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Coupling physical selection with biological selection for the startup of a pilot-scale, continuous flow, aerobic granular sludge reactor without treatment interruption

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ABSTRACT

This study removes two technical constraints for transitioning full-scale activated sludge infrastructure to continuous flow, aerobic granular sludge (AGS) facilities. The first of these is the loss of treatment capacity as a result of the rapid washout of flocculent sludge inventory and in turn the potential loss of nitrification during initial AGS reactor startup. The second is the physical selector design which currently is limited to either the complex sequencing batch reactor selection or sidestream hydrocyclones. Briefly, real wastewater data collected from this study suggested that by increasing the surface overflow rate (SOR) of an upflow clarifier to 10 m h $^{-1}$, the clarifier can be taken advantage of as a physical selector to separate flocculant sludge from AGS. Redirecting the physical selector underflow and overflow sludge to the feast and famine zones of a treatment train, respectively, can create a biological selection that not only promotes AGS formation but also safeguards the effluent quality throughout the AGS reactor startup period. This study provides a novel concept for economically implementing continuous flow AGS within existing full-scale, continuous flow treatment trains.

1. Introduction

Nearly all full-scale aerobic granular sludge (AGS) cultivation has been achieved through the application of sequencing batch selectors to wash out slow-settling bioflocs as a means to drive fast-settling AGS formation (Kent et al., 2018). Unless fully seeded with AGS, sequencing batch reactors (SBRs) or continuous flow reactors are expected to suffer poor treatment performance during startup as a result of substantial sludge washout in the course of flocculent sludge transformation into an AGS morphology (Sun et al., 2019). In full-scale applications, wastewater resource recovery facilities (WRRFs) cannot withstand such a treatment capacity interruption, even if it is temporary, and have to comply with discharge permit limits all the time. Although this problem can be mitigated by initially inoculating reactors with AGS, it is almost impossible to find adequate local supplies of seed AGS given the current state of the technology. Adding to this startup difficulty, the challenge to provide an external settling velocity selection that can promote swift aerobic granulation in full-scale continuous systems by specifically targeting the separation of large and dense AGS particles is another technical barrier. Hydrocyclones have been employed to provide such a selection pressure; however, their relatively low hydraulic capacity (e.g., 120–3360 m^3 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 (Avila et al., 2021; Partin, 2019; Regmi et al., 2022). 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 AGS (Ford et al., 2016). 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 AGS formation is still a challenge (Ford et al., 2016; Kent et al., 2018; Roche et al., 2022; Welling et al., 2015; Wett et al., 2015). For this reason, an alternative strategy should be developed to provide sufficient selection strength to promote successful continuous flow AGS formation while maintaining the startup treatment performance required for large full-scale applications. This study aimed to address these two technical

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challenges in one design illustrated in Fig. 1.

It is our hypothesis that clarifiers (or other clarifier-like structures) might be used to provide the physical selection on sludge settling velocity needed by increasing the surface overflow rate (SOR) to 10 m h^{-1}. A 10 m h^{-1} selective settling velocity has been proven to be a physical selection strong enough to drive continuous flow aerobic granulation when a sequencing batch selector was used (Sun et al., 2021, 2019). In addition to this physical selection, our previous studies also concluded that having a feast-to-famine duration ratio <0.30 is conductive to sludge settleability improvement even when physical selection strength was not strong enough (An et al., 2021b). Hence, it is also our hypothesis that the feast/famine conditions investigated in our previous studies can be taken advantage of to provide 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. Although the roles of feast/famine conditions and the settling velocity selection have been reported in previous studies (Kent et al., 2018), a smart strategy of physical and biological selection integration into one continuous flow system fed with real wastewater for achieving both AGS formation and effluent quality safeguarding during reactor startup has not been developed before. This study is aimed to provide such a novel approach to enable full-scale applications of continuous flow AGS technology with reasonable infrastructure modifications.

2. Results

2.1. Upflow selection

The effectiveness of a continuous upflow selector in separating bio-particles based on their physical settleability was investigated in this study. The mixed liquor suspended solids (MLSS) distribution along the height of the upflow selector is shown in Fig. 2A, B, C, and D, which was used as an indicator of its particle separation performance and monitored over 197 days of the PFR operation. As can be seen from Fig. 2A, on the 25th day after the startup, there was only a slight MLSS difference, e.g., 300 mg L^{-1}, between the underflow and overflow, indicating poor separation efficiency probably due to the lack of sludge densification or granulation. To drive aerobic granulation, solids retention time (SRT) was gradually reduced from 10 days to 5 days within the next two months according to Fig. 2E. It is noteworthy that other than the minor loss of solids in the final clarifier effluent, biofloc wasting through SRT control is the only outlet for bioflocs to be removed from the PFR system (Fig. 1). As a consequence, both MLSS and mixed liquor volatile suspended solid (MLVSS) concentrations in the PFR gradually decreased in Fig. 2F corresponding to the SRT decrease in Fig. 2E. This SRT reduction led to gradual improvement of the MLSS distribution along the height of the upflow selector as shown in Fig. 2B to C as flocculent sludge was preferentially wasted from the system. The accidental sludge loss events on days 36 and 85, shown in Fig. 2F, were due to tubing leakage which was quickly fixed on the same day. By day 107 after startup, the difference in MLSS between the underflow and overflow increased to 2923 mg L^{-1} with only 1080 mg L^{-1} MLSS remaining in the overflow and

