Effects of Nitrate Recycle on the Sludge Densification in Plug-Flow Bioreactors Fed with Real Domestic Wastewater

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Abstract: The impact of adding a modified Ludzack–Ettinger (MLE) configuration with Nitrate Recycle (NRCY) on continuous-flow aerobic granulation has yet to be explored. The potential negative effects of MLE on sludge densification include that: (1) bioflocs brought by NRCY could compete with granules in feast zones; and (2) carbon addition to anoxic zones could increase the system organic loading rates and lead to higher feast-to-famine ratios. Two pilot-scale plug flow reactor (PFR) systems fed with real domestic wastewater were set up onsite to test these hypotheses. The results showed that MLE configuration with NRCY could hinder the sludge granulation, but the hindrance could be alleviated by the NRCY location change which to some extent also compensates for the negative effect of higher feast-to-famine ratios due to carbon addition in MLE. This NRCY location change can be advantageous to drive sludge densification without a radical washout of the sludge inventory, and had no effects on the chemical oxygen demand (COD) and nitrogen removal efficiencies. The PFR pilot design for the MLE process with a modified NRCY location tested in this study could be developed as an alternative to hydrocyclones for full-scale, greenfield, continuous sludge densification applications.

Keywords: NRCY; MLE; granular sludge; PFR startup

1. Introduction

Process intensification through the employment of advanced cell immobilization techniques, such as activated sludge densification via aerobic granulation, provides an economical option to expand the treatment capacity of wastewater resource recovery facilities (WRRFs) with a low capital, land, and maintenance investment [1]. To achieve aerobic granulation, hydraulic selection pressure has always been utilized as a driving force to selectively return better settling sludge to the feast zone or the phase of either plug-flow reactors (PFRs) or sequencing batch reactors (SBRs). This mode of operation selectively favors the coaggregation of microbial sludge into aerobic granules by washing out their diffuse flocculent sludge counterpart [1,2]. As a result, the transition from flocculent sludge to densified sludge often came at the cost of substantial sludge washout over the course of the reactor startup. This in turn directly resulted in the loss of treatment capacity until the granular sludge inventory within the reactor was slowly re-established [2,3]. However, in full-scale applications, WRRFs cannot withstand such a treatment interruption, even if it is temporary, and have to comply with discharge permit limits all the time. Therefore, a strategy is needed to drive densified sludge formation by selectively separating granules and bioflocs, while also safeguarding the effluent quality throughout the reactor startup period without treatment degradation or interruption.

Hydrocyclones have been employed in most of the WRRFs to provide such a selection pressure; however, their relatively low hydraulic capacity (e.g., 120–3360 m³ d⁻¹) limits their application in large systems to sidestream return activated sludge (RAS), where only
a small portion, e.g., 5–7%, of the RAS flow can be processed for physical selection [4–6]. This low-strength selection pressure plus the high suspended solids content in RAS, e.g., 5000 mg L\(^{-1}\), compromised the physical selection efficiency of hydrocyclones for sludge densification [7]. As a result, a prolonged startup phase, perhaps as long as a year, was required to observe significant sludge settleability improvements in full-scale continuous-flow reactors in which densified sludge formation is still a challenge [1,7–10]. For this reason, an alternative strategy should be developed to provide sufficient selection strength to promote successful continuous-flow sludge densification, while maintaining the startup treatment performance required for large full-scale applications. To this end, [2] demonstrated a new strategy by continuously returning the sludge in the underflow and overflow of a continuous upflow gravity selector to the feast and famine zones of a plug-flow reactor, respectively, which can effectively drive a smoother transition from flocculent-activated sludge to granular sludge, without dramatic sludge concentration and effluent quality decline during the reactor startup. However, this strategy was only proven successful in conventional-activated sludge pilots without biological nitrogen removal (BNR) [2,11]. Its application in a modified Ludzack–Ettinger (MLE) configuration with Nitrate Recycle (NRCY) has yet to be explored. The potential challenges for having both MLE and continuous-flow aerobic granulation in one system include that: (1) NRCY could bring bioflocs to the feast zone to compete with granules; (2) carbon addition to anoxic zones could lift the system organic loading rates and result in higher feast-to-famine ratios. Our hypotheses are that by changing the NRCY location, bioflocs could be largely separated from densified sludge, which makes the sludge densification achievable. Meanwhile, the higher organic loading rates (OLRs) and feast-to-famine ratios in MLE systems with NRCY caused by carbon addition could hinder the full granulation process. But the hindrance might be compensated to some extent by the NRCY location change. Testing these two hypotheses will not only provide engineering solutions, but also shed light on the continuous-flow aerobic granulation mechanism by crosschecking with the previous understandings of the feast and famine selection roles [2,11,12].

