been found that the anaerobiosis in the anaerobic and anoxic zone that consisted of 37% of reactor volume could not deteriorate the sludge settling properties even at the HRT of 3.9 hours. The SVI values of sludge taken from the reactor with the HRT of 7.2 hours averaged to less than 50 mL/gr. It was postulated that a fast settling sludge in the BNR system could not always ensure production of clear effluent. The sludge with very low SVI might not achieve a complete biological flocculation potential. In addition, the degree of denitrification in the BNR system could be related to the sludge settleability.

Keywords BNR, extracellular polymer, sludge settling, SVI

Introduction The successful design and operation of biological nutrient removal (BNR) processes critically depend on the solids-liquid separation step in the final settling tank. Although the BNR system design assumes no sludge settling problems in the final settling tank, the fluctuation of daily loading and up to 40% of anaerobiosis in the BNR system would be considerable factors to determine the sludge settleability. An early survey in South Africa (Lakay et al., 1988; Blackbeard et al., 1988) indicated that sludge settling problems were occasionally encountered in BNR plant operations. The anaerobiosis of activated sludge could improve sludge settleability. Early studies by Westgarth et al. (1964) and Ford and Eckenfelder (1970) reported that the sludge settleability was improved when activated sludge was exposed to a short period of anaerobiosis. A properly designed anaerobiosis zone in the BNR system could not induce the sludge settling problems (Wanner et al., 1987a, b). However, fluctuation of influent loading and temperature may deteriorate the sludge settleability. A hypothesis of surface to volume ratio between floc formers and filamentous organisms that compete for DO and substrate would be a possible explanation for the bulking-related settling problems. Another interesting fact is that sludge that settles too fast could not produce clear and crisp effluent due to the limited flocculation of fine particulates. As a consequence, the sludge volume index (SVI) test has a limited value to evaluate the overall settling characteristics. The extracellular polymer (ECP) content in the sludge has long been considered as an indicator of biological flocculation in the activated sludge process. However, the skepticism for analytical accuracy due to the contamination of intracellular polymer (ICP) during the ECP analysis has circum-
scribed the usage in settling study. Analytical methods to determine the ECP content in sludge samples have been subject to intensive researches (Brown and Lester, 1980; Novak and Haugan, 1981; Gehr and Henry, 1983) and various modification methods have been developed. In addition, the importance of ECP in the sludge settling step was further materialized by Sheintuch and co-workers (1986; 1987) for the design of final settling step which was incorporated with sludge age and ECP content in the activated sludge process, but it is not widely accepted for the design purpose. The purpose of this study is to investigate the relations between the classical settling index of SVI and extracted biopolymers in the sludge sample from the BNR system. Regarding the importance of denitrification in the BNR system, a comparison between the sludge settleability and denitrification was also investigated.

Materials and methods

Laboratory reactor. A 5-stage laboratory BNR reactor as shown in Figure 1 was operated with various hydraulic retention times (HRT) of 3.9, 5.6 and 7.2 hours. The BNR reactor consisted of anaerobic–anoxic–3 stage oxic zones. Both anoxic internal recycle of 100% and anaerobic internal recycle of 100% were maintained on the basis of influent flow rate. External sludge recycling was also maintained at 100% of influent flow rate. The operating temperature was maintained at 20 ± 0.5°C. The operating MLSS in the reactor was maintained in the range of 2,500 ~ 3,000 mg/L.