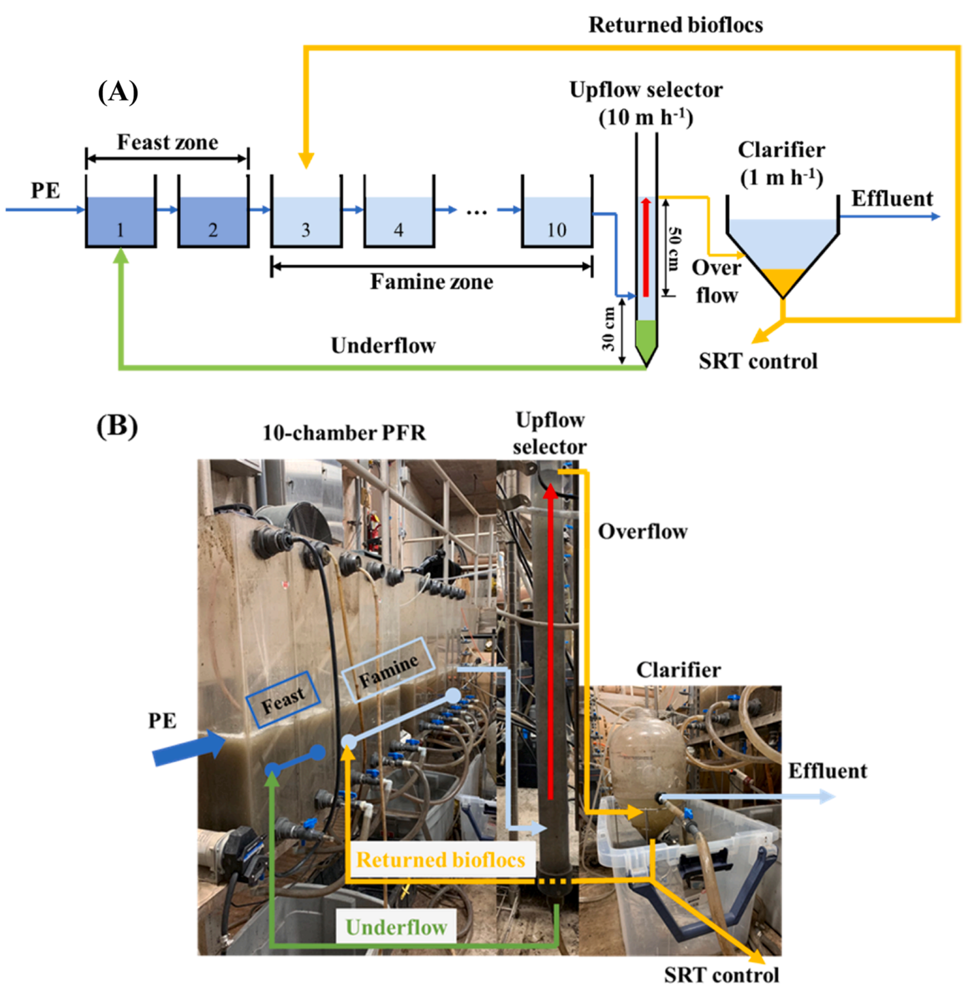

Fig. 1. (A) schematic and (B) photographic illustration of the 10-chamber plug flow reactor (PFR) equipped with a continuous upflow selector.

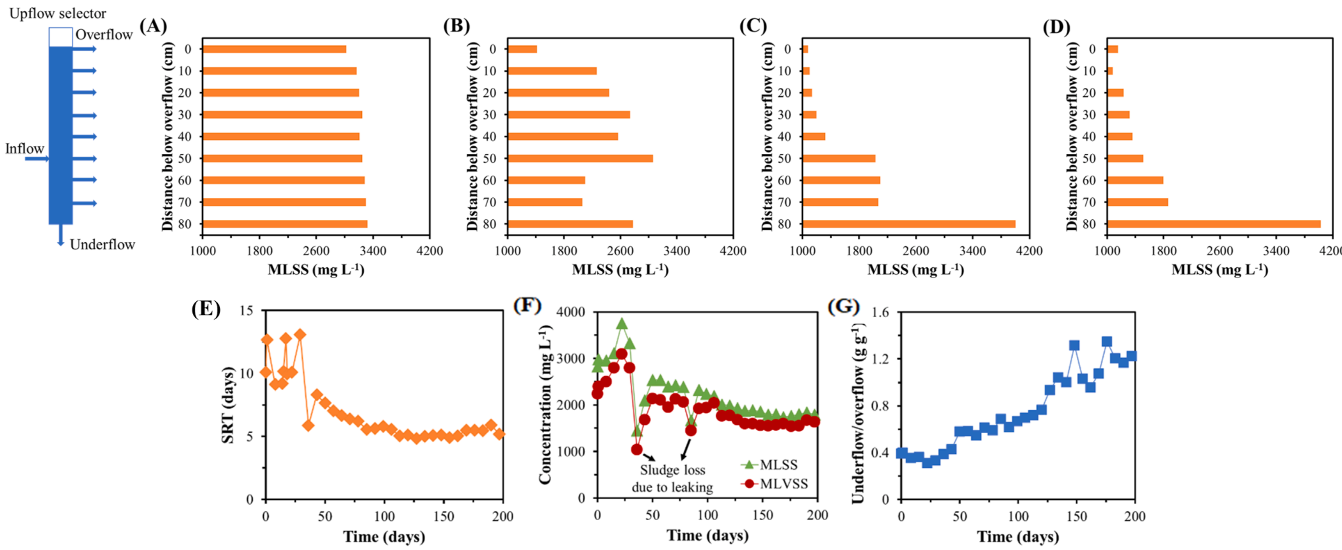

Fig. 2. MLSS concentration distribution measured at different distances below the overflow surface in the upflow selector on days (A) 25, (B) 43, (C)107, and (D) 197. Profiles of (E) SRT, (F) MLSS and MLVSS in the last chamber of the PFR, as well as (G) underflow-to-overflow biomass ratios of the upflow selector measured over the 197 days of operation.