Thus, two PFR pilots fed with real domestic wastewater were set up onsite to test the hypotheses (Figure 1). Compared to the configuration in the study by [2,11], a NRCY system along with an equalization tank was added. The results from this study would shed light on how to drive sludge densification through granulation in an MLE process, while safeguarding effluent quality during reactor startup and providing a novel approach to enable the full-scale application of continuous-flow sludge densification technology with reasonable infrastructure modifications.

**Figure 1.** Illustrative design of the PFR systems equipped without NRCY (A) and with NRCY (B).
2. Materials and Methods

2.1. Reactor Setup and Operation

Two granulation PFR pilot trains were set up as illustrated in Figure 1A,B, and operated in situ by feeding real primary effluent (PE) at the Upper Occoquan Service Authority (UOSA), a WRRF in Centreville, VA, USA. The startup strategy of a conventional-activated sludge process was tested using the PFRs configured in Figure 1A and compared to the MLE PFRs with NRCY configurations in Figure 1B. Each PFR pilot with a 140 L total working volume was made of 10 identical completely mixed chambers connected in a series to create feast/famine conditions along the flow direction. The hydraulic retention time of each pilot was controlled at 6.5 h, similar to that of the full-scale secondary process at UOSA. All chambers in Figure 1A and Chambers 4 to 10 in Figure 1B were aerated at a flow rate of 3 L min\(^{-1}\) through aeration stones to ensure dissolved oxygen (DO) > 2 mg L\(^{-1}\) and maintain homogenous mixing conditions in each chamber. In contrast, the first three chambers in Figure 1B (MLE system with NRCY) were airtight and kept anoxic by purging nitrogen gas at the beginning and recirculating headspace gas in each chamber afterward. Methanol was added to the first chamber as a carbon source for denitrification.

As shown in Figure 1A,B, a continuous upflow column with an internal diameter of 8 cm and a total working height of 80 cm was installed at the end of the PFR to mimic a high-rate clarifier. The column inflow, i.e., the mixed liquor from the PFR effluent, continuously entered at a height of 30 cm from the column bottom and then flowed up for 50 cm before overflowing from the top of the upflow column (Figure 1A,B). The upflow velocity of this column, which is equivalent to the surface overflow rate (SOR) of a clarifier, was set at 10 m h\(^{-1}\) based on the flow rate and column cross-section area, to separate the sludge particles based on their settling velocity. Theoretically, only those particles with a settling velocity greater than 10 m h\(^{-1}\) can be retained within the underflow of the velocity selector and returned to the first feast zone chamber of the PFR (Figure 1A,B). The unsettled bioflocs in the overflow were sent to another clarifier designed with a more typical SOR of 1 m h\(^{-1}\). A portion of bioflocs settled under this low SOR was wasted on a daily basis for solids retention time (SRT) control (Figure 1A,B). The remainder of the flocculent sludge was returned to the first famine zone of the PFR. The flow rate ratio of the selector overflow to underflow was set at about 1.6. An airlifting method described in a previous study was used to return sludges in Figure 1A,B [3]. In addition, an equalization tank with an HRT of about 1 h was added to the MLE system after the clarifier (Figure 1B). NRCY in Figure 1B was achieved through a peristaltic pump (Masterflex\textsuperscript{®} L/S, Cole-Parmer, Vernon Hills, IL, USA), and its flow rate to the inflow rate was set at a ratio of 2. The NRCY location was changed from Chamber 10 to the equalization tank on Day 99.

