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Enhancing bioflocculation in high-rate activated sludge increases sensitivity to changes in surface overflow rate while improving effluent quality

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Abstract

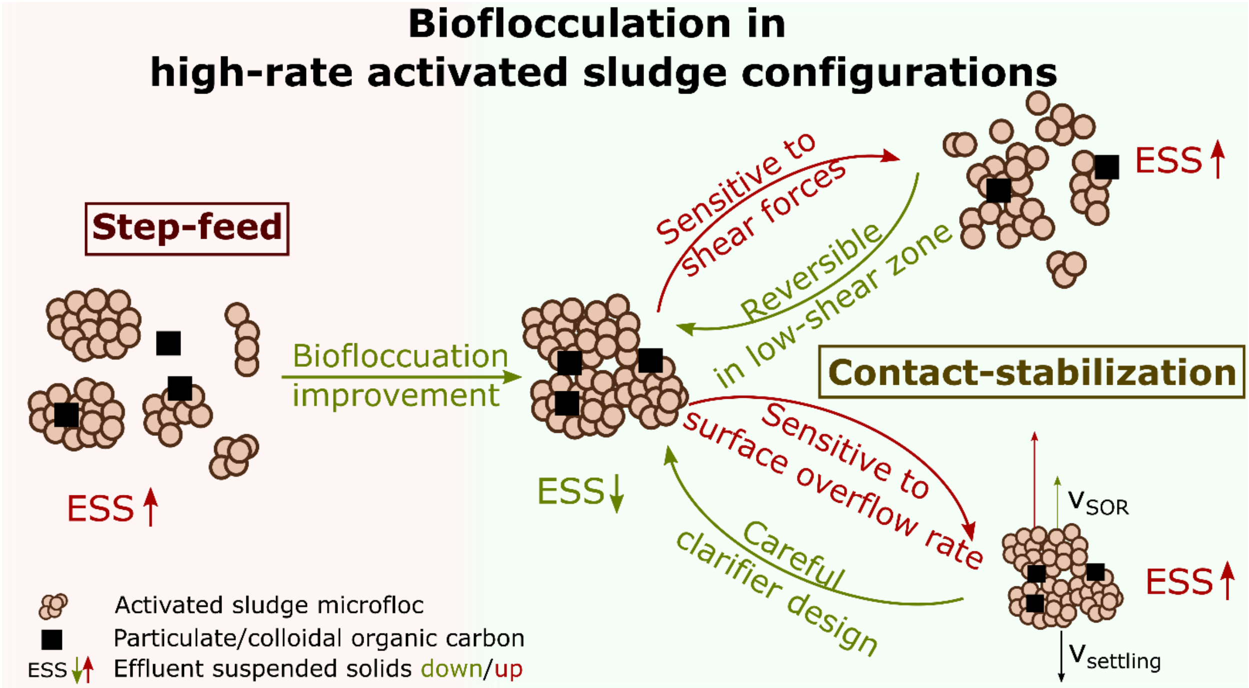
High-rate activated sludge (HRAS) depends on good bioflocculation and subsequent solid-liquid separation to maximize the capture of organics. However, full-scale applications often suffer from poor and unpredictable effluent suspended solids (ESS). While the biological aspects of bioflocculation are thoroughly investigated, the effects of fines (settling velocity < 0.6 $m^3/m^2/h$), shear and surface overflow rate (SOR) are unclear. This work tackled the impact of fines, shear, and SOR on the ESS in absence of settleable influent solids. This was assessed on a full-scale HRAS step-feed (SF) and pilot-scale contact-stabilization (CS) configuration using batch settling tests, controlled clarifier experiments, and continuous operation of reactors. Fines contributed to 25% of the ESS in the full-scale SF configuration. ESS decreased up to 30 mg TSS/L when bioflocculation was enhanced with the CS configuration. The feast-famine regime applied in CS promoted the production of high-quality extracellular polymeric substances (EPS). However, this resulted in a narrow and unfavorable settling velocity distribution, with 50% ± 5% of the sludge mass settling between 0.6-1.5 $m^3/m^2/h$, thus increasing sensitivity towards SOR changes. A low shear environment (20 s^{-1}) before the clarifier for at least one minute was enough to ensure the best possible settling velocity distribution, regardless of prior shear conditions. Overall, this paper provides a more complete view on the drivers of ESS in HRAS systems, creating the foundation for the design of effective HRAS clarifiers. Tangible recommendations are given on how to manage fines and establish the optimal settling velocity of the sludge.

Keywords: A-stage, floc formation, sewage, settling, wastewater

List of Acronyms

AWTP	Advanced wastewater treatment plant
BSA	bovine serum albumin
CEPT	Chemically enhanced primary treatment
COD	Chemical oxygen demand
CS	Contact-stabilization
CSV	Critical settling velocity
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
ESS	Effluent suspended solids
F/M	Food-to-microorganism
HRAS	High-rate activated sludge
ISV	Initial settling velocity
LB	Loosely-bound
OHO	Ordinary heterotrophs
PN	Protein
PS	Polysaccharides
SF	Step-feed
SLR	Sludge loading rate
SOR	Surface overflow rate
SRT	Solids retention time
SVI	Sludge volume index
TB	Tightly-bound
TOF	Threshold of flocculation
TSS	Total suspended solids
vOUR	Volumetric loading rate

Graphical abstract



1. Introduction

High-rate activated sludge (HRAS) is a promising technology to maximize the diversion of carbon through from the water to the solids line. The technology works on the principle that carbon present in the wastewater is converted to a harvestable form (carbon redirection) by converting incoming COD into particulate biomass in combination with adsorption of particulate and colloidal COD onto the biomass and storage of soluble COD in the cell. Both adsorption and storage, frequently lumped together as 'biosorption', are carbon removal processes with do not require oxidation and thus further increase the observed yield of the biomass (Guellil et al., 2001; Rahman et al., 2017; Ullrich and Smith, 1951). This particulate form of COD, made up out of biomass and adsorbed/stored influent organics, can be subsequently captured in the clarifier and send to the solids processing line (carbon capture) for biogas production or upgraded commodities (Alloul et al., 2018). Carbon redirection in HRAS is commonly achieved by operating an extremely short solids retention time (SRT) of 0.2 - 0.8 days and high organic loading rate (> 2 g COD/L/d) depending on the reactor configuration. This short sludge age reduces the amount of oxidation of carbonaceous organics to CO_2 and limits the endogenous respiration of the sludge contributing contribution to CO_2 formation (Cagnetta et al., 2019; De Graaff & Roest, 2012; Jimenez et al., 2015; Meerburg et al., 2015; Rahman et al., 2016). However, shortening the SRT leads to diminishing returns in terms of carbon capture due to increased stress on the bioflocculation mechanism, creating bioflocculation limitations (Rahman et al., 2016).

In this study, bioflocculation is defined as the ability of the sludge to form (macroscopic) flocs in the clarifier with a settling velocity greater than the upflow velocity imposed by the surface overflow rate (SOR) in the clarifier. Bioflocculation is thus a driver for the settling behavior of the sludge within the clarifier and is therefore directly correlated with the observed effluent suspended solids (ESS) (Murthy, 1998; Urbain et al., 1993). A bioflocculation limitation is subsequently defined as the manifestation of an increase in ESS because of inadequate floc formation. HRAS systems report out ESS numbers in range of 30 – 83 mg total suspended solids per liter (TSS/L) (Cagnetta et al., 2019; De Graaff and Roest, 2012; Rahman et al., 2019), while conventional, long SRT systems typically achieve 8-19 mg TSS/L (EPA, 2013). Additionally, SRT is not a good predictor of effluent suspended solids in practice. The full-scale A-stage reactors operated in the Netherlands all operate around the same aerobic SRT (~ 0.2 days), yet all report out different ESS (De Graaff and Roest, 2012). The reason for this poor correlation is unknown, but given that factors such as influent composition, reactor and clarifier design, shear forces, and more influences the final ESS, the effect of SRT was probably not strong enough to dictate this correlation.