4003 mg L^{-1} MLSS partitioned into the underflow (Fig. 2C). This clear MLSS gradient in the upflow selector remained throughout the rest of the experiment (Fig. 2D), demonstrating that excellent separation efficiency was achieved in the continuous upflow selector as the sludge settleability steadily improved over the course of the experiment (Fig. 1). As would be expected with separation efficiency improvement, the ratio between biomass partitioned into the upflow selector underflow and overflow gradually increased from an undesirable value of 0.3 to an acceptable value of 1.2 over the rest of the experiment (Fig. 2G). It was apparent that a large portion of the sludge biomass gradually transformed into larger and denser bioparticles that were continuously retained and returned to the feast zone (Fig. 1A).

In order to confirm this observation, particle size distributions of the sludge in the overflow and underflow of the upflow selector were compared on day 197 in Fig. 3A. As can be seen, the particle size distribution in the underflow showed two peaks with almost equal height, i.e., a peak around 250 μm representing more dispersed bioflocs and another peak around 1000 μm representing compact AGS. In contrast, the particle size distribution in the overflow only showed one peak around 250 μm, indicating only bioflocs were in the overflow. These particle size distributions were confirmed by photomicrographs of sludge morphologies sampled from the upflow selector overflow and underflow. As shown in Fig. 3E and F, large and dense AGS were comingled with bioflocs in the underflow while only bioflocs were present in the overflow. One can conclude that a 10 m h^{-1} SOR provided sufficient physical velocity selection to separate AGS from bioflocs. This good separation efficiency provides a prerequisite for dense AGS and light bioflocs to be further subjected to internal biological selection by returning them to the feast and famine zones of the bioreactor, respectively (Fig. 1).

On days 183 and 197, the carbonaceous specific oxygen uptake rates ($SOUR_{COD}$) and nitrogenous $SOUR_N$ of the sludges were measured in the upflow selector by sampling along the height of the column as shown in Fig. 4. The column inflow, i.e., upflow selection, starts at an entry point 50 cm below the overflow surface. All samples collected above this point had significantly greater $SOUR_{COD}$ than those collected below it ($p < 0.05$), indicating that the majority of bioflocs that were washed out with overflow were enriched with heterotrophic bacteria (Fig. 4A). Conversely, sludge samples collected below this entry point were the denser, more compact AGS demonstrating significantly greater $SOUR_N$ than those collected above it ($p < 0.05$), indicating the heavier bioparticles retained in the underflow were enriched with nitrifying bacteria (Fig. 4B). Since SRT was controlled by wasting the flocculent sludge in the upflow selector overflow (Fig. 1A), the “flocculent sludge” SRT is expected to have a shorter SRT than that of the bulk mixed liquor sludge and significantly shorter than that of the granular sludge which was retained almost indefinitely. This is one explanation for why the fast-growing heterotrophic microbes dominated the overflow biomass, while the slow-growing nitrifying bacteria partitioned to the underflow (Fig. 4).

2.2. Feast/famine selection

The in-situ oxygen uptake rate (OUR) and soluble COD (sCOD) profiles measured in all chambers of the PFR are shown in Fig. 5A. As can be seen, OURs in the first two chambers were substantially greater than those in the rest chambers (Fig. 5A), indicating the first two chambers were in the feast zone while the rest of the reactor chambers were more or less in famine condition. This observation is supported by the sCOD profile in Fig. 5A which shows the majority of the readily biodegradable sCOD was removed in the first two chambers, leaving mostly refractory sCOD in downstream chambers. The sCOD removal efficiency profiles in Fig. 5B measured throughout the 197 days of experiment further confirm that the great majority of the sCOD was removed in the first two chambers. These profiles evidenced a clear segregation between feast and famine zones throughout the period of reactor operation. As a matter of fact, a feast-to-famine duration ratio of 0.25, as estimated from Fig. 5A of this test, was confirmed to be an optimum feast-to-famine ratio for boosting aerobic granulation in both SBRs and PFRs (An et al., 2021b). This disparate substrate availability and microbial activity distribution between the feast and famine zones created a selective condition to favor biological selection toward AGS growth while suppressing the growth of dispersed bioflocs, particularly under the designed sludge return configuration shown in Fig. 1. In the field of aerobic granulation research, it is well-known that bioflocs are very competitive in utilizing readily biodegradable substrates for their looser structure and higher specific surface area (Tay et al., 2002). Hence, such a selective condition is required for aerobic granule sludge formation (Kent et al., 2018), i.e., returning final clarifier overflow biomass together with underflow biomass into the same feast zone would have eliminated such a selective condition and failed aerobic granulation unless a rapid washout of bioflocs is practiced which

