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1 **Introducing Bioflocculation Boundaries in Process Control to Enhance Effluent**  
2 **Quality of High-Rate Contact-Stabilization Systems**

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24 **Abstract**

25 High-rate activated sludge (HRAS) systems suffer from high variability of effluent quality, clarifier  
26 performance, and carbon capture. This study proposed a novel control approach using bioflocculation  
27 boundaries for wasting control strategy to enhance effluent quality and stability while still meeting carbon  
28 capture goals. The bioflocculation boundaries were developed based on the oxygen uptake rate (OUR) ratio  
29 between contactor and stabilizer (feast/famine) in a high-rate contact stabilization (CS) system and this  
30 OUR ratio was used to manipulate the wasting setpoint. Increased oxidation of carbon or decreased wasting  
31 was applied when OUR ratio was  $<0.52$  or  $>0.95$  to overcome bioflocculation limitation and maintain  
32 effluent quality. When no bioflocculation limitations (OUR ratio within  $0.52 - 0.95$ ) were detected carbon  
33 capture was maximized. The proposed control concept was shown for a fully automated OUR-based control  
34 system as well as for a simplified version based on direct waste flow control. For both cases, significant  
35 improvements in effluent suspended solids level and stability ( $<50$  mg TSS/L), solids capture over the  
36 clarifier ( $>90\%$ ), and COD capture (median of  $32\%$ ) were achieved. This study shows how one can  
37 overcome the process instability of current HRAS systems and provide a path to achieve more reliable  
38 outcomes.

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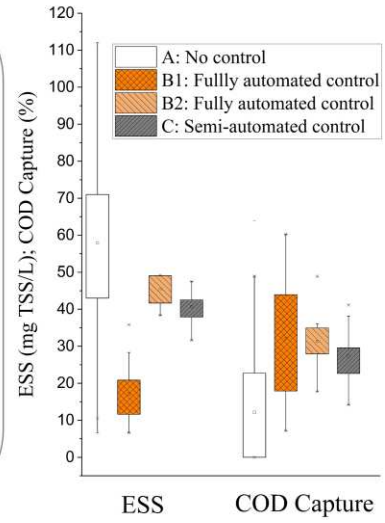
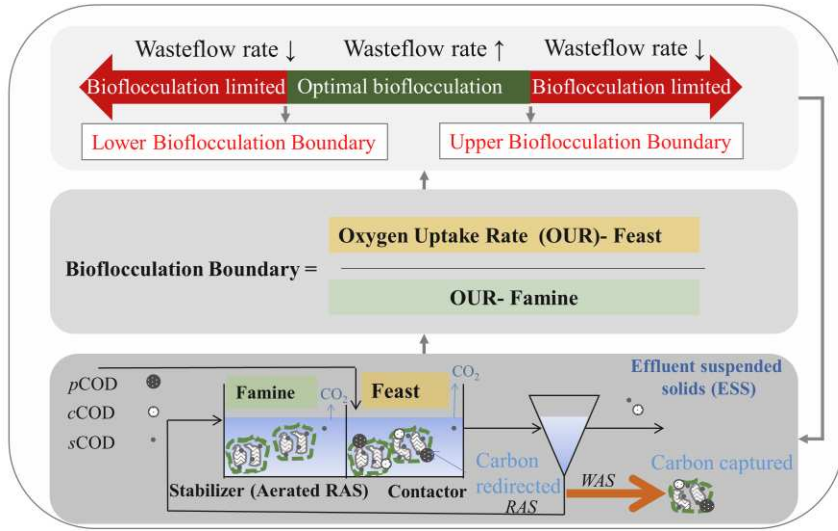
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46 **Key words:** High-rate activated sludge, Settleability, Clarifier performance, A/B process, Energy recovery

47 **Abstract Graph**



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60 **Practitioner points:**

- 61 ➤ Online bioflocculation boundaries (upper and lower limit) were defined by the OUR ratio between  
62 contactor and stabilizer (feast/famine).
- 63 ➤ To maintain effluent quality, carbon oxidation was minimized when bioflocculation was not limited  
64 (0.52-0.95 OUR ratio) and increased otherwise.
- 65 ➤ A fully automated control concept was piloted, also a more simplified semi-automated option was  
66 proposed.
- 67 ➤ Wasting control strategies with bioflocculation boundaries improved effluent quality while meeting  
68 carbon capture goals.
- 69 ➤ Bioflocculation boundaries are easily applied to current wasting control schemes applied to HRAS  
70 systems (i.e. MLSS, SRT, OUR controls).

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## 74 **1. Introduction**

75 Combined with carbon-efficient nutrient removal systems (short-cut nitrogen removal), high-rate  
76 activated sludge systems (HRAS) provide a major pathway towards energy neutrality as they allow for the  
77 redirection of 35-60% of wastewater COD towards energy recovery through anaerobic digestion (De Graaff  
78 & Roest, 2012; Rahman et al., 2020). The A-stage of the AB-process is a HRAS process with very short  
79 SRT (0.3-0.5 days) combined with a high loading rate ( $>2 \text{ kg COD kg VSS}^{-1} \text{ d}^{-1}$ ) that minimizes oxidation  
80 and maximizes sorption onto sludge. Carbon redirection denotes the transformation of organic carbon  
81 (particulates, colloids, and soluble) from wastewater into the sludge matrix through biosorption (i.e.,  
82 extracellular adsorption, enmeshment, and intracellular storage) and microbial growth phenomena (Rahman  
83 et al., 2016). Subsequently, carbon capture denotes the recovery of particulate carbonaceous organics  
84 through settling and wasting of the activated sludge, which then has the potential to be used for energy  
85 recovery in the form of biogas production in an anaerobic digester. Thus, good bioflocculation and settling  
86 behaviour in HRAS systems are essential for successful energy recovery from wastewater.