Bioflocculation is mediated by extracellular polymeric substances (EPS) produced by the biomass in the reactor (Sheng et al., 2010). For HRAS systems, the influent strength and characteristics are the main driver for EPS production. For medium to high-strength raw wastewater, EPS production is predominantly driven by the food-to-microorganism (F/M) rate (Rahman et al., 2017; Sturm et al., 2016). For low-strength wastewaters or chemically enhanced primary treatment (CEPT) effluent, a proper feast-famine regime seems to be important to drive the production of EPS as applied in the contact-stabilization (CS) configuration (Rahman et al., 2019). Contact-stabilization, first described by Coombs (1922), utilizes return activated sludge aeration (i.e. "stabilization) in the stabilizer zone, before bringing into contact with the sewage in contactor under a short hydraulic retention time. This configuration utilizes feast (contactor) and famine (stabilizer) conditions in the process, inducing EPS production (Rahman et al., 2019). An important aspect of bioflocculation is the capture of 'fines', i.e. particulate solids that settle slower than 0.6 $m^3/m^2/h$ (within flocculation time of 10 min). Fines get enmeshed into the flocs with the help of free binding spots on the EPS, thus improving effluent quality (Van Winckel et al., 2019). However, the effect of increased fines load on the ESS in continuous systems with or without proper bioflocculation is poorly understood.

While the positive impact of improved bioflocculation on process performance is known (Jimenez et al., 2015; Rahman et al., 2016), its influence on the settling velocity distribution is unknown. Additionally, the effect of shear and fines loading within the context of bioflocculation on the distribution is not well understood. Hydraulic variations in or prior to the clarifier, like changes in SOR or shear forces, can have a detrimental effect on effluent quality regardless of how well bioflocculation is maintained. HRAS systems operate at a higher SOR (0.75 – 1.4 $m^3/m^2/h$) (De Graaff & Roest, 2012) than most conventional clarifiers (0.6 – 1 $m^3/m^2/h$) (WEF, 2005). Because the typical settling velocity distribution of HRAS sludge is unknown, the effect of elevated SOR on effluent suspended solids remains unclear. HRAS could have a higher sensitivity towards SOR changes in a HRAS clarifier. Sensitivity here is defined as degree of change in effluent suspended solids induced by a change in SOR in the HRAS clarifier. A low sensitivity to SOR changes is preferred because this widens the operational range of the clarifier where no significant increase in ESS is observed. However, a severe bioflocculation limitation may lead to a low sensitivity, but a high effluent suspended number across the operational range of the HRAS clarifier. To this date, there is no clear recommendation on optimal SOR ranges a HRAS clarifier should operate in. Additionally, high shear before the clarifier can disrupt flocs and alter the settling velocity distribution, thus changing how the SOR will impact effluent quality.

Traditionally, clarifier performance in activated sludge systems is assessed using 'classical' clarifier metrics, such as sludge volume index (SVI) which measures volume of the settled sludge bed after a predetermined amount of time (typically 30 minutes) and initial settling velocity (ISV) which is the maximum settling speed of the sludge bed. However, these metrics have been shown to be unreliable as they overemphasize the importance of hindered settling and compression in a clarifier which correlates poorly with effluent quality, especially in HRAS systems (Mancell-Egala et al., 2017; Mancell-Egala et al., 2016). Moreover, Granular systems also report out great SVIs but often fail to adequately capture fines (Li et al., 2008; Rocktäschel et al., 2015). Most HRAS systems are fed with raw wastewater which potentially obscures the link between physical parameters (SOR, shear,…) and bioflocculation, driven by biological parameters. Rinas et al. (2018) determined that, on average, 15% of solids travel faster than 1.2 $m^3/m^2/h$ during dry weather condition. This can increase up to 40% during wet weather. These solids can potentially provide ballast for flocs and improve suspended solids removal without the need for flocculation. This also turns the HRAS system into a form of primary clarifier at very short SRT, further obscuring the link between effluent suspended solids with EPS production. Kinyua et al. (2017) found a low correlation between EPS and bioflocculation for a HRAS pilot treating raw wastewater. The four A-stage installations in the Netherlands all report very low SVI numbers (60 – 80 mL/g TSS), despite dealing with limitations in the clarifiers (De Graaff & Roest, 2012). No rigorous experimental work has been performed thus far on the effect of shear-induced stressors on biology itself in HRAS systems.

This paper provides a unique perspective of two HRAS configurations operated in the absence of influent solids settling faster than 0.6 $m^3/m^2/h$ (due to the presence of CEPT). This allowed for a rigorous assessment of the impact of process configuration (step-feed vs contact-stabilization), SOR and shear on bioflocculation and resulting effluent quality in absence of other settling drivers than the biology itself. Most HRAS studies work with raw sewage and thus cannot differentiate between bioflocculation-driven settling (floc formation) and other form of sedimentation (e.g fast settling influent solids and inorganics). This was achieved using batch settling tests, controlled clarifier experiments, and continuous reactor operation, which allowed for a clear view on the settling velocity distribution of HRAS sludge and impacting factors. This work therefore creates a new foundation for the design of smart and effective HRAS clarifiers, aiming at tangible recommendations on how to manage fines and establish the optimal settling velocity of the sludge.

2. Material and Methods

2.1. Operation of the reactors

In this study, two configurations of the HRAS process have been utilized and investigated: A pilot-scale high-rate contact-stabilization (CS) configuration and full-scale (high-rate) step-feed (SF) configuration, which acts as the secondary treatment step in the Blue Plain advanced wastewater treatment plant (AWTP) sewage treatment train in Washington, DC, USA.

The step-feed (SF) reactor used in this study was the East train of the full-scale secondary treatment step at Blue Plains AWTP in Washington, DC, treating about half of the on average 1.4 million cubic meters of effluent from CEPT per day. The East reactor train consisted of four individual parallel reactors each consisting out of four passes. The total volume of the reactor was 61504 m^3 (~ 16.25 million gallons). RAS was introduced in pass 1, while CEPT effluent was provided in pass 1, 2, and 3 depending on the incoming flow, and can thus be defined as a step-feed configuration. The step-feed reactor had 14 clarifiers in operation. The clarifiers were rectangular, 3.6 meters (~ 11.8 feet) deep and had a surface area of 1846 m^2 (~ 19870 ft^2) each. The SF reactor was bioaugmented with biological nutrient removal waste activated sludge (SRT ~ 20 days) from the downstream reactor (Bailey Jr et al., 2008). An annotated satellite image of the East reactor train can be consulted in Supplementary **Figure S1**.

A high-rate activated sludge pilot (volume (V) = 1000 liters), configured in a contact-stabilization configuration, was operated at Blue Plains AWTP. Fresh effluent from the full-scale CEPT process was continuously fed to contactor zone (V = 223 liters), where it came into contact with starved sludge from the stabilizer zone (V = 223 liters). After a hydraulic retention time of 36 minutes, the sludge was introduced into three parallel clarifiers (Ø = 30.5 cm, V = 302 liters each). The settled sludge flowed to the return activated sludge tank (V = 50 liters), which acted as a flow equalizer for the pilot. The sludge was then pumped into the stabilizer zone (V = 223 liter) where ample DO (> 2 mg O_2/L) was provided to oxidize sorbed organics in absence of feed. After a hydraulic retention time of 88 minutes, the stabilized sludge was reintroduced in the contactor. Mixing and aeration was achieved with coarse bubble aeration. Sludge wasting was achieved from the contactor. A schematic of the pilot can be consulted in the Supplementary **Figure S2**. Composite samples (24-hour) were automatically taken on an hourly basis from the influent, effluent and WAS for further analysis. Grab samples were used for the EPS protocol.

2.1.1. *Contact-stabilization pilot scenarios*

Three scenarios were operated in the high-rate CS reactor (**Table 1**). Scenario A was characterized by an aerobic contactor (DO > 0.5 mg O_2/L). This was achieved by providing a higher rate of coarse bubble aeration where seven seconds of continuous aeration was provided per four minutes instead of one second (**Table 2**). Scenario B and C used an anoxic contactor (DO < 0.5 mg O_2/L) with a lower shear as a result. Scenario C has a significantly higher sludge waste rate, resulting in a lower SOR compared to scenario A and B given that sludge wasting was performed from the mixed liquor.