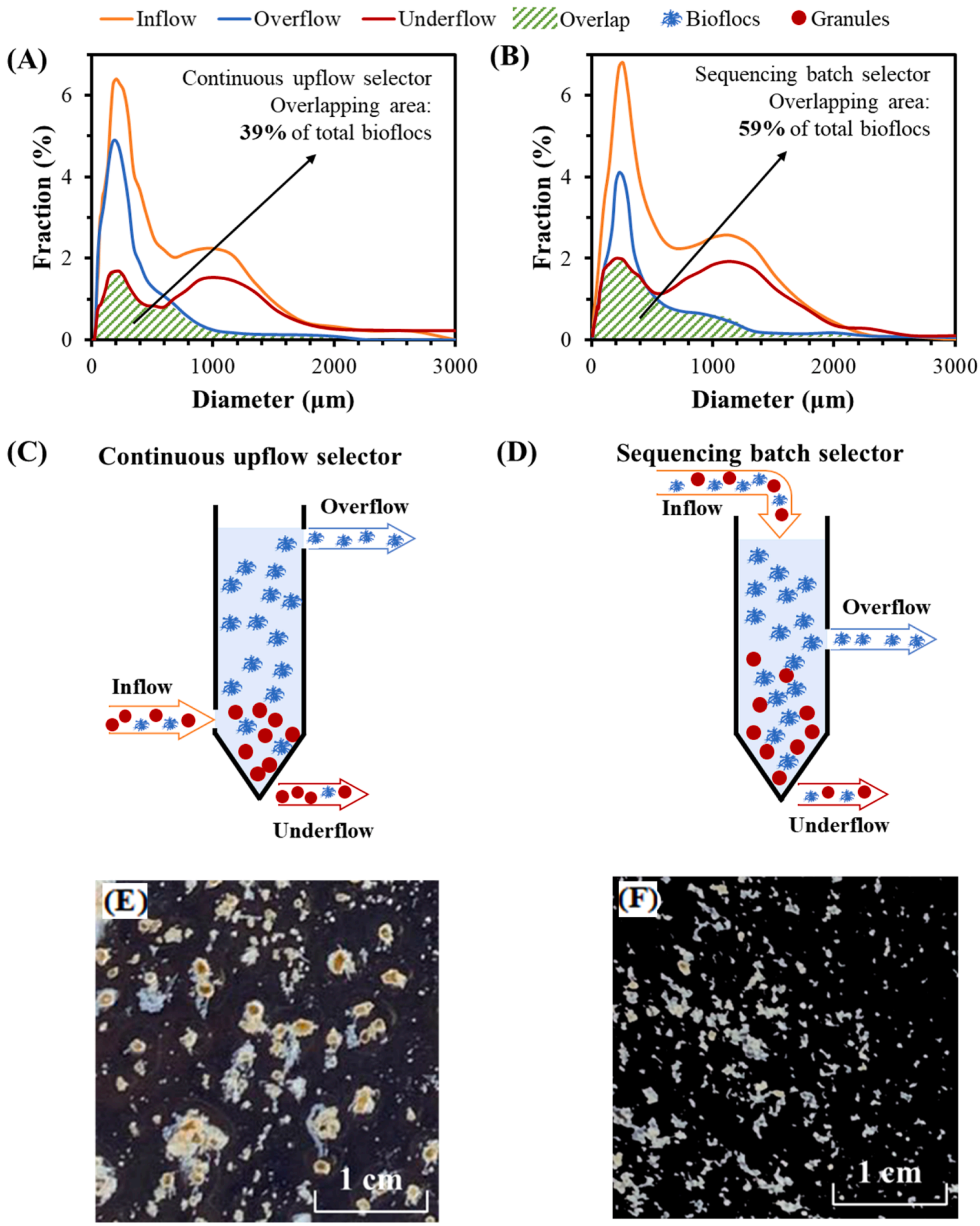

Fig. 3. Particle size distribution in the inflow, overflow, and underflow of (A) a continuous upflow selector and (B) a sequencing batch selector reported in a previous study (An et al., 2021a); Illustration of bioflocs escaping physical selection pressure in (C) a continuous upflow selector and (D) a sequencing batch selector; Morphologies of flocculent sludge and AGS in the (E) underflow and (F) overflow of the upflow selector on day 197.

unfortunately led to the loss of treatment capacity during startup (Sun et al., 2019). One can see from Fig. 2G that by segregating upflow selector return sludge to the feast zone and the final clarifier return sludge to the famine zone, the ratio of biomass particitioned into the underflow over overflow quickly increased. Over time more of the sludge inventory became heavier and was retained in the upflow selector underflow as it was returned to the feast zone where it benefited from a competitive substrate advantage while dispersed flocculent sludge was outselected through starvation and sludge wasting (Fig. 1).

2.3. The evolution of sludge settleability

Fig. 5C shows that the zone settling velocity (V_{zs}) of seed sludge was only 2 m h $^{-1}$. Unlike the radical V_{zs} improvement achieved in a previous study without bioflocs return (Sun et al., 2019), the SRT control practiced in this study showed a slower improvement in sludge settleability. After 162 days of sludge acclimation to the experimental conditions, V_{zs} gradually increased to 10 m h $^{-1}$, which is the designed SOR of the upflow selector (Fig. 5C). The sludge volume index, namely SVI_{30} and SVI_5/SVI_{30} have been collectively used to evaluate the extent of

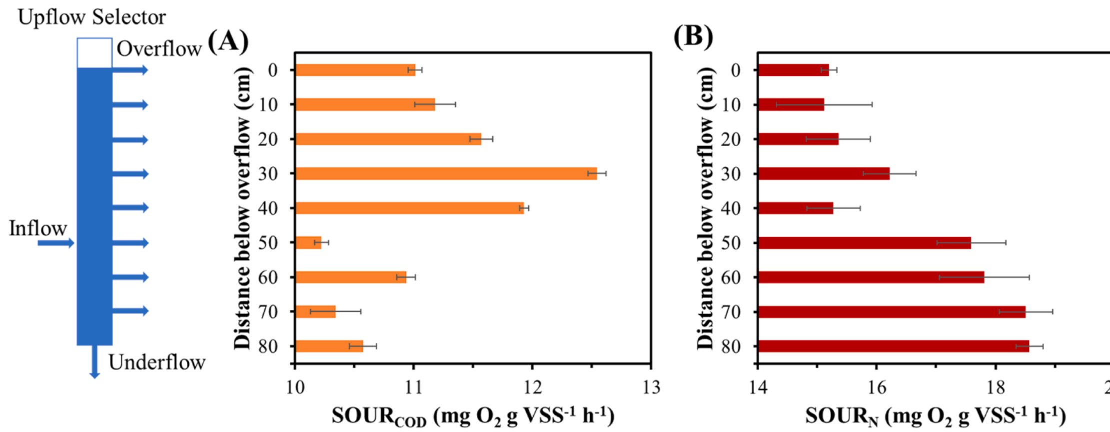

Fig. 4. (A) $SOUR_{COD}$ and (B) $SOUR_N$ distribution along the height of the upflow selector averaged from the data collected on days 183 and 197. The error bars represent the standard error derived from five sets of replicate data.