87 Reported carbon capture from HRAS varied between 21-55% of total incoming COD (Dai et al.,  
88 2018; Dolejs et al., 2016; H. Guven et al., 2017; Jimenez et al., 2015; Meerburg et al., 2015; Rahman et al.,  
89 2020) and effluent suspended solids (ESS) from 10 - 120 mg TSS/L (H. Guven et al., 2017; Jimenez et al.,  
90 2015; Ngo et al., 2021a; Rahman et al., 2016, 2019; Rahman et al., 2020), indicating that bioflocculation is  
91 variable and a function of sludge retention time (SRT), organic loading rate, wastewater composition,  
92 reactor configuration, and environmental conditions (Rahman et al., 2020). Recently, high-rate contact-  
93 stabilization (CS) was able to mitigate this issue by imposing a feast-famine regime through recycled  
94 activated sludge (RAS) aeration (famine) before contacting the sludge with the wastewater (feast). The  
95 latter triggered an extracellular polymeric substances (EPS) response, improving bioflocculation, which led  
96 to improved carbon capture, lower ESS, and lower threshold of flocculation (TOF) (Rahman et al., 2017).  
97 In addition, a high-rate CS configuration showed lower carbon oxidation and thus energy input to achieve  
98 similar carbon capture than conventional HRAS systems when operated under similar organic loading rate

99 and SRT (Rahman et al., 2019). Recently, high-rate CS was implemented in secondary treatment at Blue  
100 Plains Advanced Wastewater Treatment Plant providing significant improvement in effluent quality,  
101 clarifier performance, and energy efficiency (Ngo et al., 2021b). Wett et al. (2020) have also implemented  
102 this approach of contact stabilization process in existing primary clarifiers in the AAA process by operating  
103 the contact and stabilization parts in a time-sequenced mode. This approach has led to enhanced  
104 bioflocculation relative to a more conventional A-stage process or primary clarifiers. Furthermore, the  
105 approach of feeding through a settling blanket in the contact mode also achieved desired physical contact  
106 to enhance flocculation and achieved 75% COD capture (Wett et al., 2020).

107 In addition, bioflocculation has shown to deteriorate at shorter SRT (Dai et al., 2018; Faust et al.,  
108 2014; Jimenez et al., 2015; Rahman et al., 2020; Van Dierdonck et al., 2012), and when the food to microbe  
109 (F/M) ratio drops below an unknown critical threshold (Rahman et al., 2016; Stum & De Clippeleir, 2020).  
110 In contrast, the current practice relies on a standard aerobic SRT target of 0.2 days (De Graaff & Roest,  
111 2012), which does not result in optimized bioflocculation performance or energy balances. The high  
112 variability in the effluent quality of HRAS systems is one of the reasons why primary clarifiers are often  
113 preferred over HRAS systems despite their lower carbon capture.

114 Despite many advances in process control for nutrient removal (Le et al., 2018; Palatsi et al., 2021;  
115 Regmi et al., 2014, 2015; Zhu et al., 2017), process control for HRAS systems has been basic and not  
116 optimized for optimal energy or carbon management. Recent studies mainly focused on SRT control (Lee  
117 et al., 2007; Olsson et al., 2015; Seuntjens et al., 2020; Wu et al., 2011; YSI, 2014), but the latter is barely  
118 used in practice due to the need for several TSS probes and thus added complexity versus the widely used  
119 mixed liquor suspended solids (MLSS) control. Miller et al. (2017) explored the possibility of using MLSS-  
120 based wasting control to minimize variability in the COD removal efficiencies and bioflocculation. The  
121 study concluded that maintaining an MLSS concentration setpoint rather than having an SRT target  
122 delivered a more effective approach in maximizing COD removal efficiencies (up to 90%) at DO  
123 concentrations of 0.5-1.3 mg O<sub>2</sub>/L (Miller et al. 2017). However, the study showed high variability in

124 effluent quality, especially in colloidal COD, and thus indicated that such controls do not optimize for  
125 bioflocculation.

126 As an alternative, the use of oxygen uptake rate (OUR) as a control variable for management of  
127 sludge wasting rather than SRT or MLSS targets was shown to result in more direct control of energy input  
128 and thus COD oxidation management, which further increased the energy efficiency of plants (Van  
129 Winckel, 2019; Van Winckel et al., in review). Moreover, when the OUR setpoint was chosen correctly,  
130 bioflocculation was better managed, resulting in stable COD redirection and capture. However, given that  
131 the loading rate and environmental conditions dictated the biomass inventory and degree of bioflocculation,  
132 these parameters fluctuated dramatically even at stable OUR (Van Winckel, 2019; Van Winckel et al., in  
133 review).

134 Previous studies showed bioflocculation is a dynamic process (Ngo et al., 2021a; Rahman et al.,  
135 2016). Thus, one specific SRT, MLSS, or OUR setpoint for wasting control cannot be applied widely over  
136 different wastewater treatment plants to achieve similar and stable effluent quality. In addition, daily,  
137 weekly, or seasonal changes in wastewater characteristics and operational conditions (MLSS, oxygen  
138 levels, temperature, surface overflow rates, solids loading rates) will significantly impact the floc formation  
139 and the capture within the clarifiers. Thus, optimal wasting targets or setpoint for these different wasting  
140 control strategies will have to be adjusted noticeably in the function of the conditions driving  
141 bioflocculation. So far, no online control concept exists that directly controls and manages bioflocculation,  
142 and this was therefore the focus of this study.

143 The study aimed to develop an online bioflocculation control by determining online detection of  
144 bioflocculation limitations to manage wasting target. The proposed control concept prioritizes meeting  
145 effluent TSS targets before enhancing carbon redirection and energy balances. The hope is that such a  
146 control approach can better stabilize effluent quality from HRAS systems and enhance process reliability.  
147 This study included the following aspects: (i) the bioflocculation limitation indicators were developed based  
148 on 600 days of HRAS pilot runs under different OUR with various bioflocculation conditions; (ii) the



149 process control concept was laid out; (iii) proof of principle of the proposed concept was performed on the  
150 OUR-based wasting control and simplified waste flow rate control to show global applicability and impact  
151 on effluent total suspended solids (ESS), clarifier capture efficiency, and carbon capture achieved. This  
152 study shows for the first time an online control approach directly tailored to managing the settling behaviour  
153 of activated sludge.

## 154 **2. Materials & Methods**

### 155 *2.1 Pilot system*

156 A high-rate activated sludge pilot ( $V = 1402$  liters) was operated at Blue Plains Advanced  
157 Wastewater Treatment Plant in Washington (AWWTP), DC, USA. The pilot was operated in a contact-  
158 stabilization (CS) configuration and continuously fed with fresh chemically-enhanced primary treatment  
159 (CEPT) effluent originating from the full-scale plant at Blue Plains. The CEPT effluent was pumped  
160 continuously to the pilot to keep composition as close as possible to the full-scale facility and avoid storage.  
161 Details of wastewater influent characteristics are described in table 1. The system was inoculated with high-  
162 rate secondary sludge from the Blue Plains AWWTP, which operated under an average SRT of  $1.5 \pm 0.8$   
163 days aimed to maximize carbon capture to anaerobic digester for methane gas. The CEPT effluent was fed  
164 into the contactor ( $V = 223$  liters) reactor for a total HRT of 36 minutes and targeted DO at  $0.5 \text{ mg O}_2/\text{L}$ .  
165 From the contactor, the sludge was sent into the three clarifiers ( $\emptyset = 30.5$  cm and  $V = 302$  liters each). The  
166 underflows were combined in a return activated sludge (RAS) tank ( $V = 50$  liters) for 20 minutes and  
167 subsequently pumped into the stabilizer column ( $V = 223$  L) with the RAS recycle ratio at 60%. Here, the  
168 sludge was provided ample DO ( $> 2 \text{ mg O}_2/\text{L}$ ) and HRT of 96 minutes to oxidize all remaining absorbed  
169 organics. Sludge wasting was conducted from the contactor MLSS. Details on configuration and setup are  
170 described in Rahman et al., (2016), Van Winckel, (2019), and Van Winckel et al., (in review).