2.2. Analytical Methods

The chemical oxygen demand (COD), nitrate, nitrite and ammonia concentrations were analyzed using TNTplus\textsuperscript{®} 820, 835, 839 and 840 vials in a spectrophotometer (Hach, Loveland, CO, USA). Nitrate, nitrite, ammonia and soluble COD (sCOD) samples were measured with the filtrate from 0.45 \(\mu\)m syringe filters (EZFlow\textsuperscript{®}, Old Saybrook, CT, USA). The total inorganic nitrogen (TIN) concentration was calculated by summing nitrate, nitrite and ammonia nitrogen concentrations. DO was measured using an HQ40D meter (Hach, Loveland, CO, USA). The sludge volume index (SVI) was calculated after diluting the mixed liquor samples to total suspended solids (TSS) concentrations of 1000 mg/L to avoid hindered settling [13].
3. Results
3.1. Negative Impacts of NRCY on Granulation

Figure 2 allows for a comparison of the settleability improvement in the last chamber of the two PFRs in Figure 1A,B. One (Figure 1A) is a conventional-activated sludge process without a BNR, and the other (Figure 1B) is an MLE process with NRCY and external carbon (methanol) addition. In general, the former appeared to achieve better granulation than the latter. Figure 2A shows that after a 141-day operation, the SVI30 of the systems without and with NRCY dropped to more or less the same level around 74 mL/g, even though the SVI30 of the system without NRCY somehow started at a much higher level than that of the system with NRCY (114 mL/g). In addition, SVI30 surges in the system without NRCY were observed on Days 36 and 71 due to accidental sludge loss events (Figure 2A). Meanwhile, SVI5/SVI30 evolution in Figure 2B demonstrated that the system without NRCY had much lower values than the system with NRCY. For example, after about a 140-day operation, the SVI5/SVI30 of the system without NRCY plummeted to around 1.5, while the one with NRCY stayed around 2.1. It was reported that the SVI5/SVI30 of successful granulation should approximate 1 [3]. Thus, the system without NRCY likely achieved more granulation than the other with NRCY. This could also be evidenced by the V2S profiles in Figure 2C. It can be seen that after around a 140-day operation, the V2S of the system without NRCY rose to 5.4 m/h, which was 42% higher than that of the system with NRCY.

As discussed above, the system appeared to achieve better granulation and thus, better sludge settleability when NRCY was not employed. In other words, the NRCY configuration could be disadvantageous to the aerobic granulation. The potential reasons for this were twofold. Firstly, NRCY located at the end of the aerobic zone could bring bioflocs to the feast zone competing with granules, which defeated the purposes of the pilot design (Figure 1). The pilot design herein can take advantage of feast/famine conditions in PFRs to provide a biological selection internal to the bioreactors by redirecting the underflow and overflow of the physical velocity selector to the feast and famine zones of the treatment train, respectively [12]. Therefore, the introduction of bioflocs to feast zones might be responsible for the inferior sludge settleability. This challenge was addressed by changing the NRCY location from the last aerobic chamber containing significant MLSS biomass to an equalization tank after biomass separation in the clarifier on Day 99, as shown in Figure 1B. The hypothesis of the recirculated flocculent biomass to the feast zone will be tested and discussed in the following sections. Even so, sludge settleability in the system with NRCY was still inferior to the one without NRCY. This could be ascribed to a second reason, i.e., the higher OLRs and feast-to-famine ratios caused by carbon addition. To denitrify NO3−–N brought by NRCY in the anoxic zone, external carbon (methanol) was added to the first anoxic chamber, as shown in Figure 1B, which led to a higher OLR.
than the one without NRCY (Figure 1A). According to Zhang et al. [14], a decreased OLR enhances the formation and stability of aerobic granular sludge because granular sludge cultivated under a decreased OLR maintains a good balance between polysaccharides and protein in the extracellular polymeric substance (EPS) content. Similarly, Ghangrekar et al. [15] reported that for a reactor started at a higher OLR, the SVI of the sludge became higher even if the SVI of the inoculum was lower. In addition, average sCOD concentrations in each chamber of the two systems were shown in Figure 3. One can see that without NRCY, a majority of the readily biodegradable sCOD (rbsCOD) was removed in the first two chambers, leaving mostly a refractory sCOD in downstream chambers. A feast-to-famine duration ratio of 0.25, as estimated from Figure 3, was deemed to be beneficial to aerobic granulation in PFRs [12]. In contrast, with the NRCY configuration and external carbon addition, rbsCOD was not consumed until Chambers 5 (before NRCY relocation) and 7 (after NRCY relocation), resulting in feast-to-famine duration ratios of 0.67 and 1.50, respectively, which were considered less favorable for successful granulation [12]. Therefore, the higher organic loading rates and feast-to-famine ratios in the MLE system with NRCY (Figure 1B) could explain its inferior granulation process to the one without NRCY (Figure 1A).