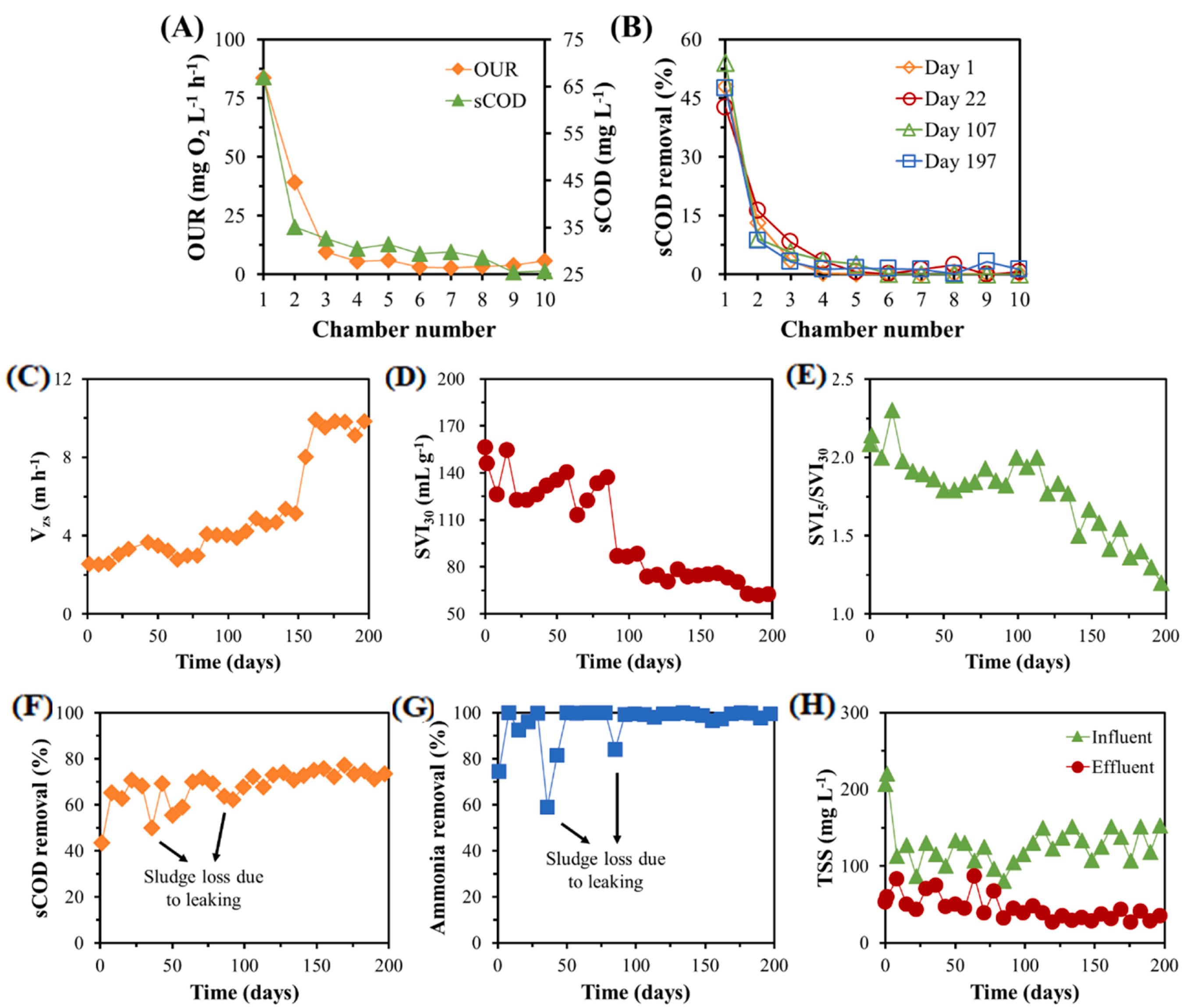

Fig. 5. (A) OUR and sCOD profiles measured in each chamber of the PFR on day 197, and (B) sCOD removal efficiency measured in each chamber on days 1, 22, 107, and 197; Profiles of (C) V_{zs}, (D) SVI_{30}, and (E) SVI_5/SVI_{30} measured in the last chamber of the PFR over the 197 days of operation; Pofiles of (F) sCOD removal, (G) ammonia removal, and (H) TSS in the influent and effluent of the PFR over 197 days of operation.

granular sludge formation (Kent et al., 2018; Pronk et al., 2015). Technically, granular sludge should possess $SVI_{30} < 60$ mL g $^{-1}$ and a SVI_5/SVI_{30} ratio approaching 1.0, respectively. Fig. 5D shows that SVI_{30} was stabilized around 60–65 mL g $^{-1}$ after 120 days, which was half the SVI_{30} value of the initial seed sludge. The SVI_5/SVI_{30} ratio also decreased from 2.0 to 1.2 after 197 days (Fig. 5E), evidencing good progression of successful continuous flow AGS.