### 171 *2.2 Online OUR measurement*

172 The online oxygen uptake rates (OUR) of the contactor and stabilizer reactors were automatically  
173 calculated via small online ex-situ setups consisting of aeration (1.5 liters) and measurement vessels (300  
174 ml). Every 20 minutes, the sludge from the contactor and stabilizer reactors was pumped for 400 seconds  
175 into the setup, filling up both vessels. At the 300 second mark, the vessels were aerated for 100 seconds to  
176 increase DO to a minimum of 5 mg O<sub>2</sub>/L. It should note that sludge in both vessels were mixing continued  
177 and isolated with the ambient air (Figure S4). Afterwards, both air and feed pumps were turned off, and a  
178 declining DO curve was generated. The DO data points were captured for 750 seconds via a membrane  
179 probe (Atlas Scientific, USA) and were converted to digital signals using the Atlas Scientific EZO™ DO  
180 chip, which communicated with an Arduino Mega through a UART protocol. The signals were then sent to  
181 MATLAB R2019b, where the corresponding OUR from the logged declining DO slope was calculated.  
182 Data quality was checked by calculating the slope for multiple ranges of DO i.e. 5 to 4 mg O<sub>2</sub>/L, 4 to 3 mg  
183 O<sub>2</sub>/L, 3 to 2 mg O<sub>2</sub>/L, 4 to 2 mg O<sub>2</sub>/L, and so forth (1 – 0 mg O<sub>2</sub>/L was not considered due to rate limiting  
184 conditions). The slope with the highest R<sup>2</sup> was selected. If the R<sup>2</sup> of all slopes was similar, the 4 – 2 mg  
185 O<sub>2</sub>/L slope was chosen. If all slopes had a R<sup>2</sup> lower than 0.8, the value of the previous measurement cycle  
186 was fed to the controller. More information could be found at Van Winckel, (2019), Van Winckel et al., (in  
187 review) and supplemental Figure S4.

### 188 *2.3 Controller*

189 The pilot system was operated under OUR-based wasting control (scenario A), OUR-based wasting  
190 control with bioflocculation boundaries (Scenario B), and wasting flow control with bioflocculation  
191 boundaries (Scenario C). Scenario A was used to identify bioflocculation limitation boundaries and acted  
192 as the baseline scenario. Detailed control logic overviews are provided in Figure 3.

#### 193 *2.3.1 Baseline testing without bioflocculation boundaries (Scenario A)*

194 During this scenario, the wasting pump was controlled to meet a given OUR setpoint for the  
195 contactor (Figure 3A). The calculated OUR value (measured variable) was subjected to a one-hour rolling  
196 average, then compared to the control setpoint and fed to the proportional/integrative (PI) controller in

197 Matlab Simulink. The PI controller calculated the number of seconds the sludge waste pump had to run per  
198 hour in order to achieve a target averaged waste flow rate (control variable). This control approach was first  
199 tested by Van Winckel et al. (2019) and Van Winckel et al. (in review), where more details can be found.  
200 When the OUR setpoint was lower than the contactor OUR, the waste flow rate increased. Details of the  
201 test conditions are addressed in table 1 and table 2.

202 The measured OUR of the contactor reactor was first subjected to a one-hour rolling average before  
203 sending to the PID controller to allow for smoothening out of the signal. P, I, and D values used during the  
204 scenario were 750, 0.025, and 0, respectively, resulting in low errors between OUR measured and OUR  
205 target. During this scenario, OUR setpoints were changed on a regular basis to push the system and  
206 understand when bioflocculation limitations occurred.

### 207 2.3.2 Dynamic wasting control with bioflocculation boundaries (scenario B and C)

208 The control system adjusted OUR setpoints or waste flow setting based on bioflocculation behavior  
209 as depicted in Figure 3B and Figure 3C. When the OUR ratio between contactor versus stabilizer was within  
210 the upper and lower limit, no bioflocculation limitation was detected, and OUR setpoints were decreased  
211 (Figure 3B), or waste flow setpoints were increased (Figure 3C). Outside this OUR ratio range, an opposite  
212 trend in OUR or waste flow rates were applied to allow more carbon oxidation and enhance bioflocculation  
213 first (Figure 3B and 3C).

214 The degree of OUR setpoint change depended on how far one operated from the bioflocculation  
215 boundaries, and a linear gain factor was applied to control rate of change in case of the OUR control  
216 example (Figure 3B). As sludge wasting was a quicker process than microbial growth, a quicker response  
217 for decreasing wasting (increased gain factor A versus B) was deemed appropriate to minimize the chance  
218 of a complete sludge washout when bioflocculation was limited. The gain factors A and B were 2 and 0.5,  
219 respectively for scenario B1. Both A and B were set to 1 during scenario B2. Scenario B1 and B2 followed  
220 the same control logic but represented different wastewater characteristics (Table 1). For scenario C, a

221 simplified concept was tested where a step change in waste flow rate setting of 80 L/d was proposed that  
222 represented a potential change in SRT of approximately 0.1 days. This change was only made on a daily  
223 basis and based on a triplicate OUR ratio measurement during the day rather than online OUR ratio data  
224 collection. Scenario C was tested to see if the concept could be scaled down to a semi-online concept if  
225 online implementation of OUR ratios was deemed too complicated in practice.

226 For Scenario B, OUR setpoints were adjusted every 20 minutes as new OUR data was provided.  
227 The OUR ratio signal was smoothed out by taking an one hour rolling average of the contactor data to avoid  
228 instabilities in control response. It should be noted that P and I parameters were set at 75 and 0.025,  
229 respectively, based on two weeks of tuning before the start of the B scenario (data not shown). The fast  
230 response of the system to control targets was confirmed under the given tuning conditions.