2.2. Batch settling tests

Batch settling test were performed to test the impact of shear and the resulting settling velocity distribution (as assessed with different critical settling velocities) on the TSS capture of sludge. Fresh sludge was sourced from the full-scale SF reactor and diluted to ~ 350 mg TSS/L with final effluent from the full-scale plant to preserve the ionic balance. The diluted sludge was poured into a modified Nalgene® 4L graduated cylinder (ø = 10 cm) and mechanically mixed at a fixed or alternating shear rate for 10 minutes with an IKA Eurostar 60 (IKA, USA) mixer equipped with two 4-bladed axial flow impellers, which are suitable for flocculation over a wide range of RPMs in HRAS applications (Balemans et al., 2020). Ten minutes was chosen because this was sufficient for the rate of floc formation and breakup to meet steady state conditions in previous studies (Biggs & Lant, 2000; Mancell-Egala et al., 2017; Wahlberg et al., 1994). After 10 minutes, the sludge was baffled with Plexiglas stick to quickly dissipate the kinetic energy and flocs were allowed to settle for a predetermined amount of time. After the designated settling time, clamps located five centimeters below the liquid level were opened, and sludge was allowed to rapidly drain into a sample cup within about five seconds or less. The TSS collected represented the fraction of total TSS that settled slower than target CSV.

The two degrees of freedom tested with the methodology described above were (1) shear rate and (2) the critical settling velocity (CSV) of the sludge. Shear rate was mediated through mixing speed, whereas CSV was varied with the settling time.

The investigated shear rates were 20, 55, 90, and 180 s^{-1}, which corresponded to 100, 185, 260 and 410 revolutions per minute (RPM) respectively. These shear rates were fixed for ten minutes as described above. The effect of alternating shear was also tested. Here, the shear rate was automatically and instantaneously switched between 20 and 90 s^{-1} every one or five minutes for a total of ten minutes.

To obtain a settling velocity distribution of the sludge at different shear rates, a range of critical settling velocities were investigated. A CSV of 9, 3, 1.5, 0.6, 0.3, and 0.15 $m^3/m^2/h$ was chosen which corresponded to 0.33, 1, 2, 5, 10, and 20 minutes before releasing the sampling clamps.

2.3. Clarifier experiments

A pilot-scale clarifier (V = 99 liter, ø = 20 cm, height = 305 cm) constructed out of transparent acrylic was operated in a continuous manner to determine the effects of SOR and shear in a controlled environment which was representative of a full-scale clarifier. Fresh sludge was hauled from the CS or SF reactor, diluted with plant process water and put in a stirred vessel which acted a flow equalization tank. The sludge was then gravitationally introduced in a 20L bucket, where shear was applied for 10 minutes under controlled conditions with an IKA Eurostar 60 (IKA, USA) mixer equipped with two 4-bladed axial flow impellers. Next, the sludge was introduced into the clarifier with Masterflex® peristaltic pumps at the midpoint of the column (152 cm). The overflow and underflow of the clarifier were reintroduced in the stirred flow equalization tank. The sludge's temperature was kept between 15 – 20 °C, as this was the normal operational temperature of the pilot. Samples were periodically taken (at least in triplicate) after at least three HRT to ensure steady state conditions.

The SOR (0.6 and 1.5 $m^3/m^2/h$) was changed by changing the recirculation speed over the clarifier. Given the closed-loop nature of this experiment, the liquid volume (and thus liquid height) in the shear bucket had to be varied to ensure a 10-minute retention time given a specific SOR. As the liquid height changes the relation between RPM and the resulting shear, the RPM was changed in relation with the change liquid height to ensure equal average shear throughout the experiment.

2.4. Analytical methods

2.4.1. Measurement of the particle size distribution

Flocs sizes were determined by taking photographs of the formed flocs in the acrylic pilot-scale clarifier. The digital camera (Canon T3i) was equipped with a 60mm f/2.8 USM macro lens and focused about 1 cm deep in the clarifier. Sizes were calibrated with an adhesive ruler which was put on the clarifier. Photos were taken at full resolution (18 megapixels) and analyzed with Image-J 1.52n. First, contrast was enhanced by spreading the histogram so that 1% of the pixels were saturated. Next, a Boolean image was created using the threshold tool where the grayscale intensity was chosen at 10% of distribution. Last, the Feret diameter was determined on the isolated particles, ignoring those smaller than 0.01 mm^2, as they would be most likely noise. At least 10 pictures were analyzed per sludge sample, or until the particle size distribution did not change anymore.

2.4.2. Other methods

Total suspended solids and total Kjeldal nitrogen were measured following the standard methods (APHA, 2005). COD, ammonium and phosphorus was determined using Hach® (Loveland, Colorado, USA) kits following the manufacturer's instructions. COD was fractionated into total COD (no pretreatment), filtered COD (sample filtered through Wattman 1.5 micrometer filter), and filter-flocculated COD (flocculation-filtration with $ZnSO_4$ according to Mamais et al. (1993)). Extracellular polymeric substances (EPS) were determined using a heat extraction following the procedure described in Van Winckel et al. (2019). Protein content of the EPS was determined using the modified Lowry protein assay kit (Thermo Fisher, USA) (Lowry et al., 1951) with bovine serum albumin (BSA) as the standard. Polysaccharide level was determined using the DuBois method with glucose as the standard (DuBois et al., 1956). Volumetric oxygen uptake rate (vOUR) was determined by taking a fresh sludge sample, aerating it briefly to 5 mg O_2/L DO, and measuring the declining slope in the absence of atmospheric oxygen up to a DO of 1 mg O_2/L. The resulting OUR was normalized to 20 °C with the standard Arrhenius coefficient (1.04) (Metcalf & Eddy, 2003)

2.5. Calculations

Average the average velocity gradient (G, s^{-1}) was calculated using equation 1 as postulated by Camp and Stein (1943):

$$G = \sqrt{\left(\frac{\epsilon}{\nu}\right)} \quad (1)$$

Where ϵ (m^2/s^3) is the average energy dissipation rate and ν (m^2/s) the kinematic viscosity (of water). The average energy dissipation rate from an impeller could further be calculated with Equation 2 by Godfrey et al. (1989):

$$\epsilon = \frac{P_o N^3 D^5}{V} \quad (2)$$

Where P_o (-) is the impeller's power number, which was calculated empirically based on the work of Furukawa et al. (2012), N the impeller's rounds per minute (RPM), D the impeller's diameter (m) and V (m^3) the volume of the container.

2.6. **Statistical analysis**

Two-way Student t-tests with unequal variances were performed in Microsoft excel to establish statistical significance. When the p-value was smaller than 0.05, the two (normally distributed) variables were considered significantly different from each other.

3. Results

3.1. Continuous reactor operation

The high-rate contact-stabilization (CS) pilot reactor was operated on CEPT effluent for three distinct scenarios A, B, and C lasting 25, 31, and 22 days (28, 31, and 44 times the SRT) respectively. The key differences between the scenarios were (1) measurable DO in the contactor in scenario A, leading to (2) an increased shear regime in the same scenario due to more frequent coarse bubble aeration, and (3) the difference in SOR (0.9 and 0.6 $m^3/m^2/h$ for scenario B and C respectively) due to wasting from the mixed liquor rather than the return activated sludge (**Table 2**). DO in the contactor could be ruled out based on previous work by Rahman et al. (2017), who determined that a 0.5 mg O_2/L was required to induce an EPS response in this configuration. Lower DO should have negatively impacted effluent quality based on the hypothesis that absence of DO leads to a decrease in EPS production, discouraging bioflocculation. However, the effluent suspended solids in scenario A were on average better than scenario B and C (**Table 1**), discrediting the potential impact of an aerated contactor on bioflocculation.

Influent characteristics were typical for CEPT effluent and did not significantly differ between scenarios (**Table 1**). Particulate COD was low compared to ty pical A-stage HRAS systems which range from 200 – 400 mg particulate COD/L (De Graaff & Roest, 2012; Jimenez et al., 2015). Colloidal COD was comparable to the A-stages in the Netherlands (De Graaff & Roest, 2012). CEPT potentially lowered the seeding rate of active ordinary heterotrophic organisms from the influent into the reactor compared to an A-stage fed with raw wastewater (Rahman et al., 2016), although no measurements were performed to confirm this. The three scenarios achieved similar effluent qualities in terms of soluble and colloidal COD. Effluent suspended solids (**Figure 1-IV**) and particulate COD (**Table 1**) were significantly different lower in scenario C than A ($p = 0.002$ and 0.013 respectively), with scenario B falling in between the two. This resulted in significant differences in C/N/P capture because no capture was achieved in scenario A (**Figure 1-VI/VII/VIII**). Scenario B was able to capture 18% ± 12% of the incoming COD, the highest COD capture (36% ± 16%) was achieved in scenario C. (**Figure 1-IV**).