2.4. *Effluent quality*

In Upper Occoquan Service Authority (UOSA), a high-lime process is used to flocculate phosphorus in tertiary treatment, and nitrate needs to be discharged to the downstream Occoquan reservoir to maintain an oxidized environment in the bottom waters for eutrophication prevention. For this reason, only sCOD and ammonia removal are targeted in the secondary treatment process investigated in this study. Fig. 5F and G show that both sCOD and ammonia removal efficiencies were maintained at acceptable levels, e.g., 75% and 100%, throughout 197 days of the AGS PFR startup period. There were two exceptions resulting from unintended sludge inventory losses on two individual days because the tubing leakage (Fig. 2F). Similarly, the final clarifier effluent total suspended solids concentration (TSS) was also controlled in an acceptable range throughout the 197 day test duration and stabilized around 30 mg L $^{-1}$ (Fig. 5H). The wall effect in the small clarifier used in the study (Fig. 1) might have aggravated the effluent TSS and can be avoided in full-scale clarifiers. Moreover, the tertiary lime flocculation and clarifiers in full-scale application will also further strengthen the TSS removal. One remarkable observation was that a five-day bulk SRT would normally be considered too short to sustain reliable nitrification in a bioreactor (Liu and Wang, 2014; Poduska and Andrews, 1975); yet 100% nitrification efficiency was achieved throughout the experiment (Fig. 5G). It can be inferred that a majority of the nitrifying bacteria were immobilized in the AGS returned from the upflow selector underflow, resulting in a much longer SRT for the denser sludge retained in the system. Observations from Fig. 4B are in-line with this inference.

2.5. *Continuous upflow selector versus sequencing batch selector*

Sequencing batch selector operation has been commonly used to provide the physical selection needed to drive AGS formation (Kent et al., 2018). In a previous study, a sequencing batch selector was used in concert with continuous flow feast/famine selection to achieve successful continuous flow AGS formation (An et al., 2021a). The sequencing batch selector was made of a column similar to the one used as a upflow selector in this study (Fig. 1), but it was operated in a sequencing batch mode with a cycle of one minute of feeding, four minutes of settling, one minute of overflow discharge, followed by another minute of underflow discharge. The volume exchange ratio of the sequencing batch selector was set at 50% with a discharging height of 65 cm to provide a selective settling velocity of about 10 m h $^{-1}$, which is same as the SOR of the upflow selector used in this study. In an attempt to compare the effectiveness of the two types of physical selectors in separating desired bioparticles under the same selective settling velocity, the particle size distributions for the two selector inflows, overflows, and underflows were plotted in Fig. 3A and B. In order to make a fair comparison, results based on a similar inflow particle size distribution were selected and presented. Technically, the selector separation efficiency should be inversely related to the overlapping area between the overflow and underflow, i.e., the same size particles ending up in both overflow and underflow indicates poor separation efficiencies; conversely, a 100% separation efficiency between bioflocs and AGS would leave zero overlapping area in particle size distribution between the two streams (Fig. 3A and B). Because the inflow entry point was located at 30 cm above the bottom of the continuous selector used in this study, a portion of bioflocs may escape upflow selection through gravity settling into this 30 cm stagnant bottom space as illustrated in Fig. 3C. Likewise, it is known that an SBR with a 50% volume exchange should have at least 50% bioflocs unselected due to gravity settling within the space below the effluent port as illustrated in Fig. 3D. As a result, those bioflocs escaping the selector overflow contributed to the overlapping area in Fig. 3A and B. Because a continuous upflow selector can be designed with an inflow entry point closer to the bottom, its particle separation should be more complete with less bioflocs escaping the selection. This assumption is supported by the results in Fig. 3A and B, i.e., the bioflocs escaping the physical selectors made up 39% and 59% of the total bioflocs that should have been washed out with the overflow from the continuous upflow selector and sequencing batch selector, respectively. These results indicate a much better separation efficiency in the former than latter. Theoretically, this separation efficiency in a continuous upflow selector could be improved by moving the inflow entry point further down to the column bottom in full-scale design (Fig. 3C).

3. Discussion

3.1. *The synergy between physical and biological selection*

There is a consensus that AGS formation is driven by selection pressure that selectively reduces the persistence of bioflocs in a bioreactor (Kent et al., 2018). Since SRT control is the only managed sludge outlet in the reactor shown in Fig. 1, and the flocculent sludge was the only type of sludge wasted, the successful continuous AGS demonstrated in Fig. 2 through 5 was driven by the SRT controlled wasting that imposed outselection pressure only on the flocculent sludge portion of the biomass inventory. This can be seen from the SRT-dependent key metrics for AGS such as SVI_{30}, SVI_5/SVI_{30}, V_{zs}, and selector underflow/overflow biomass ratios in Fig. 6. It appears that only when the bulk SRT was less than 5.5 days, could AGS formation be achieved. For example, only after the reactor SRT was controlled below 5.5 days, the SVI_{30} and SVI_5/SVI_{30} started to drop to 60 mL g $^{-1}$ and 1.1, respectively, which are typically considered as the characteristics of AGS (Figs. 2E, 5D-E and 6A). One should be aware that the actual SRT of the flocculent sludge portion of the biomass should be much shorter than 5.5 days while that of the AGS portion retained in the system should be longer, both of which were factored in the overall bulk SRT calculated for the reactor biomass inventory. This conclusion is supported by the distinctive distribution of heterotrophic and nitrifying microorganisms in the overflow and underflow in Fig. 4. The dominance of nitrifying bacteria in the underflow of hydrocyclone system by selectively overflow sludge wasting for SRT control has also been observed in one recent study (Regmi et al., 2022). The enrichment of nitrifying population in the granules through SRT control < 5.5 days could also explain the settleability improvement in terms of SVI_{30}, SVI_5/SVI_{30} and V_{zs} in Fig. 6. The slow-growing microorganisms were reported to facilitate the formation of granules (Liu et al., 2004; Xia et al., 2007). Since the SRTs of dense granules and bioflocs were not able to be differentially controlled in previous granulation studies in SBRs, especially during the reactor startup, the SRT was usually not considered as a decisive factor for aerobic granulation (Li et al., 2008). However, the novel arrangement in the Fig. 1 setup offered the possibility for SRT deviation of AGS and flocculant sludge. The short SRT of flocculent sludge realized significant outselection of this type of biomass while the retention and recirculation of AGS resulted in their persistence and dominance in the sludge bed (Fig. 2G). Therefore, the SRT control provided sufficient selection pressure resulting in the continuous flow AGS formation by selectively retaining the slow-growing bioparticles for granule settleability improvement.