Figure 3. Average sCOD concentration profiles of the PFR pilot without NRCY (using data from Days 120 to 134), with NRCY and before its location change (using data from Days 43 to 57), and after its location change (using data from Days 120 to 134).

3.2. Effects of NRCY Location on Solid Concentrations

As mentioned in the previous section, it is hypothesized that the introduction of bioflocs to the feast zone by NRCY could be avoided by relocating NRCY from the last aerobic chamber to an equalization tank after the clarifier. This hypothesis was tested, and the results were revealed in the following sections.

From Day 1 to 98, NCRY was located at the 10th aerobic chamber, which was similar to the full-scale MLE process, as shown in Figure 1B. Day 1 to 36 was benchmarked as the startup period, as shown in Figure 4. After the startup, the MLVSS profiles of each chamber stabilized during Day 37 to 98 under the combined effects of NRCY and sludge inventory redistribution (Figure 4). Along with NO$_3^-$–N, a huge amount of flocculent biomass in the NRCY stream was returned to the first feast chamber, namely Chamber 1 (C1). It is well known that bioflocs are very competitive in utilizing readily available substrates for a number of reasons. They can outcompete granules because of their looser structure and higher specific surface area that promotes fast substrate diffusion and utilization [16]. Therefore, returning both flocculent and granular sludges in the NRCY to the same feast zone, as is conventionally practiced is counterproductive to the progression and stabilization of
continuous-flow sludge densification. After realizing this, the NRCY location was changed to the equalization tank after the clarifier on Day 99 (Figure 1B). There were very few suspended solids in the equalization tank after the clarifier. Thus, almost no bioflocs were returned to the feast zone after the location change. Consequently, a substantial drop of the MLVSS in all feast chambers was observed, as shown in Figure 4. For example, the average MLVSS concentration in C1 plummeted from 1403 to 498 mg/L after the location change. Conversely, MLVSS concentrations in the famine chambers were not subjected to the NRCY location change, and thus barely changed. This was because a large amount of bioflocs from the clarifier were returned to the first famine chamber, namely Chamber 7, by internal sludge inventory redistribution shown in Figure 1B, regardless of the NRCY location change.

Figure 4. MLVSS profiles in different chambers over the course of the pilot operation with NRCY.

With all this being said, sludge concentrations in the entire PFR actually decreased after the switch to the new NRCY source location. This was ascribed to effluent solid concentration (TSS and VSS) increases (Figure 5A) and an actual SRT decrease (Figure 5B). When the NRCY was relocated from the last aerobic chamber to the equalization tank (Figure 1B), the SOR of the clarifier rose from 1 to 1.7 m/h because the additional recycle flow went through the clarifier. Given this, it was anticipated that effluent TSS concentrations would increase, the degree of which was not well accounted for, from 27 mg/L (Day 106) to 73 mg/L (Day 148), after NRCY location change (Figure 5A). The SRT was calculated based only on the waste-activated sludge (WAS) being purposely discharged, while the actual SRT was impacted by the sludge lost in the clarifier effluent. In retrospect, when the SOR of the clarifier was small (≤1 m/h), the WAS was deemed as the only significant sludge leaving the system, but with the NRCY location change and the increase in SOR, the sludge loss through the effluent ramped up considerably, dropping the actual SRT after Day 99 to as low as 3 days on Day 141 (Figure 5B). Similar to the clarifier, the upflow velocity of the upflow selector also increased from 10 to 17 m/h with the NRCY location change (Figure 1B). Less densified sludge was retained in the underflow and more flocs were washed out, which caused the drop in MLVSS concentrations in the underflow of the upflow selector (Figure 6A). As a result, the dry mass ratios of underflow over overflow also declined (Figure 6B).
3.3. Effects of the NRCY Location on Mixed Liquor Settleability

As mentioned in the previous section, NRCY relocation facilitated the separation and redistribution of heavier densified sludge and lighter bioflocs to the feast and famine zones, respectively. Biofloc out-selection was further boosted by subjecting bioflocs to low substrate availability. As a result, an improvement of sludge settleability, especially in feast chambers, was observed after the NRCY location change.