Biomass activity, measured with volumetric OUR, was similar for all tested scenarios once corrected for temperature. Scenario C had a low mixed liquor concentration compared to Scenario B but fell within the uncertainty of scenario A (**Figure 1-III**). This resulted in a comparatively shorter SRT for scenario C (**Table 2**), leading to a slightly higher redirection percentage (**Figure 1-V**). However, all three scenarios had comparable amounts of extracellular polymeric substances (EPS) measured with similar quality as given by the protein over polysaccharide ratio (PN/PS) (**Table 2**). EPS amount and quality has been shown to be linked with the sludge's potential to bioflocculate (Sheng et al., 2010). Therefore, the operational differences observed had no impact on the bioflocculation potential. Given that all biological parameters impacting bioflocculation were similar between the scenarios and DO has been ruled out, the difference in effluent quality was potentially explained by SOR, shear or a combination of the two.

3.2. Impact of different shear regimes on TSS capture

A feast-famine regime, as provided by the CS configuration, has been shown to be a major driver for bioflocculation (Rahman et al., 2017). A step-feed configuration, which operates with smaller substrate gradients and lacks proper feast-famine, should therefore have less favorable bioflocculation potential. To test this, the impact of different shear regimes was tested on a full-scale step-feed (SF) HRAS system treating the same CEPT effluent as the CS pilot. These tests were performed with diluted sludge (350 mg TSS/L) to limit the number of collisions, magnifying any bioflocculation limitations present. Percentage of TSS captured was used instead of absolute ESS numbers as the latter do not reflect true clarifier conditions in continuous systems. Batch tests do provide valuable insights in relative floc formation dynamics in a controlled environment. TSS capture decreased with increasing velocity gradient regardless of the shear regime applied (**Figure 2**). **Figure 2-I** shows the impact of continuous shear for 10 min ranging from 20 s^{-1} to 180 s^{-1}. When a velocity gradient of 90 s^{-1} was applied, the final TSS capture significantly decreased at a critical settling velocity (CSV) of 0.6 $m^3/m^2/h$ and below. This indicated that a continuous high shear regime had a disruptive effect on the entrapment of fines (fraction of TSS < 0.6 $m^3/m^2/h$) within the floc. At 1.5 $m^3/m^2/h$, the SOR typically associated with floc formation (Mancell-Egala et al., 2017), all velocity gradients above 20 s^{-1} resulted in the same TSS capture, indicating that low shear was required for the creation of flocs that settle well. At a CSV of 3 $m^3/m^2/h$, TSS capture was exclusively driven by settling velocity as all datapoints converged (**Figure 2-I**). For this reason, 3 $m^3/m^2/h$ was not tested in **Figure 2-II/III**.

The shear regime in the pipes or channels leading to the clarifier is rarely constant. Mixing in the channel prior to the clarifier in combination with bends, turns and other sources of head loss in the piping lead to changing and often unpredictable shear forces. To account for this, the effect of alternating velocity gradients, 20 and 90 s^{-1} reflected in **Figure 2-II/III** as black and blue respectively, was investigated. One minute at 20 s^{-1} was enough to restore the floc formation to similar levels achieved at 20 s^{-1} with continuous shear in **Figure 2-I** (19% ± 4% and 22% ± 4% TSS capture respectively) (**Figure 2-II**). When the final velocity gradient of 20 s^{-1} was increased from one to five minutes, the fraction of flocs settling faster than 1.5 $m^3/m^2/h$ increased (36% ± 4%) (**Figure 2-II**). This indicated that high shear forces followed by sufficiently a long low-velocity gradient could help by (1) encouraging the formation of faster settling flocs by potentially by rearranging floc structures, (2) promoting the formation of bigger flocs, and (3) improving enmeshment of fines within the flocs. When a final velocity gradient of 90 s^{-1} was applied for five minutes, the TSS capture at 1.5 $m^3/m^2/h$ was similar to the continuous shear experiment (11% ± 3% vs 10% ± 2% respectively). The capture was completely lost (0%) when 90 s^{-1} was applied for one minute at the end. This indicated that high shear forces applied for even a brief amount of time can have detrimental effects on solids separation without adequate time under low shear conditions to reverse the floc breakage.

3.3. Continuous clarifier tests to understand effects shear and SOR

While batch experiments are a good tool to fingerprint the impact of shear on the settling velocity distribution, they fail to provide representative effluent suspended solids one might expect in a full-scale system due to scale constraints. Additionally, batch settling experiments are not entirely representative for continuous flow systems, given that no underflow exists for batch settling. For this reason, the impact of high (250 s^{-1}) and low (19 s^{-1}) shear, SOR, and sludge loading rate (SLR) on effluent suspended solids was tested in a pilot-scale, continuous, clarifier (**Figure 3-III**). Two sludge types were tested. One originated from the full-scale step-feed reactor operating at an average SRT of 1.5 ± 0.5 days, F/M ratio of 3.0 ± 0.8 kg COD/g VSS/d, and SOR of 1.4 ± 0.2 $m^3/m^2/h$. The other sludge was sampled from the contact-stabilization pilot, which was subjected to an average SRT of 1.4 ± 0.5 days, F/M ratio of 11.2 ± 3.7 kg COD/g VSS/d, and SOR of 1.1 ± 0.1 $m^3/m^2/h$. **Table 3** gives the EPS quality and quantity associated with the two sludge types. CS had a significantly higher amount of EPS and was of better quality than SF. CS also produced larger flocs within the clarifier as 25% of the CS flocs had a Feret diameter larger than 3.4 mm, compared to 1.6 mm for SF flocs (**Figure S3**).

Under low shear conditions (19 s^{-1}), CS sludge achieved a significantly lower effluent suspended solids (38 ± 1 mg TSS/L) than SF sludge (69 ± 4 mg TSS/L) at 0.6 $m^3/m^2/h$. Only when the clarifier solids inlet concentration was increased to 869 mg TSS/L (i.e. increasing the amount of collisions), was SF able to achieve a similar effluent quality (38 ± 4 mg TSS/L) (**Figure 3-III**). When the SOR was increased to 1.5 $m^3/m^2/h$, the effluent suspended solids increased on average with 9 and 42 mg TSS/L for SF and CS testing, respectively. This indicated that most CS flocs settled between 0.6 – 1.5 $m^3/m^2/h$, which is the typical operational range of HRAS clarifiers, while the sensitivity toward SOR changes was larger for CS. The former was confirmed by batch settling experiment in **Figure 3-I/II**, where under bioflocculation promoting conditions (19 s^{-1}), 26% ± 13 % of SF settled between 0.6-1.5 $m^3/m^2/h$ compared to 50% ± 5% for CS (**Figure 3-II**). A similar trend could be observed without mixing energy (**Figure 3-I**). The increase was also in line with what was observed in the pilot during continuous operation, as the resulting ESS fits the curve well (**Figure 4**).

When sludge was subjected to harsh shear (250 s^{-1}) for 10 minutes prior to being introduced to the clarifier, a significant increase in effluent suspended solids was observed for all scenarios (**Figure 3-III**). At an SOR of 0.6 $m^3/m^2/h$, applying a velocity gradient of 250 s^{-1} induced a 10 ± 3 mg TSS/L increase in effluent suspended solids of regardless of sludge type or feed concentration. This indicated desorption of fines rather than floc formation limitation. When the SOR was increased to 1.5 $m^3/m^2/h$, the CS sludge experienced a significantly bigger impact of shear compared to the SF system. Whereas SF increased by 20 ± 7 mg TSS/L, the 250 s^{-1} velocity gradient induced a 45 ± 3 mg TSS/L increase for CS at 1.5 $m^3/m^2/h$, indicating an increased sensitivity to changes in SOR when high shear was applied. Shear management is thus imperative when bioflocculation is managed in CS system.