#### 231 *2.4 Experimental methods*

232 Twenty-four-hour composite samples were collected for the influent and effluent, and grab samples  
233 were collected from the contactor and stabilizer on a daily basis. Influent, effluent and contactor samples  
234 were analyzed for total (tCOD), particulate (pCOD), colloidal (cCOD), and filter-flocculated COD (ffCOD)  
235 fractions. The ffCOD was assessed by following Mamais et al. (1993) method (ZnSO<sub>4</sub> flocculated and 0.45  
236  $\mu\text{m}$  filtered). The ffCOD was considered a true soluble COD, and the difference between ffCOD and 1.5  
237  $\mu\text{m}$  glass microfiber filtered COD indicated the colloidal COD (cCOD) fraction. In contrast, the difference  
238 between tCOD and 1.5  $\mu\text{m}$  glass microfiber filtered COD indicated the particulate COD (pCOD) fraction.  
239 COD, total phosphorus (TP), ortho-phosphorus (OP), and Ammonia (NH<sub>3</sub>-N) levels were tested via HACH  
240 test kits and HACH DR 2800 spectrophotometer (HACH, Loveland, CO, USA). In addition to the  
241 mentioned samples, grab samples for RAS, stabilizer, and ESS from three clarifiers were also collected to  
242 assess the total suspended solids (TSS) measured by standard methods (APHA, 2005).

#### 243 *2.5 Mass balance calculations*

244 For COD mass balance, particulate COD leaving the system was categorized as biomass instead of  
245 the particulate substrate (Meerburg et al., 2015, Rahman et al., 2016), and carbon redirection was assessed  
246 from the carbon mass balance over the reactor from the gathered daily performance data. The formula used  
247 for COD fractionations was similar to the calculations used in the studies of Rahman et al. (2016) and Van  
248 Winckel et al. (2019). Detail of the calculations are described in the equation 1 to 5 in supplemental  
249 information S5.

## 250 *2.6 Statistical analysis*

251 The statistical differences among the three bioflocculation parameters and different pilot runs were  
252 calculated using an unpaired t-test. T-tests with a p-value < 0.05 were identified as statistically significant.

## 253 **3. Results & Discussion**

### 254 *3.1 Defining bioflocculation targets for HRAS systems (scenario A)*

255 The main objective of the initial operation was to push the system towards increased carbon  
256 redirection, decreased OUR, and decreased SRT. By doing so, bioflocculation was challenged as previously  
257 observed (Jimenez et al., 2015; Rahman et al., 2016), resulting in a decreasing trend of carbon capture and  
258 TSS capture in the clarifier and an increasing trend in effluent suspended solids (ESS) concentration. Failure  
259 of bioflocculation was characterized by the inability to waste and thus a complete loss of COD capture even  
260 though organic loading rates were maintained and wasting control was activated. Given the diverse  
261 environmental and loading conditions during the 600 days (534 data points) high-rate CS pilot run (Table  
262 1) and the constant push of the system towards failure by setting changes in OUR targets, a broad range of  
263 conditions that led to bioflocculation limitation were captured in the dataset and shown in Table 1. This led  
264 to an average  $12 \pm 15\%$  COD capture,  $58 \pm 24$  mg TSS/L ESS and  $73 \pm 22\%$  TSS capture over the clarifier  
265 (Table 2 and Figure 1). It must be noted that the fundamental interest of the iterative approach was not to  
266 achieve a good and consistent bioflocculation but rather to understand and determine the critical conditions

267 that induce bioflocculation limitation. By acknowledging these conditions, early indicators can be identified  
268 and thus may be used to develop online bioflocculation control approaches.

269 The obtained data was further used to identify the definition for this system of good and limited  
270 bioflocculation conditions. To do so carbon capture, which is directly dependent on the bioflocculation  
271 behavior and entails the main function of the high-rate system (Rahman et al., 2020), was used as the main  
272 criteria to differentiate the bioflocculation conditions. Only at a carbon capture target of 31%, a significant  
273 impact on ESS ( $p < 0.05$ ) and TSS capture over the clarifier ( $p < 0.1$ ) was observed (Figure 1, Table 2). At  
274 carbon capture above 31%, average of ESS levels were lower at  $38 \pm 18$  mg TSS/L instead of  $61 \pm 23$  mg  
275 TSS/L, and average of TSS capture over the clarifier was  $84 \pm 11$  instead of  $79 \pm 16\%$  (Figure 1). Boxplot in  
276 Figure 1 also showed a clear differentiation between in ESS and TSS capture in clarifier at the threshold of  
277 COD capture at 31%. In addition, the good bioflocculation dataset was characterized by significantly  
278 ( $p < 0.05$ ) shorter SRT, lower carbon oxidation, MLSS, effluent total, and particulate COD (Table 1 and 2).  
279 Conventional feast-famine parameters defined on either soluble or total COD over biomass present were  
280 not sensitive enough to be directly linked to bioflocculation conditions ( $p < 0.05$ , Table 1) despite literature  
281 emphasizing the importance of feast-famine for improved settleability (Sturm & De Clippeleir, 2020). It  
282 should be noted that the probability of achieving bad bioflocculation was high in the overall data set, and  
283 good bioflocculation only represented 15% of the used data set (Table 1).

284 Overall, the following differentiating criteria were defined to achieve good bioflocculation: (i)  
285 COD capture above 31%, (ii) effluent suspended solids (ESS) below 48 mg TSS/L as defined by the overlap  
286 of the 75<sup>th</sup> percentile of the good and the 25<sup>th</sup> percentile of the bad bioflocculation data, and (iii) a TSS  
287 capture over the clarifier above 78%, as defined by the 25<sup>th</sup> percentile of good bioflocculation dataset  
288 (Figure 1). It should be noted that the COD capture criteria for the pilot system was lower compared to data  
289 from full-scale HRAS systems treating raw sewage with typical carbon capture around 25-74% COD  
290 capture. The latter is a result of the difference in wastewater characterization (Rahman et al., 2020). For the

291 effluent solids and clarifier capture criteria, targets defined based on the pilot system fall within full-scale  
292 results reported by De Graaff & Roest (2012) and are thus more globally applicable.