As shown in Figure 7, the SVI30 of all the chambers dropped after the NRCY location change on Day 99. Among those, the SVI30 of the feast chambers dropped more than that of the famine chambers. For example, the SVI30 values of Chambers 2, 3 and 4 (C2, C3 and C4) dropped from 73, 74 and 90 mL/g to around 51, 49 and 54 mL/g, respectively, around 50 days after the NRCY location change (Figure 7). The research of [1] suggested an upper limit of 60 mL/g SVI30 for successful sludge densification or granulation. With that being said, successful sludge densification has been achieved in feast chambers such as C2 and C3, which demonstrated the effectiveness of the upflow selector and sludge redistribution system. Similarly, the drops in SVI5/SVI30 values in feast chambers were also observed (Figure 8). For example, the SVI5/SVI30 of C2, C3 and C4 dropped from 2, 2 and 1.8 to 1.6, 1.3 and 1.5 mL/g, respectively, 50 days after the NRCY location change. It should be pointed out that these SVI5/SVI30 values were still off from 1, which is a theoretical value when successful aerobic granulation was achieved [17], indicating the incomplete sludge granulation process. The improved settleability was also evidenced by VZS profiles in Figure 9. It can be seen that the VZS of the sludge in all the feast zones
increased substantially. Among those, the values of $V_{ZS}$ in C1, C2, C5 and C6 became greater or equal to the upflow velocity of the selector (17 m/h) 50 days after the NRCY location change, which indicated that sludges in these feast chambers were adapted to the reactor’s gravity selection pressure.

Figure 7. SVI$_{30}$ profiles in different chambers over the course of the pilot operation.

Figure 8. SVI$_5$/SVI$_{30}$ profiles in different chambers over the course of the pilot operation.
In contrast, the improvement of sludge settleability in the famine chambers after the NRCY location change was very limited in terms of SVI$_{30}$ (Figure 7), SVI$_5$/SVI$_{30}$ (Figure 8) and V$_{ZS}$ (Figure 9), which indicated that only part of the sludge inventory was densified through the granulation, unlike the system without NRCY mentioned in Section 3.1. It appears that the system was headed in the right direction, but as a result of unfavorable conditions for the entire system granulation, more time would have been needed to nurture the granular sludge formation and weed out the flocculent sludge from the system. One of the bottlenecks is the high feast-to-famine duration ratio due to supplemental carbon addition to the MLE system with NRCY (Figure 3).