3.4. Impact of fines loading on effluent suspended solids

At Blue Plains advanced wastewater treatment plant, the waste originating from pre-dewatering (solids processing return, SPR) is sent back to the secondary reactors for treatment. This waste is a combination of dissolved air flotation effluent and centrifuge reject liquid. The stream thus contains everything that was not captured in these processes, thus inherently rich in fines. The average TSS in the SPR stream within the timeframe of **Figure 5** was 885 ± 401 mg TSS/L. From January 16 until 21, the SPR flow to the SF reactor was on average 0.12 ± 0.03 kg TSS_{SPR}/kg

MLSS/d, where after it was fully redirected to the parallel "West" reactor on January 21 (**Figure 5**). After a brief upset in the reactor due to wet weather, the effluent suspended solids decreased significantly with an average of 10 mg TSS/L from 40 ± 5 to 30 ± 6 mg TSS/L ($p = 0.003$).

4. Discussion

4.1. Role of biomass and EPS on capturing fines

Fines were defined as suspended solids that settle slower than 0.6 $m^3/m^2/h$, and therefore will not be separated in a clarifier unless enmeshed into a bioflocculated floc. Bioflocculation is mediated though EPS (Sheng et al., 2010), which can be provided through the active biomass fraction in the wastewater, microbial growth (Rahman et al., 2019), and supplemented by bioaugmentation of BNR sludge (Van Winckel et al., 2019). In this study, CEPT removed a large amount of settleable solids and active heterotrophic fraction, thus EPS could only be formed through growth (CS + SF) or seeded with bioaugmentation (SF). Additionally, solids removal could only be achieved with proper bioflocculation given the lack of fast settling solids fed from the influent. The higher EPS concentration found in contact-stabilization are in line with previous studies, where a higher EPS production was found when sludge was subjected to a feast-famine regime (Meerburg et al., 2016a; Rahman et al., 2017). An increase in EPS amount and quality (PN/PS-ratio) was correlated with an increase in capture of fines, as CS configuration had both higher EPS quantity/quality and better capture of fines as indicated by the lower ESS obtained at 0.6 $m^3/m^2/h$ (**Figure 3**). This was also in agreement with previous work on a similar wastewater composition (Van Winckel et al., 2019). However, Kinyua et al. (2017) did not find a similar correlation for an A-stage HRAS configuration, presumably because of the nature of the influent (raw sewage vs CEPT-effluent). As such, for systems operating on CEPT-effluent, fines were best managed by promoting EPS formation through going from step-feed to plug-flow (F/M increase) or by shifting to contact-stabilization (feast-famine). Whether the bioflocculation differences between CS and SF can be attributed to the loosely-bound (LB) or tightly-bound (TB) fractions of the EPS remains unclear. This EPS fractionation was not performed in this study, as no agreed definition of what defines the LB and TB fractions exist and are moreover highly dependent on the extraction method. Existing research has therefore conflicting evidence, where some cite a negative correlation between bioflocculation and LB (Li and Yang, 2007), while other mainly attribute bioflocculation to the TB fraction (Liu et al., 2010). Additionally, EPS is also important for the capture of colloidal COD, which has the same trend as fines (Jimenez et al., 2015).

Bioflocculation can also be improved by increasing the mixed liquor concentrations in the reactor, essentially increasing the total number of collisions in the clarifier, negating the impact of poorer collision efficiency. The effluent suspended solids observed for SF in **Figure 3** was achieved with a TSS-limited clarifier. Increasing the concentration improved the effluent quality as more collisions led to higher chance of fines enmeshing in the flocs. At fixed influent characteristics and loading rate, increasing the mixed liquor in the reactor is synonymous to increasing the SRT, which translates into sacrificing some carbon redirection to ensure carbon capture. The balance between carbon redirection and capture will depend on influent characteristics and temperature and is thus plant specific. Controlling on oxygen uptake rate, rather than SRT, takes these conditions into account (Van Winckel et al., 2018). Additionally, the degree of bioflocculation (collision efficiency) can be measured with the threshold of flocculation (TOF) parameter (Mancell-Egala et al., 2017), which can be helpful in determining the bioflocculation impairment is present without relying solely on ESS numbers. Furthermore, Ngo et al. 2021 determined that a relationship exists between TOF and ESS which can be implemented in a process model, helping future modelling efforts to optimize HRAS clarifiers.

4.2. Role of SOR and shear on effluent suspended solids

The SOR is a critical yet often overlooked design parameter for HRAS systems. The SOR applied at the high-rate contact-stabilization pilot and full-scale step feed were in range of the typical SOR applied in the A-stage reactors in the Netherlands (0.75 - 1.4 $m^3/m^2/h$) (De Graaff & Roest, 2012). An elevated SOR puts pressure on the flocs, requiring an overall faster settling velocity distribution if one wants adequate effluent quality. SOR itself can only be lowered by increasing the number of clarifiers, requiring large capital investments and the required land to do so. Managing the settling velocity distribution of the sludge thus is the only way to manage the impact of SOR on effluent quality.

The effect of shear on the settling velocity distribution was dependent on the velocity gradient applied prior to introduction into the clarifier. At low shear, flocculation will be promoted thus providing a more favorable and faster settling velocity distribution. At high shear, floc breakup will be dominant, leading to an overall slower distribution. This was especially true when bioflocculation was adequate, as inducing high shear on the CS sludge led to a stark shift in settling velocity distribution, deteriorating the effluent suspended solids as a result. In essence, shear cannot be easily separated from SOR because changes in settling distribution will lead to different impacts of SOR on the effluent suspended solids. Maximizing carbon capture therefore should include creating the most optimal settling velocity distribution possible through EPS-mediated bioflocculation and maintain that distribution when the sludge is introduced into the clarifier.

A common hypothesis postulates that applying a high SOR will over time will shift the settling velocity distribution to the right and might eventually lead to granulation (Beun et al., 1999). However, no evidence of selection of fast settling aggregates in HRAS systems due to elevated SOR have been observed in this study. One hypothesis may be that HRAS' microbiome is too fast growing for this selection to take place and the SOR is too low to be selective enough. Significant differences in microbiome between a high-rate and low-rate activated sludge were found in the Netherlands (Meerburg et al., 2016b). Physical selectors, like a screen of cyclone using for deammonification, could artificially increase the selection pressure on the sludge without jeopardizing effluent quality (Wett et al., 2015). There are indications that this hold true for selective retention of phosphate accumulating bacteria (Ford et al., 2016). However, further research will have to show the design and effectiveness of a physical selector for continuous HRAS applications.

4.3. Technology solutions to manage fines capture and optimize the settling velocity distribution

The capture of fines and the resulting final settling velocity distribution of the sludge can be managed by smart clarifier inlet design which minimizes inlet shear with or without flocculation zones to ensure full flocculation. Furthermore, sludge introduced to the clarifier can be fed through the sludge blanket. Multiple technologies exist that actively try to mitigate effluent suspended solids. While most are designed for conventional systems, they should be transferable to HRAS-type of applications.

Computational fluid dynamics (CFD) is a tool which can help designing proper clarifier geometry and pick the right inlet and baffle design (Samstag et al., 2016). When linked to population balances, the impact of hydrodynamic shear on floc formation/breakup can be estimated (Nopens et al., 2015). Hydraulic flows can also be assessed with acoustic doppler measurements to identify high shear areas in-situ and validate potential changes (Kinnear & Deines, 2001). The inlet preferably includes a flocculation zone, which provides ample low shear conditions prior to entering the clarifier in combination with smart baffle design helps in retaining the maximum settling velocity distribution, especially after higher shear conditions in the channel (**Figure 2-III**). Special considerations should further be given to appropriate baffle design to fully dissipate the kinetic energy. This will be especially true for high-rate activated sludge systems that deal with higher flow rates. Double perforated baffles could help dissipate the energy in rectangular tanks (Lee, 2017). Circular clarifiers could on the other hand benefit from an energy-dissipating inlet design (Shaw et al., 2005).