Wastewater quality analysis. All analytical procedures for wastewater qualities were performed in accordance with the Standard Methods (1995), except biopolymer content in sludge. Analytical methods to determine the ECP content in sludge samples have been subject to intensive researches (Brown and Lester, 1980; Novak and Haugan, 1981; Gehr and Henry, 1983) and various modifications have been developed. In this study, the biopolymer content was measured by the centrifugation and sonication followed by gravitational analysis as follows. The sludge samples from oxic reactor were used for SVI test. At the same time, centrifuge 50 mL of sludge sample for 15 minutes at 2,700 G (Marathon 21 K). Discard the supernatant in order to remove the slime ECP. Re-suspend the centrate with the deionized water. Repeat the step. The re-suspended centrate is then exposed to the sonication (Cole-Parmer Model CV 26). Centrifuge the sample for 10 minutes. Remove the supernatant and mix to 1:1 with a solvent (ethyl alcohol + acetone). The mixture is then cooled overnight at 4°C. Observe the white to straw color precipitates. Filter the precipitates with a glass fiber filter paper (Whatman 934 AH), and dry at 80°C for 2 hours. Measure the residues with an analytical balance. Calculate the polymeric content in the original sludge samples in grPolymer/grMLVSS. Although the extracted materials would be mostly biopolymers from the sludge sample, it is more accurate to call them “solvent insoluble matters (SIM)” from the sludge. In addition, the specific surface area and specific mean diameter of particulate in the supernatant of 30 minutes settled-sludge samples were measured by the particle size analyzer (Mastersizer MAF 5001, Malvern Instrument, Ltd).

![Figure 1 Layout of laboratory BNR unit](https://iwaponline.com/wst/article-pdf/42/3-4/283/428141/283.pdf)
Wastewater and effluent qualities. A settled sewage from the domestic wastewater treatment plant was stored at 4°C, and fed to the lab BNR reactor. The operational results of various operating HRTs are shown in Table 1. The nitrification and denitrification were not fully developed at short HRTs. Biological phosphorus removal was also not fully developed in the laboratory reactors probably due to lower content of readily biodegradable organic in the wastewater.

Results and discussion

Verification of biopolymer extraction method. According to the previous studies on ECP analysis (Brown and Lester, 1980; Gehr and Henry, 1983), most of the analytical error is caused by the extraction method itself. Since the ECP extraction method commonly uses strong chemicals so that it eventually leads to an eruption of protoplasm that contains intracellular polymers. For instance, both extracellular polymer and intracellular polymer have similar chemical characteristics when analyzing the biopolymeric contents. In these cases, the resulting ECP data could include the intracellular polymers with ECP. It means that the extensive chemical treatment and physical stripping methods must not be used for the ECP analysis. In order to verify the biopolymer data, a cell DNA test was performed for the sonicated samples. If the sample in this study was exposed to extensive sonication and high degree of G forces, both intracellular polymers and DNA of cells could be released. If cell DNA is detected, it is an analogue of intracellular polymer eruption. The gel-electrophoresis was used to detect the DNA in the sonicated samples to determine the ICP contamination. The DNA screening method to prevent ICP contamination could improve the reproducibility of ECP data. Preliminary experiment was performed with various sonication times at a given sonicator output power (12% of full power for Model CV 26) to detect the cell DNA contamination. It was found that significant amount of cell DNA was detected at more than 10 minutes of sonication time. Based on the experimental result, all experiments for the SIM of sludge polymer test were performed with the cell DNA tests.