### 3.4. Effects of NRCY Location on COD and Nitrogen Removal

As discussed in the previous section, after the change of NRCY location, better sludge settleability was observed, especially in the feast chambers. However, limited sludge settleability improvement was achieved in the famine zone, which indicated that only part of the sludge inventory was densified through granulation. The potential reason for this could be the relatively higher feast and famine ratios of the system after the NRCY location change (Figure 1B). As shown in Figure 3, before the NRCY relocation, the majority of the rbsCOD was removed in the first four chambers, leaving mostly refractory sCOD in downstream chambers, which resulted in a feast-to-famine duration ratio of 0.67. After NRCY relocation, the MLVSS of the first several chambers declined substantially, as mentioned in Section 3.2. Consequently, under the same OLR, rbsCOD was not used up until the seventh chamber after the change of NRCY location, which led to a feast-to-famine duration ratio of 1.5 (Figure 3). It was reported by [12] that to achieve successful continuous sludge densification in PFRs, a feast-to-famine duration ratio should be equal to or less than 0.6 in addition to an adequate external gravity selection pressure. With this being said, feast-to-famine duration ratios of 0.67 and 1.5 before and after NRCY relocation, respectively, were both considered unfavorable for successful sludge densification in PFRs. However, the change of NRCY location promoted the sludge densification, especially in feast chambers in terms of sludge settleability, despite the unfavorable feast-to-famine duration ratio. One can see that a biological selection internal to the reactor imposed by separately returning heavier densified sludge and lighter bioflocs to the feast and famine zones with the aid of NRCY relocation, and the increased gravity selection pressure in terms of the upflow velocity in the selector could compensate for the increase in feast-to-famine duration ratios to some extent.
Influent tCOD and sCOD removal efficiencies remained around 75% and 65%, respectively, and they were uninterrupted throughout the entire operation (Figure 10A). Similarly, ammonia removal efficiencies also remained stable (Figure 10B), regardless of NRCY location change, excluding some fluctuations from Days 71 to 92 due to SRT alteration (Figure 5B), which indicated the good nitrification performance over the course of the operation. These could be explained by the fact that floc recirculation from the bottom of the clarifier allowed for the retention of an adequate amount of sludge inventory. Figure 11A,B show the nitrogen profiles on Day 57 (before NRCY location change) and Day 127 (after NRCY location change), respectively. The NRCY location change seemed to have no effect on the system TIN removal, as both locations resulted in around 70% TIN removal and met the discharge limit of TIN < 10 mg/L. Specifically, system TIN removals mainly relied on the methanol-driven denitrification in anoxic chambers, especially in C3 (Figure 11A,B). As shown in Figure 12, DO concentrations were above 1 mg/L in C1 and pilot operation. Figure 10A,B show the nitrogen profiles on Day 57 (before NRCY location change) and Day 127 (after NRCY location change), respectively. The NRCY location change seemed to have no effect on the system TIN removal, as both locations resulted in around 70% TIN removal and met the discharge limit of TIN < 10 mg/L. Specifically, system TIN removals mainly relied on the methanol-driven denitrification in anoxic chambers, especially in C3 (Figure 11A,B). As shown in Figure 12, DO concentrations were above 1 mg/L in C1 and C2, but dropped to around 0.6 mg/L in C3 on both Days 57 and 127. In other words, the chambers did not turn strictly anoxic until C3, although methanol was added in C1, and aeration was not employed in the first three chambers. The DO concentrations in C1 were even higher than the influent. This could be explained by the fact that NRCY and granule recirculation streams to C1 were entrained with large amounts of DO. As mentioned in Section 2, these air-uplifted granule streams from the underflow of the upflow selector contained air. The DO was not consumed by ordinary heterotrophic organisms (OHOs) until the third chamber (C3). As a result, most of the denitrification took place in C3, as shown in Figure 11 A,B. The TIN drop in C1 could be ascribed to the dilution caused by the mix of influent, NRCY and granule recirculation. Nitrification started as aeration began in C4 and basically, all of the NH$_4^+$–N residuals were oxidized to NO$_3^−$–N before C9.

![Figure 10](image1.png)

**Figure 10.** Profiles of (A) influent COD and (B) ammonia removal efficiencies over the course of the pilot operation.

![Figure 11](image2.png)

**Figure 11.** Nitrogen concentration profiles in different chambers on Day 57 before NRCY location change, (A) and Day 127 after NRCY location change (B). Chamber 0 refers to the influent.
4. Discussion