Feeding through a sludge blanket filters the feed through a sludge bed, maximizing the number of collisions that take place. This would mean that any floc breakage that occurred prior to clarifier will be nullified as the sludge will very rapidly reflocculate within the blanket. This allows for instantaneous capture of fines and disconnects the SOR from the sludge's (flocculent) settling velocity distribution. Multiple technologies are commercially available. The Hydrograv Adapt technology utilizes an adaptable feed height so that it always feeds below sludge blanket (Benisch et al., 2018).

The Hydrograv Adapt however relies on a sufficiently high blanket level present, which requires longer residence times within the clarifier. Given that high-rate systems operate at very short SRT and HRT, extending the residence time in the clarifiers may lead to increased total sludge age, thus inaccurately estimating the SRT in the reactor. In addition, desorption may happen due to increased anaerobic residence times. This issue can be mitigated by shifting to a AAA™ (Alternated Activated Adsorption) process. AAA is a retrofitted primary clarifier which is act as primary settler and A-stage combined in one tank where the feed is introduced through the settled sludge bed (Wett et al., 2020).

The main culprit of sludge bed feeding is the sensitivity to wet-weather flow. Sudden changes in flow may lead to turbulent conditions which can upset the bed, causing elevated effluent suspended solids or in extreme cases clarifier failure. The Hydrograv mitigates this issue by having a dynamic feed height which, e.g. through CFD, could be calibrated to certain heights given a specific sludge blanket height (Benisch et al., 2018). The clarifier was able to handle up to 700 gal/ft^2/d (~ 1.2 m^3/m^2/h) without sacrificing on effluent quality, which could make it appropriate for high-rate applications. Ultimately, novel innovations in clarifier design will be necessary to accommodate the shear-prone, slower settling velocity distribution created by HRAS and ensure excellent year-round performance in terms of effluent quality.

5. Conclusion

Overall, this work clarified the impact of process configuration, SOR, and shear on effluent suspended solids in high-rate activated sludge systems solely driven by bioflocculation. The following can be concluded from this study:

- Managing the loading and capture of fines (< 0.6 $m^3/m^2/h$) to high-rate activated sludge systems can significantly improve (i.e. lower) effluent suspended solids levels. An estimated 10 mg TSS/L of the effluent suspended solids was attributed to fines in the plug-flow configuration. A switch to contact-stabilization could potentially decrease the effluent suspended solids up to 31 mg TSS/L. Clarifier development focusing on sludge blanket filtration can further improve ESS under dry weather conditions.
- High-rate activated sludge systems with a good quantity and quality of extracellular polymeric substances (like the contact-stabilization process) are efficient in capturing fines but create flocs with a narrow settling velocity distribution with a low average velocity. This makes the system sensitive to surface overflow rate changes, because 50% ± 5% of the mass has a settling velocity between 0.6-1.5 $m^3/m^2/h$.
- Allowing for orthokinetic flocculation at a low velocity gradient (20 s^{-1}) for at least 1 minute before clarification can safeguard the optimal the settling velocity distribution independent of tank shear conditions. This highlights the importance of feed well design and 3D distribution profile of clarifiers.

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Tables

Table 1. Influent and effluent characteristics of the contact stabilization pilot reactor runs. All average (and standard deviations) were calculated based on n > 14, except total Kjeldal N which was calculated based on n > 3.

	Influent			**Effluent**			
	Scenario A	***Scenario B***	***Scenario C***	***Scenario A***	***Scenario B***	***Scenario C***	
Total COD	**218** ± 61	**237** ± 39	**232** ± 18	**149** ± 39	**141** ± 23	**143** ± 27	mg COD/L
Particulate COD	**98** ± 31	**108** ± 36	**112** ± 28	**83** ± 23	**63** ± 23	**58** ± 27	mg COD/L
Colloidal COD	**50** ± 17	**85** ± 113	**46** ± 21	**13** ± 14	**21** ± 23	**24** ± 13	mg COD/L
Soluble COD	**85** ± 17	**72** ± 12	**74** ± 17	**57** ± 19	**57** ± 7	**58** ± 11	mg COD/L
Total Kjeldal N	**41** ± 5	**41** ± 8	**68** ± 14	**43** ± 10	**42** ± 4	**58** ± 3	mg N/L
Ammonia N	**35** ± 3	**34** ± 6	**37** ± 4	**34** ± 2	**34** ± 5	**36** ± 4	mg N/L
Total Phosphorus	**2.4** ± 0.6	**2.3** ± 0.5	**2.7** ± 0.6	**2.1** ± 0.8	**1.6** ± 0.4	**1.9** ± 0.7	mg P/L
Ortho-Phosphorus	**1.2** ± 0.4	**0.7** ± 0.4	**1.1** ± 0.3	**0.5** ± 0.3	**0.2** ± 0.2	**0.9** ± 0.3	mg P/L
Effluent suspended solid	**57** ± 9	**64** ± 19	**61** ± 13	**62** ± 19	**49** ± 11	**40** ± 15	mg TSS/L

Table 2. Process, clarifier parameters and extracellular polymeric substances (EPS) characteristics from the three scenarios operated on the contact-stabilization pilot reactor. The average of the process and clarifier parameters were calculated based on n > 14. EPS was calculated based on n > 4. PN = protein, PS = polysaccharide.

	Scenario A	*Scenario B*	*Scenario C*	
	Process parameters			
Solids retention time	**0.9** ± 0.5	**1.0** ± 0.5	**0.4** ± 0.3	d
Temperature	**14.8** ± 2.3	**15.1** ± 1.0	**19.3** ± 1.5	°C
Dissolved oxygen	**0.8** ± 0.8	**0.0** ± 0.1	**0.0** ± 0.1	mg O_2/L
Coarse bubble duration	**7** ± 0	**1** ± 0	**1** ± 0	s
Coarse bubble frequency	**4** ± 0	**4** ± 0	**4** ± 0	min^{-1}
Coarse bubble air flow rate	**4** ± 0	**4** ± 0	**4** ± 0	m^3/h
Organic loading rate	**5.5** ± 0.4	**5.4** ± 0.8	**5.0** ± 0.3	kg COD/m^3/d
F/M rate (total COD)	**26** ± 17	**17** ± 3	**30** ± 9	kg COD/kg VSS/d
F/M rate (soluble COD)	**9.0** ± 5.6	**5.1** ± 1.1	**9.4** ± 3.7	kg COD_{ff}/kg VSS/d
	Clarifier Parameters			
Surface overflow rate	**1.0** ± 0.0	**0.9** ± 0.1	**0.6** ± 0.2	m^3/m^2/h
Solids loading rate	**9.2** ± 4.8	**9.3** ± 2.7	**2.9** ± 1.2	kg TSS/m^2/d
	Extracellular polymeric substances (EPS)			
Total EPS	**286** ± 42	**233** ± 79	**241** ± 67	mg COD/g VSS
Total EPS protein	**65** ± 21	**32** ± 3	**34** ± 20	mg BSA/g VSS
Total EPS polysaccharide	**36** ± 14	**21** ± 6	**25** ± 6	mg glucose/g VSS
Total EPS PN/PS ratio	**1.8** ± 0.2	**2** ± 0	**1.4** ± 0.5	mg BSA/mg glucose

Table 3. Characteristics of the extracellular polymeric substances (EPS) of the full-scale step-feed and pilot-scale contact-stabilization reactor at the time of the clarifier experiments (n = 3, PN = protein, PS = polysaccharide).