### 293 *3.2 Development of bioflocculation boundaries for limitation detection*

294 It was hypothesized that bioflocculation would remain efficient even under low SRT when a  
295 sufficient feast-famine regime is applied. Increased feast-famine was shown to induce EPS production in  
296 high-rate CS systems (Meerburg et al., 2016; Rahman et al., 2017) and A stage systems (Jimenez et al.,  
297 2015; Rahman et al., 2019). In addition, the EPS response as a function of the feast-famine regime was  
298 directly linked to the formation of denser sludge and granule formation (Sturm & De Clippeleir, 2020).  
299 Feast-famine was defined in the latter study by the added readily biodegradable COD (rbCOD) compared  
300 to the present biomass concentration (in VSS). As online rbCOD measurements are not available, online  
301 control to a specific feast-famine level is not practically feasible. In addition, data based on scenario A did  
302 not clearly show a link between traditional feast famine parameters and bioflocculation behavior (Table 1).  
303 This indicated that measuring a direct microbial response might entail better information. This study  
304 proposed the use of the ratio between contactor and stabilizer OUR as an online but analog signal for the  
305 activity in the contactor (feast) versus stabilizer (famine). This OUR ratio represented the microbial  
306 response more directly and was hypothesized to represent the feast famine condition of the system and  
307 would therefore play a direct role in bioflocculation (Sturm and De Clippeleir, 2020). Rahman et al. (2017)  
308 showed the link between this OUR ratio and the EPS response from stabilizer to contactor and its impact  
309 on the amount of carbon captured. This work aimed to translate this observation into a viable online control  
310 system tailored to optimizing bioflocculation. The overall control concept of inclusion of bioflocculation  
311 boundaries on top of wasting control strategies is shown in Figure 3. The upper and lower bioflocculation  
312 boundaries were defined based on baseline data available during scenario A.

#### 313 *3.2.1 Upper bioflocculation limit*

314 A high OUR ratio would indicate a high feast-famine regime and thus beneficial conditions for  
315 bioflocculation. However, the OUR ratio can also increase substantially when the active biomass fraction

316 or MLSS drops to lower numbers. Biomass limitation can lead to decreased COD removal and/or EPS  
317 production as well as decreased bioflocculation due to the limited number of collisions. The pilot often  
318 experienced MLSS limitation due to low-strength wastewater fed to the pilot and limited the organic loading  
319 due to hydraulic limitation as a result of limited clarifier surface. This resulted in a median MLSS  
320 concentration during scenario A of 350 mg TSS/L. Table 1 also shows slightly increased OUR ratios in the  
321 limited bioflocculation group compared to the good bioflocculation group, indicating that an upper OUR  
322 ratio boundary might be needed to avoid such flocculation limitations (Table 1).

323 The probability of achieving good bioflocculation (COD capture > 31%, ESS < 48 mg TSS/L, and  
324 TSS capture in clarifier > 78%), as defined in section 3.1, given a maximum OUR ratio target, is presented  
325 in Figure 2A. The chance of achieving the desired bioflocculation decreased at OUR ratios above 0.95. The  
326 latter OUR ratio target coincided with a decrease in median MLSS levels (Figure 2B) and thus confirmed  
327 potential biomass limitation. All analyses considered, the upper bioflocculation was set at 0.95, and this  
328 target was implemented within the control scheme (Figure 3).

### 329 3.2.2 Lower bioflocculation limit

330 Wet weather events decreased the OUR ratio by diluting the readily biodegradable COD in the  
331 influent and decreasing the biomass activity in contactor. The pilot system emphasized the latter events as  
332 it operated under constant hydraulic load rather than increased hydraulic load during full-scale wet weather  
333 events. Without a lower bioflocculation boundary, the controller would push down the OUR setpoint and  
334 thus increase wasting in an attempt to increase feast famine regime, and by doing so risk biomass washout  
335 due to a lack of substrate availability (feast potential) in the wastewater. The wet weather modus operandi  
336 in full-scale plants entails protecting biomass inventory by bypassing certain reactor zones and/or  
337 decreasing airflow rates during aeration (EPA, 2014). In this pilot influent flow rates were kept constant,  
338 and thus surface overflow rates on the clarifiers did not change, neither did the operational strategy change  
339 during those events. Based on the dataset of various rain events 132 data points of the OUR ratios at dry  
340 and wet weather events were separated described in Figure 2C. Wet weather event periods were detected



341 by plant influent flows to the full-scale WRRF below and above 1400602 m<sup>3</sup>/d (370 MGD), respectively.  
342 An OUR ratio of 0.52 separated out the OUR ratios obtained between dry and wet weather events, and such  
343 low OUR ratios were thus resulting from diluted wastewater loading (75<sup>th</sup> of during wet weather event,  $p <$   
344 0.05 with before wet weather event (Figure 2C)). An OUR ratio of 0.52 was chosen as the lower OUR  
345 boundary, and this target was implemented within the control scheme shown in Figure 3.

### 346 *3.3 OUR control with bioflocculation boundaries (Scenario B): full automated example*

347 This scenario was a fully automated scenario in which all parameters were collected, and all process  
348 control decisions were made automatically every 20 minutes (Figure 3). It was envisioned that although  
349 tested here on an OUR-based wasting strategy, the same concept could be applied for MLSS-based wasting  
350 (Miller et al., 2017) or SRT-based wasting (Olsson et al., 2015; YSI, 2014) using online TSS probes.  
351 Scenario B was split up into two scenarios based on wastewater characterization. The organic loading rate  
352 during scenario B1 was statistically lower than reference scenario A due to diluted wastewater  
353 characteristics (Table 1, Figure 4D, and 5B). The decreased total COD loading rate might have impacted  
354 the bioflocculation behavior during scenario B1, and the lower overall solids loading might have favored  
355 decreased effluent suspended solids (Rahman et al., 2016; Van Winckel et al., 2018). Scenario B2, although  
356 representing a shorter period, had similar wastewater characteristics than the reference scenario A and  
357 overcame this potential impact (Table 1).