4.1. The Synergy between Physical and Biological Selection Pressures

Granulated cells were not able to compete with dispersed cells (bioflocs) for the limited substrate availability because bioflocs have looser structures and a higher specific surface area that promotes fast substrate diffusion and utilization [16]. There is a consensus that sludge densification is normally driven by physical selection pressure that selectively reduces the persistence of bioflocs in a bioreactor [1]. For this reason, extremely low SRTs, e.g., 0.3–0.4 days, had to be employed with such a conventional approach to rid the biomass inventory of bioflocs in a radical way [3,18], because the SRTs of dense granules and bioflocs were not able to be differentiated in previous granulation studies. As an undesirable consequence, the MLVSS in a bioreactor subjected to such a radically short SRT will also have a dramatic reduction in performance, resulting in poor effluent quality [3,18]. This performance loss resulting from the drastic physical out-selection pressure could be resolved by incorporating a strategy that manages to separate flocculent sludge and granule SRTs, while incorporating reactor internal biological selection through controlling feast and famine regimes for the distinctly different sludge morphologies, i.e., selective densified and biofloc returns to feast and famine zones, respectively. As shown in Figure 1B, an upflow selector with a selection velocity of 10–17 m/h offered the possibility for SRT deviation of densified and flocculant sludge. It is noteworthy that other than the loss of solids in the final clarifier effluent, biofloc wasting from the bottom of the clarifier through SRT control is the only outlet for bioflocs to be removed from the PFR system (Figure 1B), and bioflocs were the only type of sludge wasted. The short SRT of bioflocs realized a significant out-selection of this type of biomass, while the retention and recirculation of densified sludge resulted in their persistence and dominance in the sludge bed of feast chambers, as mentioned in Section 3.3. In contrast to the physical selection provided by the upflow selector external to the bioreactors, biological selection internal to the reactor imposed by separately returning a heavier densified sludge and lighter bioflocs to the feast and famine zones further boosted biofloc out-selection by subjecting bioflocs to low substrate availability. To this end, an innovation on the NRCY location of an MLE process in a PFR pilot train was conducted in this study, as mentioned in the previous sections, i.e., returning nitrate-rich mix liquor from the equalization tank after the clarifier instead of the aeration tank to avoid returning bioflocs to the feast chambers. This is a novel application of internal biological selection pressure that can boost a continuous-flow sludge densification and has not been widely accepted in full-scale applications. Consequently, the densified sludge MLSS gradually built up in the PFR, obtaining the advantage from their growth in the feast zone, while bioflocs were steadily starved out.

Figure 12. DO concentration profiles in different chambers on Day 57 before NRCY location change, and Day 127 after NRCY location change. Chamber 0 refers to the influent.
4.2. Implications of Full-Scale Application

At present, the most popular continuous flow selectors employed in MLE processes in WRRFs are hydrocyclones, which are currently limited to sidestream wasting applications due to technology sizing limitations and associated costs [5,9]. UOSA has also employed hydrocyclones to successfully enhance its full-scale activated sludge settleability. As designed, only 5% of the RAS was processed through the hydrocyclones. Consequently, only moderate sludge settleability improvement was observed over 23 months of operation. In contrast, in this study, it only took 148 days to achieve sludge densification in C2, C3 and C4, as indicated by SVI$_{30}$ profiles in Figure 7. The SVI$_{30}$ of C2, C3 and C4 dropped to below 60 mL/g on Day 148, while the SVI$_{30}$ of the rest of the chambers still kept above 60 mL/g (Figure 7). If we assume that biomass in C2, C3 and C4 with SVI$_{30}$ < 60 mL/g was densified sludge and the biomass in other chambers (C5 to C10) was bioflocs, as suggested by [1], then around 32% sludge inventory was densified in terms of the MLVSS shown in Figure 4. According to [10], pursuing full granulation in full-scale application is unnecessary, and only a smaller quantity of aerobic granular sludge proportion, as low as 20%, is sufficient to maintain the SVI below 100 mL/g, and could solve a lot of operational problems for plants suffering acute recurring seasonal bulking or subjected to hydraulic overloading beyond original clarifier design, while experiencing storm-induced peak events.

In addition, the design of the upflow selector and an equalization tank incorporated in Figure 1B is achievable in WRRFs. For example, the upflow selector mimics a conventional clarifier with a very high SOR, so there may be, in some cases, the flexibility to fit upflow physical selector structures within WRRFs. Therefore, the PFR pilot design (Figure 1B) for MLE process with a modified NRCY location tested in this study could be developed as an alternative to hydrocyclones for full-scale, greenfield, continuous sludge densification applications. However, the effluent TSS increase and sludge loss issues mentioned in Section 3.2 after the NRCY location change should be further addressed in future studies.

5. Conclusions

The following concluding remarks can be drawn from this study:

- MLE configuration with NRCY could hinder the sludge granulation, but the hindrance could be alleviated by the NRCY location change from the aerobic chamber to the equalization tank after the clarifier.
- MLE configuration tended to have higher feast-to-famine ratios, the negative effects of which on the sludge densification could be compensated by NRCY location change to some extent.
- The NRCY location change in conjunction with floc recirculation and continuous upflow selection can be advantageous to drive sludge densification without a radical washout of the sludge inventory.
- The NRCY location change had no effects on the COD and nitrogen removal efficiencies.
- Higher feast-to-famine ratios of the MLE system with NRCY could be the bottleneck for full sludge granulation processes.

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