Parameter	**Contact-stabilization reactor**			**Step-feed reactor**			Unit
Total EPS	**235**	±	22	**177**	±	11	mg COD/g VSS
Total EPS protein	**66**	±	3	**42**	±	21	mg BSA/g VSS
Total EPS polysaccharide	**28**	±	3	**23**	±	11	mg glucose/g VSS
Total EPS PN/PS ratio	**2.4**	±	0.3	**1.8**	±	0.3	mg BSA/mg glucose

Figures

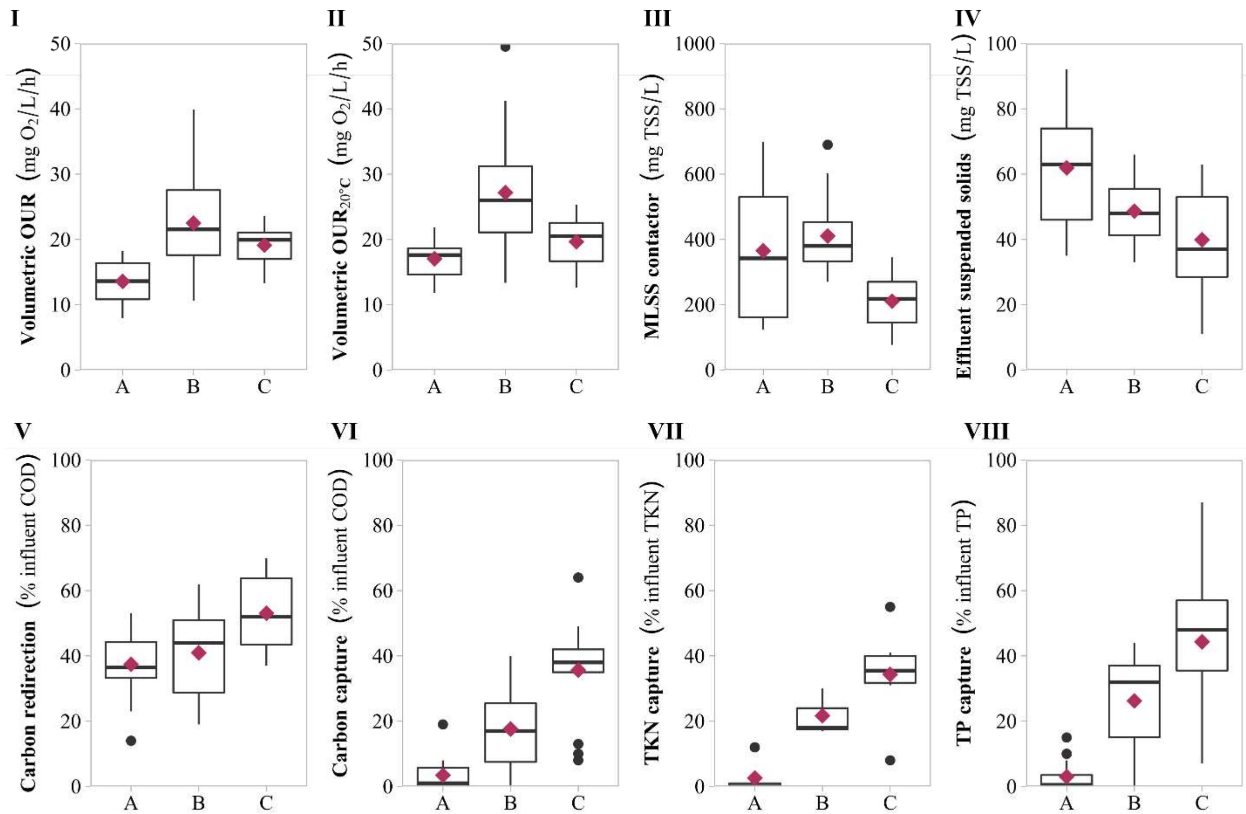

Figure 1. High-rate contact-stabilization pilot: boxplots of key biological and performance parameters of the three scenarios A, B and C (diamonds representing the average) The purple triangles indicate the data's average. **(I)** Volumetric OUR measured in the contactor. **(II)** Volumetric OUR in contactor corrected for temperature using the standard Arrhenius coefficient (1.04). **(III)** Mixed liquor concentration in the contactor. **(IV)** Effluent suspended solids. **(V)** Observed carbon redirection based on COD mass balancing. **(VI)** Percentage influent COD captured in the waste activated sludge. **(VII/VIII)** Percentage of TKN/TP capture.

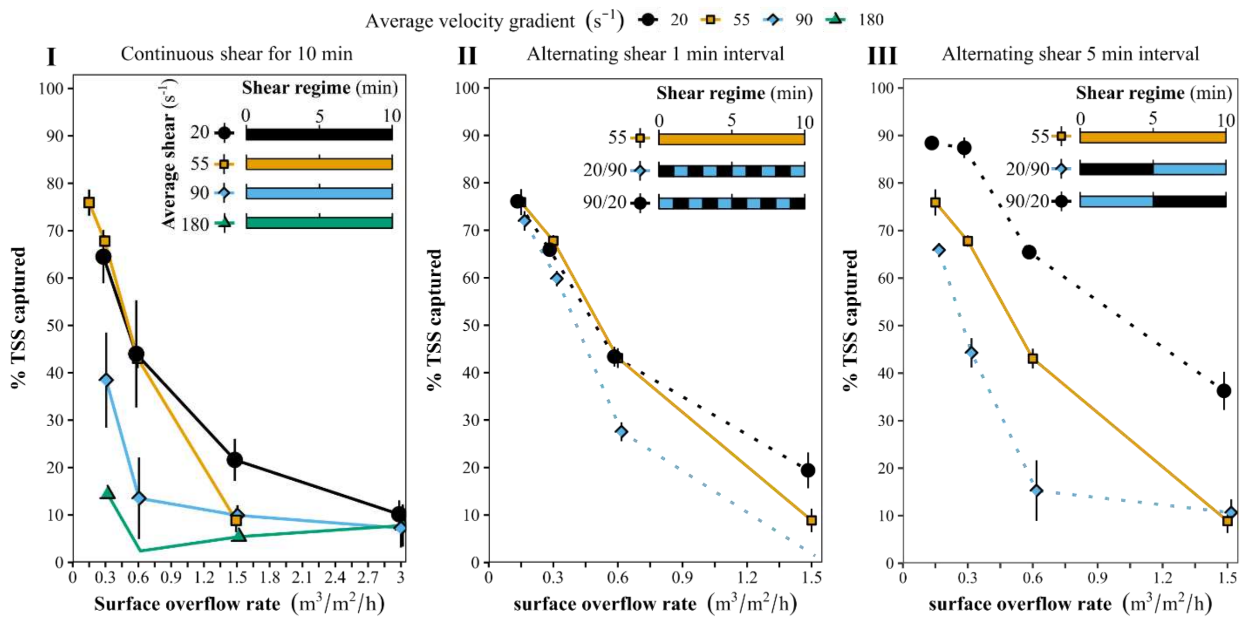

Figure 2. Effect of surface overflow rate on TSS capture in batch clarifier at different shear regimes. Sludge obtained from full-scale plug-flow reactor. **(I)** continuous shear for 10 minutes, **(II)** shear interval where the velocity gradient was alternating between 20 s^{-1} and 90 s^{-1} every minute for 10 minutes, and **(III)** shear interval with alternating velocity gradients every 5 minutes for 10 minutes. The 55 s^{-1} line from panel A was repeated in panel B and C because its total energy (G x t) was equal to the alternating shear regimes.