358 Figure 4 shows the detailed trends in OUR ratios during scenario B1 leading to detection of  
359 bioflocculation limitations, and as a result, the control responses in terms of OUR setpoint and wasting flow  
360 rate. From day 652 to 656 and 667 to 670 OUR ratios were mostly above the upper bioflocculation boundary  
361 (OUR ratio  $> 0.95$ ), thus control eventually increased the OUR setpoint up to the maximum (Figure 4A and  
362 4B). This action allowed the system to slow down wasting (Figure 4C) and thus allowed for increased  
363 carbon oxidation from 11 % on day 667 to 53% on day 669 (Figure 4E). The latter maintained good effluent  
364 quality ( $< 30$  mg TSS/L) and TSS capture ( $> 90\%$ ) despite bioflocculation limitation (Figure 4G and 4H).  
365 Rahman et al. (2020) pointed that optimal carbon oxidation is needed for achieving good effluent quality,

366 and TSS capture as a certain amount of oxidation may be required to provide a sufficient level of EPS  
367 production and as a consequence floc structure (Jimenez et al., 2015; Rahman et al., 2016). Effluent would  
368 have deteriorated without the increased carbon oxidation as observed during reference scenario A. On the  
369 other hand from day 671 to 686, OUR ratios were frequently at optimal range (OUR ratio within 0.52-0.95),  
370 so the controller decreased OUR setpoint to target a lower optimal range of carbon oxidation (Figure 4A),  
371 resulting in more carbon capture and capture efficiency (Figure 4E and 4F), while ESS and TSS capture  
372 were maintained in a good range (Figure 4G and 4H). Overall, this approach was able to meet the target  
373 good bioflocculation criteria with low ESS of  $17 \pm 6.9$  (mg TSS/L), improved TSS capture of  $95 \pm 3.9$ (%),  
374 and COD capture of  $31 \pm 15$  (%) (Table 2). This was on all three fronts a significant improvement compared  
375 to the same control OUR wasting control approach without the bioflocculation boundaries (Table 2).

376         Given the lower organic loading during scenario B1, system output was collected for a second  
377 period (scenario B2) with higher organic loading similar to the baseline scenario A, with the exception of  
378 an increased pCOD/tCOD during scenario B2 ( $p < 0.05$ ). Operational details are shown in Figure S1 and  
379 Table 1. Overall, all bioflocculation criteria were also met during this run (Table 2). While both the good  
380 bioflocculation data set for scenario A and scenario B2 observed the similar carbon redirection level, the  
381 high fraction of pCOD/tCOD in the influent potentially caused poorer effluent quality and COD capture in  
382 scenario B2 (Table 2, Figure 7). Increased particulate COD loading to HRAS systems was previously  
383 described to accelerate saturation of sorption spots (Van Winckel et al., 2018) and thus might impact  
384 bioflocculation and bioflocculation limitation significantly. Even though pCOD was driving some of the  
385 ESS levels, even under more challenging conditions in scenario B1, significantly lower ESS and better TSS  
386 capture were obtained under the newly proposed control scheme versus the OUR-based wasting control  
387 without bioflocculation boundaries (Scenario A) (Table 1, Figure 7). This result confirmed the need for  
388 dynamic wasting targets in the function of bioflocculation conditions to achieve more controlled outputs in  
389 terms of effluent quality. The achieved better bioflocculation could potentially reduce energy demand  
390 through better sorption of colloids and surfactants (Garrido-Baserba et al., 2020), which can further enhance

391 the energy mass balance of the high-rate CS system. In addition, good and stable effluent quality also  
392 created increased stability for downstream nutrient removal processes as more stable effluent COD/N ratios  
393 were established.

#### 394 *3.4 Wasting flow control with bioflocculation boundaries (scenario C): simplified control example*

395 Even though PID control has long been developed and has been accepted as standard practice, an  
396 IWA report showed that about 50% of the WRRF run their systems in manual mode rather than turning on  
397 the PID control loops due to the complexity of tuning, poor sensor choice, high maintenance, and/or the  
398 human aspect of the control systems (Olsson et al., 2015). Therefore, a simplified version of the online  
399 bioflocculation control was tested to see what benefits could be obtained when a simplified control approach  
400 was applied (Figure 3C).

401 The pilot system was operated for 16 SRT cycles under this control scheme (Figure 5). Influent  
402 characterization (concentration or COD fractions of PE) was similar between scenario C, scenario B2, and  
403 the baseline scenario A, as well as the good bioflocculation dataset within scenario A ( $p > 0.05$ , Table 1,  
404 Figure 5C). During scenario C, optimal bioflocculation conditions (OUR ratio within 0.52-0.95) were  
405 observed for the first six days (Figure 6A); hence the control increased wasting flow rate, allowing more  
406 carbon capture, capture efficiency and lower carbon oxidation (Figure 5C and 5D). However, the system  
407 was pushed more and more towards bioflocculation limitation from day of 734 to 739 (OUR ratio  $> 0.95$ ,  
408 Figure 5A) as a result of increased wasting and a sudden increase in particulate COD loading (Figure 5B),  
409 again indicating the sensitivity of bioflocculation for oversaturation of sorption spots (Van Winckel et al.,  
410 2018) and limitation in EPS (Jimenez et al., 2015; Rahman et al., 2016). As the OUR ratio reached the  
411 maximum boundary, the control slowed down the wasting flow rate permitting more carbon oxidation to  
412 enhance bioflocculation, and bioflocculation was restored at the end of the experiment (Figure 5A, 5B, 5C,  
413 and 5D). It should be noted that the system took seven days to reach optimal bioflocculation levels again  
414 (Figure 5A) as a result of the slow decision-making (every 24 hours) and offline OUR measurement (3 grab  
415 samples). An increased step-change might have accelerated the recovery; however, this might have

416 decreased control stability (Nise et al., 2011; Olsson et al., 2015). Alternatively, the frequency of grab  
417 sampling and decision-making could have been accelerated. However, this would put a larger workload on  
418 the operator and make this approach impractical when no online OUR sensor is installed. It should be noted  
419 that OUR measurements taken for flocculation boundary measurements were based on an ex-situ declining  
420 DO experiment and one can explore the use of calculated OUR from field airflow and DO data as an  
421 alternative.

422 Despite the simplification and slow control adjustments compared to scenario B, improved  
423 consistency and quality in ESS, TSS capture, and COD capture were achieved (Figure 5, 6 and Table 2). In  
424 addition, scenario C scored better in all three bioflocculation criteria than the baseline scenario A (Figure  
425 6). This showed that the concept was strong enough to work under simplified and slower control action.