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 heavier AGS and lighter bioflocs to the feast and famine zones, respectively, further boosted biofloc outselection by subjecting bioflocs to low substrate availability (Fig. 5A and

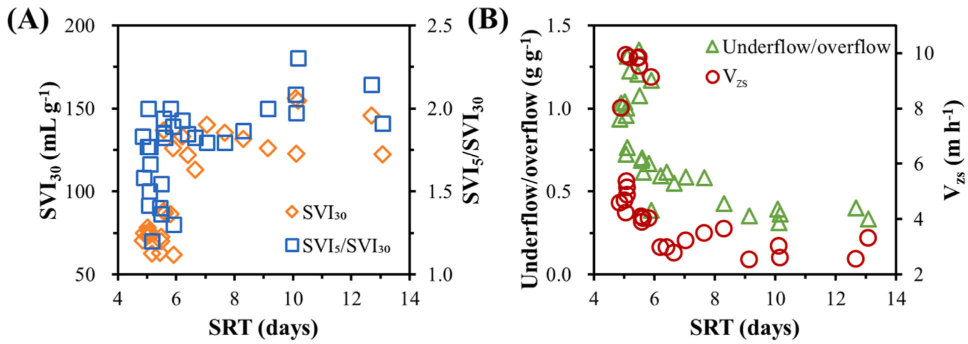

Fig. 6. SRT-dependent (A) SVI_{30}, SVI_5/SVI_{30} and (B) underflow/overflow biomass ratios, as well as V_{zs} over 197 days of operation.

B). This is a novel application of internal selection pressure has not been widely accepted in full-scale applications. To our knowledge, existing hydrocyclone-installed treatment trains return a mixture of dense sludge (hydrocyclone underflow) and lighter bioflocs (final clarifier sludge return) to feast zones without attempting to differentiate their substrate availability (Avila et al., 2021; Partin, 2019; Regmi et al., 2022). According to Fig. 4A, the physical upflow selector overflow was mainly comprised of fast growing heterotrophic bacteria. Theoretically, it would be more difficult and take much longer time to outselect these bioflocs if they were provided a competitive growth advantage by exposing them to the same high substrate availability as that of the AGS which grow and develop at a much lower rate due to mass diffusion limitation. If this more conventionally applied bioflocs-to-AGS transition setup were applied, the external physical selector would be the only driving force to transform flocculent sludge to AGS, and thus a much more radical biofloc washout type of short SRT would have to be employed (Sun et al., 2019). Even with the sidestream hydrocyclone installation, only 5–7% of the RAS can be subjective to physical selection (Avila et al., 2021; Partin, 2019; Regmi et al., 2022), leaving majority of the bioflocs and AGS unseparated in the RAS returned to the feast zone where bioflocs gain more growth advantage over AGS, which explains the slow startup period, e.g., one year, needed to observe sludge settleability improvement (Ford et al., 2016; Kent et al., 2018; Roche et al., 2022; Welling et al., 2015; Wett et al., 2015).

One may propagate the approach to only return hydrocyclone underflow to the feast zone, deferring the rest RAS to the famine zone. Given the minor sludge inventory that can be produced from the hydrocyclone underflow, the viability of this approach to establish a feast zone solely for AGS's advantage is questionable. In contrast, the setup with continuous upflow selector used in this study through the increase of clarifier SOR is capable of providing holistically physical selection on all existing sludge inventory, which explains the prompt startup of the continuous granulation system (Fig. 5C, D and E).

It is well-known that bioflocs are very competitive in utilizing readily available substrates for a number of reasons. They can outcompete AGS because of their looser structure and higher specific surface area that promotes fast substrate diffusion and utilization (Tay et al., 2002). Therefore, returning both sludges to the same feast zone as conventionally practiced to date is counterproductive to the progression and stabilization of continuous flow AGS systems. For this reason, extremely short 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 (Cofré et al., 2018; Sun et al., 2019). 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 (Cofré et al., 2018; Sun et al., 2019). For example, an SRT as short as 0.3 days had to be imposed to drive continuous flow AGS formation in one of our previous studies, resulting in an initial MLVSS as low as 300 mg L^{-1} and poor effluent quality associated with only 31% COD and 6% ammonia removal (Sun et al., 2019). This performance loss resulting from the drastic outselection pressure necessary for AGS formation can be resolved by incorporating a strategy that manages to separate the SRTs of flocculent sludge and AGS while incorporating reactor internal biological selection through controlling F:M regimes for the distinctly different sludge forms (i.e., selective AGS and biofloc returns to feast and famine zones, respectively). This strategy is shown schematically in Fig. 1. Superimposing this second selection pressure to a bioreactor allowed aerobic granulation to be achieved at a relatively higher SRT and MLVSS levels to safeguard the effluent quality throughout the reactor startup phase without treatment interruption (Fig. 5F, G, and H). The concept of leveraging such feast/famine conditions should be of interest to designers considering a transition from conventional activated sludge processes to continuous flow AGS systems. In particular by examining if existing bioreactors can accommodate the methods developed herein and described in a previous study (An et al., 2021b).