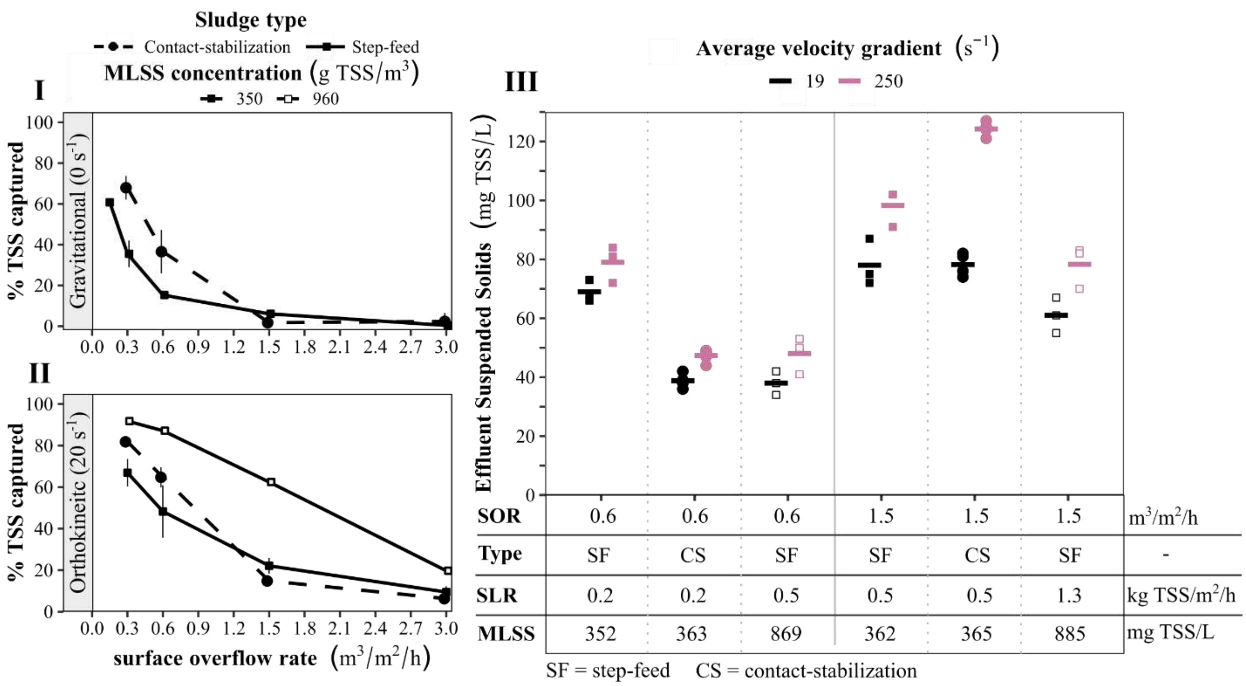

Figure 3. Continuous clarifier experiment with corresponding batch settling tests**. (I/II)** TSS capture obtained in batch settling tests at time of the continuous clarifier experiment in function of surface overflow rate in a gravitational (0 s^{-1}) **(I)** and orthokinetic (20 s^{-1}) **(II)** environment tested at 350 and 960 mg TSS/L. **(III)** Pilot-scale continuous clarifier experiment where the effluent suspended solids was investigated at 2 different surface overflow rates (0.6 and 1.5 $m^3/m^2/h$) and velocity gradients (19 s^{-1} vs 250 s^{-1}).

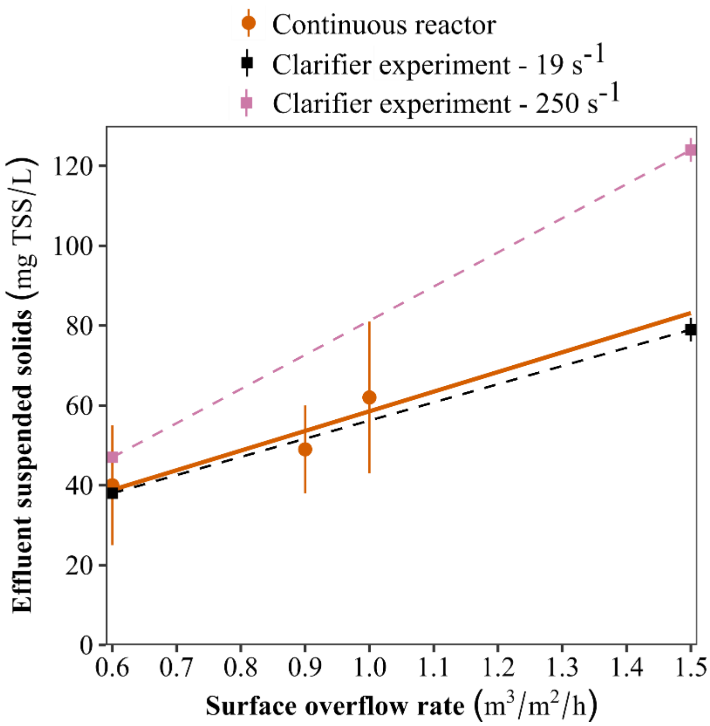

Figure 4. Effluent suspended solids obtained from the controlled clarifier experiment (squares) and continuous reactors (orange dots) of the contact stabilization reactor. The orange line denotes the linear regression trendline of the three points obtained from the continuous reactor. Dashed lines are for clarity only and not indicative of trends between the two datapoints.

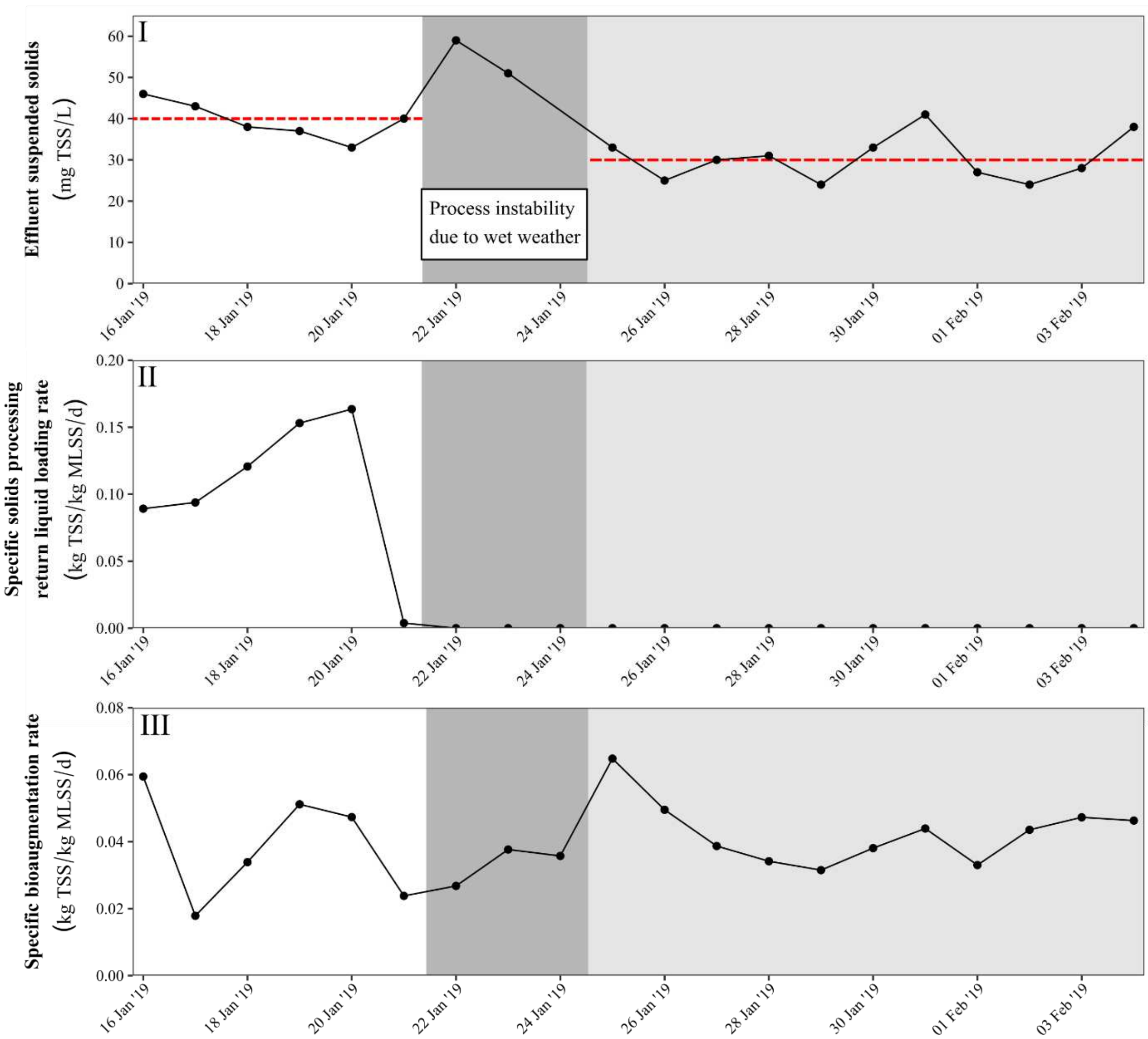

Figure 5. Full-scale step-feed (SF) high-rate activated sludge reactor: Impact of colloid loading and bioaugmentation rate on the profiles of effluent suspended solids during a three-week period **(I)**. The profiles are split up in a first period where the reactor received solids processing return liquid (levels in panel **II**; white background), with on average 40 ± 5 mg TSS/L in the effluent. In the last period, the SF reactor did not receive return liquid, and following wet weather-related instability, the average ESS level was 30 ± 5 mg TSS/L (light gray background). The bioaugmentation rates are shown in **(III)**.