### 426 *3.5 Impact of bioflocculation boundaries on control of high-rate CS systems*

427 Data from full-scale and pilot-scale systems showed high variability in bioflocculation outcomes  
428 with ESS and COD capture fluctuating in a range of 10 – 120 mg TSS/L (H. Guven et al., 2017; Jimenez  
429 et al., 2015; Ngo et al., 2021a; Rahman et al., 2016, 2019; Rahman et al., 2020) and 21-55% (Dai et al.,  
430 2018; Dolejs et al., 2016; H. Guven et al., 2017; Jimenez et al., 2015; Meerburg et al., 2015; Rahman et al.,  
431 2020), respectively. This lined up with the significant variation of ESS (7 to 113 mg TSS/L), TSS capture  
432 in clarifier (51 to 98%), and COD capture (median at 12% and varied from 0 to 49%) in scenario A with  
433 no bioflocculation control (Figure 6). The proposed control approach with bioflocculation boundaries  
434 showed both in fully automated (scenario B) as well as a simplified version (scenario C) improved ESS  
435 levels (< 50 mg TSS/L) and reliability of ESS numbers (Figure 6). Similarly, TSS (> 90%) and COD capture  
436 (median at 32%) were improved and stabilized as well (Figure 6). The increased carbon oxidation applied  
437 when bioflocculation limitation was detected (outside bioflocculation boundaries) did not increase overall  
438 carbon oxidation levels on average basis, but rather increased overall carbon capture levels as  
439 bioflocculation limitation were resolved quickly. Depending on the variability of the bioflocculation  
440 efficiency, the range of carbon capture was wider or smaller; however, this study showed that a minimum

441 level of carbon recovery could be maintained at all times based on the proposed control approach in contrast  
442 to control systems without bioflocculation boundaries (Figure 6). Table 2 also illustrated that higher total  
443 COD removal was observed when control applied bioflocculation boundaries to compare with baseline  
444 scenario. Overall, effluent tCOD of scenario B1 ( $81 \pm 14$  mg/L), B2 ( $127 \pm 31$  mg/L), and C ( $118 \pm 31$   
445 mg/L) with bioflocculation control captured lower range and more stable to compare with scenario A ( $170$   
446  $\pm 53$  mg/L) (Table 2). The latter helps WRRF achieve the needed energy recovery (Sancho et al., 2019). In  
447 addition, it should be noted that the lower and more reliable ESS has an additional energy benefit as  
448 biodegradable COD is slowly added to the downstream biological nutrient removal system where oxygen  
449 might be used to oxidize (part of) it (Sancho et al., 2019).

450 From a control standpoint, scenario B and C showed increased process stability compared to  
451 scenario A, especially in terms of clarifier performance. Increased process efficiency and stability generated  
452 in this study shows similarities with nutrient removal control systems. For example, when AvN control, a  
453 control principle focused on process efficiency, is used by itself, it showed efficient total nitrogen removal,  
454 but effluent quality could not be guaranteed (Regmi et al., 2015). In contrast, ammonium-based aeration  
455 control (ABAC) guarantees effluent quality but not necessarily process efficiency (Rieger et al., 2014).  
456 When ammonium boundaries were added to AvN control, nitrogen removal was optimized as long as  
457 ammonium limits were met and both efficiency and effluent goals were optimized (communication with  
458 Bernhard Wett). The proposed approach of adding bioflocculation boundaries to wasting strategies  
459 provided similar benefits of achieving effluent quality while reaching the best COD mass balance. In  
460 addition, the latter hybrid control systems showed increased control stability compared to the OUR control  
461 only (Van Winckel, 2019; Van Winckel et al, in review) or AvN control only (communication with  
462 Bernhard Wett).

463 It should be noted that the transferability of the exact boundary levels (0.52-0.95) as used in this  
464 study will need to be evaluated when applied to a new condition or systems. In addition, depending on the  
465 response time of the system as well as system dynamics, the gain factors will need tuning in new systems.

466 One challenge of the proposed approach was the high maintenance needs for OUR measurements. We  
467 chose to measure OUR based on an external measurement loop to allow for a standardized OUR  
468 measurement independent of diffusers or oxygen transfer changes inside the system (Van Winckel, 2019;  
469 Van Winckel et al, in review). In addition, to allow for fast response, a membrane probe was used rather  
470 than an LDO probe, which required more frequent cleaning due to the sensitivity of the membrane to fouling  
471 (Hach LDO manual, 2006; Atlas scientific membrane EZO manual, 2017). Due to the smaller setup, no  
472 automatic cleaning (mechanical or air sparge) was feasible. Probes were maintained on every 12 to 24 hour  
473 to maintain reliable data collection. Future work will need to look at online OUR measurement probes with  
474 improved self-cleaning and calibration (APS-TOX manual, 2019) or alternative ways of measuring the  
475 bioflocculation boundaries or OUR. This could include calculation of OUR in-situ, based on applied airflow  
476 rates, or by applying intermittent aeration. The latter approaches were applied in other studies to improve  
477 nitrogen removal (Baeza et al., 2002; Jubany et al., 2009; Surmacz-Gorska et al., 1996), but never tested  
478 for HRAS where faster OUR rates and more dynamics in oxygen transfer rates might exist (Garrido-Baserba  
479 et al., 2020). Alternatively, other factors that can detect the feast famine level and microbial response similar  
480 to OUR might result in the same level of bioflocculation limitation detection. Overall, identifying a reliable  
481 bioflocculation boundary detection method is crucial step to bring this concept to practical application.

#### 482 **4. Conclusion**

483 This study proposed a control strategy using bioflocculation boundaries for wasting control strategy  
484 to enhance effluent quality and effluent stability while still meeting carbon capture goals. The  
485 bioflocculation boundaries were based on feast famine detection in the high-rate contact stabilization  
486 through measurements of the OUR ratio between contactor and stabilizer. Carbon oxidation was minimized  
487 within bioflocculation boundaries of 0.52-0.95 OUR ratio and increased outside these boundaries to  
488 maintain effluent quality. Both an online dynamic OUR-based wasting control with bioflocculation  
489 boundaries (a fully automated application ) as well as a wasting flow control with bioflocculation  
490 boundaries (a simplified application) were evaluated. A significant improvements in ESS stability and level

491 (< 50 mg TSS/L), TSS capture (>90%), and COD capture (median at 32%) were achieved with this new  
492 control approach. It should note that bioflocculation boundaries can be easily applied to current wasting  
493 control schemes applied to HRAS systems (i.e. MLSS, SRT, OUR controls) and thus might have broad  
494 applicability. The translation of this concept to a practical application will depend on finding a reliable  
495 bioflocculation boundary detection method and this will need to be the focus of future work. This study  
496 shows how one can overcome the process stability challenges of current HRAS systems and provide a path  
497 to achieve more reliable outcomes.