3.2. Implication on full-scale physical selector design

At present, the most popular continuous flow physical selector employed in WRRFs is hydrocyclones which are currently limited to sidestream wasting applications due to technology sizing limitations and associated costs (Partin, 2019; Wett et al., 2015). UOSA in the U.S. 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. With such a weak selection, only moderate sludge settleability improvements were observed over 23 months of operation; however, a true shift to a purely continuous flow AGS system has not been observed. In contrast, it only took 197 days to achieve true measures of a continuous flow AGS formation with the same primary effluent (PE) in this pilot study. The key difference can be attributed to subjecting 100% of the mainstream mixed liquor flow to the selection pressure of the continuous upflow selector shown in Fig. 1.

The continuous upflow selector tested in this study demonstrated that a mainstream physical selector can be developed for full-scale, continuous AGS applications, particularly for green-field sites. Upflow selectors are more suitable and practical than the sequencing batch selectors tested in a prior study (Sun et al., 2019), especially for high flow rate, continuous flow wastewater treatment systems (Kent et al., 2018). The concept of continuous upflow selector is not new as it has been used in full-scale upflow anaerobic sludge blanket (UASB) reactors for decades (Frankin, 2001). However, it was for the first time fully explored in this study to apply it for strategic biofloc outselection using a managed SRT control approach to drive continuous flow AGS formation without performance loss during the startup phase (Figs. 1 and 2). This study has helped the field understand that an external continuous upflow selector provides excellent solid separation efficiency and may be a good choice for full-scale applications where stronger selection pressure is needed for faster transitions from conventional activated sludge to AGS (Fig. 5C to E). The upflow selector incorporated into the

Fig. 1 design mimics a conventional clarifier with a very high SOR so there may, in some cases, be the flexibility to rearrange and repurpose existing clarifier structures within WRRFs to act as external physical selectors, subject to available water head. Following the same concept, lamella plate settlers may also be considered for high flow, continuous flow, settling velocity selection in the mainstream application (Faraji et al., 2013; Li et al., 2014). Results from this study suggest that a high-capacity physical, external, upflow selector should be explored for full-scale applications to be used in concert with biological feast/famine selection internal to the bioreactors. This may provide the added selection pressure that designers are looking for to expedite the transition from conventional activated sludge to continuous flow AGS in full-scale wastewater treatment trains.

4. Conclusions

The following concluding remarks can be drawn from this study:

1) An external continuous upflow selector that incorporates managed SRT control, coupled with internal feast/famine bioreactor selection, proved effectiveness in promoting continuous flow AGS formation in real domestic wastewater without treatment interruption during AGS reactor startup.
2) SRT control through managed wasting of the flocculent sludge in the overflow from a upflow selector was successfully used as a selection pressure to drive continuous flow AGS without compromising system performance or final clarifier effluent quality.
3) Heterotrophic and nitrifying microorganisms dominated the upflow selector overflow and underflow biomass, respectively. This was explained by the actual SRT difference between the wasted flocculent sludge portion of the biomass and the AGS portion of biomass retained in the system.
4) Intelligently routing the sludge returns of AGS to feast zones and flocculent sludge to famine zones resulted in biological selection internal to the bioreactor. This biological selection was applied to boost the physical selection pressure obtained with the upflow selector, which also safeguarded the effluent quality throughout the reactor startup period without treatment performance degradation or interruption.
5) In existing WRRFs considering AGS application, secondary clarifiers hold the potential to be reconfigured to provide external physical, settling velocity selection by manipulating SORs.

5. Materials and methods

5.1. Reactor design and operation

A PFR with the design and setup illustrated in Fig. 1 was operated onsite in UOSA, a WRRF located in Centreville, VA, USA. The PE from the primary clarifiers of UOSA was fed to the PFR through gravity flow without any pretreatment. The PFR with a 140 L total working volume was made of 10 completely mixed chambers connected in series to create feast/famine conditions along the gravity flow direction (Fig. 1). The hydraulic retention time of the PFR was controlled at 6.5 h, similar to that of the full-scale secondary process at UOSA. All chambers were aerated with dissolved oxygen (DO) > 2 mg L^{-1} at a flow rate of 3 L min^{-1} in each chamber. Meanwhile, aeration also provided homogenous mixing in each chamber. As shown in Fig. 1, 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 (Fig. 1A). The SOR of this column was set at 10 m h^{-1} 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 upflow selector and returned to the first feast zone chamber of the PFR (Fig. 1A). 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 SRT control (Fig. 1A). The remainder of the flocculent sludge was returned to the first famine zone of the PFR as shown in Fig. 1A. The flow rate ratio of selector overflow to underflow was set at about 1.6. An airlifting method was used to return sludges by blowing the settled sludge through tubings into either the feast or famine zones according to the flow design in Fig. 1 (Sun et al., 2019).

5.2. Analytical methods

DO was measured using an HQ40D meter (Hach, Loveland, CO, USA). The SVI, V_{zs}, MLSS, MLVSS, as well as the SOURs were analyzed according to standard methods (Baird and Bridgewater, 2017). The gluocose and ammonia chloride equivalent to 100 mg L^{-1} COD and 30 mg L^{-1} NH_3—N were added as the sole electron donors in $SOUR_{COD}$ and $SOUR_N$ tests, respectively. The in-situ OUR in each chamber was measured without external electron donor addition. The 5 min and 30 min SVI were measured using a standard 2 L settlemeter and recorded as SVI_5 and SVI_{30}, respectively. COD and ammonia concentrations were analyzed using TNTplus® 820 and TNTplus® 840 vials in a spectrophotometer (Hach, Loveland, CO, USA). The ammonia and sCOD samples were measured with the filtrate from 0.45 μm syringe filters (EZFlow®, Old Saybrook, CT, USA). The particle size distribution was determined using a particle size analyzer (Horiba, LA-950, Kyoto, Japan). A microscope (Nikon Eclipse, E200, NY, USA) was utilized for sludge morphology imaging.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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