Effects of reactor retention times. It is known that most extracellular polymeric materials that are extracted from activated sludge are reported to be polysaccharides (Friedman et al., 1969). The polysaccharide materials would be represented by both the hydrophilic nature in the bulk liquid and negative surface charge on the bacterial surface. An activated sludge process operated at longer sludge retention times generally provides better settling sludge
corresponding to a high extracellular polymer production (Tenny and Stumm, 1965; Bisgoni and Lawrence, 1971; Gulas et al., 1979). Although the extracted materials from the sludge samples are actually solvent insoluble matters (SIM), the extract could contain mainly biological polymers. Regarding the settling characteristics in the laboratory BNR system, effects of HRT on sludge settling parameters (SVI and SIM) are plotted in Figure 2. The relations between HRT and sludge settleability have a complex nature. In general, the sludge settleability improves with longer HRT as shown in other studies (Tenny and Stumm, 1965; Bisgoni and Lawrence, 1971; Gulas et al., 1979). However, biopolymer contents, or SIM, in sludge samples can hardly be correlated with the SVI data within an operating reactor HRT. It seems that the sludge settleability measured by SVI decreases with higher SIM content, especially at the HRTs of 7.2 and 5.6 hours. Similar to the claim of Gehr and Henry (1983), SIM data may not give a clear picture of sludge settling behavior. We observed that the sludge settleability measured by SVI was mostly less than 150 mL/gr even at the reactor HRT of 3.9 hours. It is noted that a control activated sludge reactor comparatively operated at 3.0 hours of HRT showed a severe bulking. Since the control unit did not have nutrient removal features (e.g., internal recycles and anaerobiosis) and operated at slightly different SRT, a parallel comparison cannot be made. However, considering the fact that the oxic zone accounts for 67% of reactor volume (or 2.6 hours of HRT), the anaerobiosis in the lab BNR system with low HRT of 3.9 hours had a positive effect on the sludge settleability.

Figure 3 represents the relations between effluent SS and SVI with corresponding SIM data in a 3-D plot. The correlation between SVI and SIM data was insignificant. For instance, the SVI values in the oxic tank sludge, generally below 50 mL/gr at HRT of 7.6 hours, indicated the development of very fast settling sludge in the lab reactor. However, SS concentration in the final effluent from the reactor with 7.6 hrs HRT was not markedly improved compared to reactors with shorter HRTs. A positive relation between biological flocculation behavior in the activated sludge and terminal settling velocity of a floc could exist but it may not correspond with the effluent SS quality. A low average SS concentration of 9.9 mg/L was observed at the reactor with 7.6 hours of HRT, but at the same time, we also observed very fine particulates in the effluent. The final effluent from the reactors with 7.6 hours of HRT showed a low average SS concentration of 9.9 mg/L, but we also noticed the existence of very fine particulates in the effluent. The specific surface area (S.S.A.) and surface mean diameter (S.M.D.) values of particles were analyzed with the supernatant of 30 minutes-settled sludge taken from the anaerobic (An), anoxic (Ax) and oxic (Ox) tanks in the laboratory BNR units. Figure 4 shows S.S.A. in m²/grSS and S.M.D. in µm of the sludge samples from the BNR reactor. Unexpectedly, it was very hard to determine the relation between operating HRTs and the physical parameters of SSA and SMD values. Although it is premature to conclude that the effluent SS concentration cannot be related to SVI and both bio-

![Figure 2](https://iwaponline.com/wst/article-pdf/42/3-4/283/428141/283.pdf)
chemical-physical parameters of sludge samples, the sludge with very low SVI might not achieve the complete biological flocculation potential due to low ECP values.

Effects of sludge settleability in denitrification. The degree of nitrate removal was observed during the overall experimental periods. Figure 5 shows the 3-dimensional plot of nitrate removal rate in percentile over SVI and SIM data. The tendency of nitrate removal increased with better settling sludges with longer HRTs. According to the plot, the degree of denitrification in the BNR system could be related to sludge settleability.

Conclusions
The ECP contents in the sludge sample were measured by the slime-washing step followed by high-G centrifugation and sonication. The gel-electrophoresis to detect the DNA in the sonicated samples could be used to determine the contamination of intracellular polymers during the extraction. It has been found that the anaerobiosis in the anaerobic and anoxic zone that consisted of 37% of reactor volume could not deteriorate the sludge settling properties even at the HRT of 3.9 hours. The SVI values of sludge taken from the reactor with the HRT of 7.2 hours showed mostly less than 50 mL/gr. It was postulated that a fast settling sludge in the BNR system could not always ensure a clear effluent. The sludge with very low SVI might not achieve a complete biological flocculation potential. In addition, the degree of denitrification in the BNR system could be related to the sludge settleability.
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