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651 **7. Table and Figure**

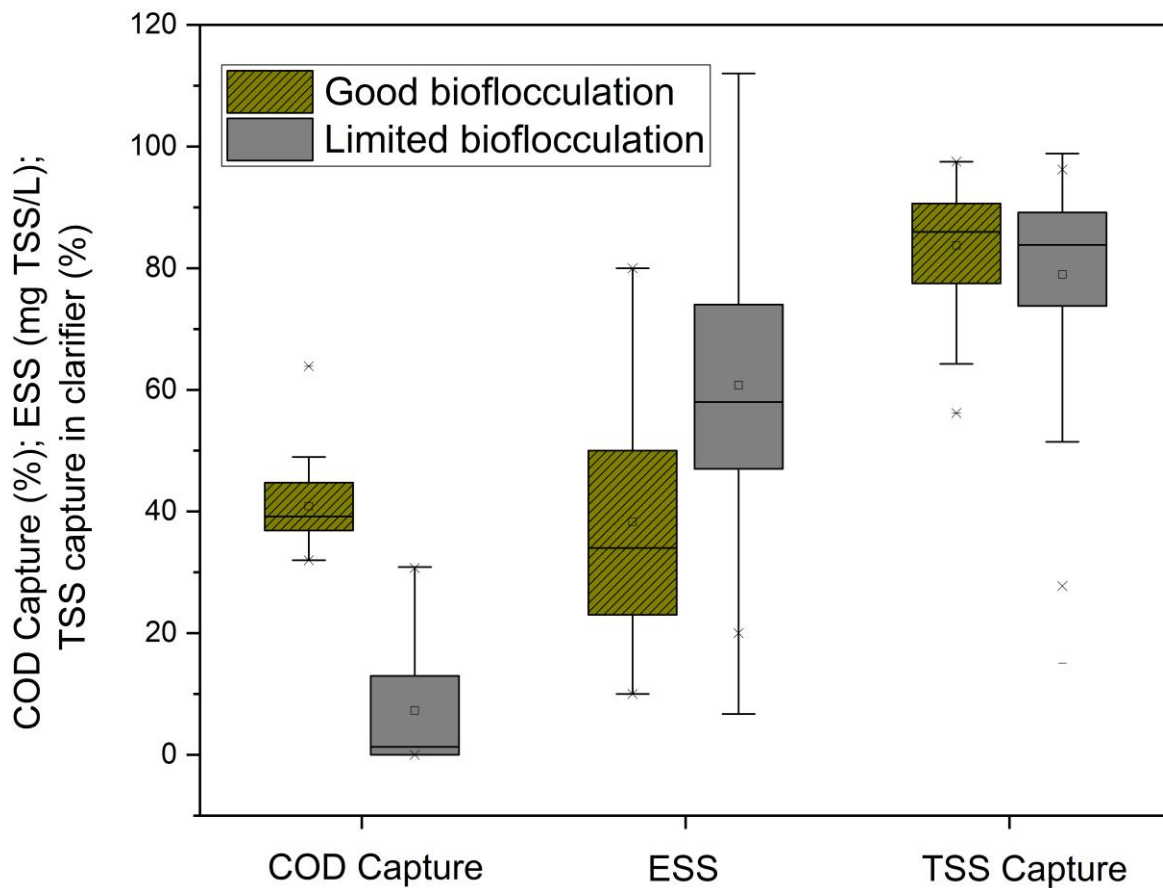
652 **Table 1:** Overview of influent characterization, operational conditions, oxygen uptake rate (OUR) data,  
653 and clarifier performance data for the baseline scenario (A) operated with OUR-based wasting control  
654 strategy, Scenario B with OUR-based wasting control strategy with added bioflocculation boundaries, and  
655 scenario C with a simplified version of control relying on direct waste flow control with bioflocculation  
656 boundaries. The details of the control logic are shown in Figure 3. Scenarios with statistically different  
657 parameters ( $p < 0.05$ ) share the same letter in superscript.

Characterization	Scenario					
	A [Baseline: OUR-based wasting control without bioflocculation boundaries (BB)]			B1 (Dynamic OUR-based wasting control with BB)	B2 (Dynamic OUR-based wasting control with BB)	C (Wasting flow control with BB)
	All	Limited (85%)	Good (15%)			
<b>Influent Characterization</b>						
tCOD (mg/L)	258 ± 69 <sup>a</sup>	261 ± 71	243 ± 55 <sup>b</sup>	158 ± 22 <sup>ab</sup>	271 ± 52	230 ± 57
pCOD (mg/L)	131 ± 54 <sup>a</sup>	135 ± 54	114 ± 49 <sup>b</sup>	66 ± 17 <sup>ab</sup>	147 ± 32	130 ± 37
cCOD (mg/L)	45 ± 28	44 ± 28	55 ± 28	42 ± 16	50 ± 18	30 ± 21
sCOD (mg/L)	81 ± 21 <sup>a</sup>	82 ± 21	75 ± 23 <sup>b</sup>	50 ± 9 <sup>ab</sup>	75 ± 20	70 ± 20
pCOD/tCOD (%)	51 ± 12 <sup>ab</sup>	51 ± 11 <sup>c</sup>	45 ± 10 <sup>bc</sup>	42 ± 9 <sup>a</sup>	54 ± 7 <sup>b</sup>	57 ± 8
cCOD/tCOD (%)	18 ± 9 <sup>ab</sup>	17 ± 8 <sup>c</sup>	23 ± 11 <sup>b</sup>	36 ± 9 <sup>a</sup>	18 ± 5	12 ± 8 <sup>b</sup>
sCOD/tCOD (%)	32 ± 7	32 ± 7	32 ± 8	32 ± 6	27 ± 5	31 ± 6
TSS (mg TSS/L)	77 ± 51 <sup>a</sup>	79 ± 53	65 ± 32 <sup>b</sup>	38 ± 10 <sup>ab</sup>	73 ± 22	76 ± 22 <sup>ab</sup>
NH <sub>4</sub> <sup>+</sup> (mg NH <sub>3</sub> -N/L)	30 ± 8 <sup>a</sup>	30 ± 8 <sup>c</sup>	34 ± 8 <sup>bc</sup>	20 ± 7 <sup>ab</sup>	28 ± 8	22 ± 6 <sup>b</sup>
OP (mg PO <sub>4</sub> -P/L)	1.2 ± 0.7 <sup>a</sup>	1.2 ± 0.6	1.3 ± 0.5 <sup>b</sup>	0.8 ± 0.2 <sup>ab</sup>	1.7 ± 0.6	1.6 ± 0.6 <sup>ab</sup>
Soluble volumetric loading rate (kg sCOD/m <sup>3</sup> /day)	2 ± 0.6 <sup>a</sup>	2 ± 0.7 <sup>c</sup>	1.7 ± 0.6 <sup>bc</sup>	1.2 ± 0.2 <sup>ab</sup>	1.8 ± 0.5 <sup>a</sup>	1.5 ± 0.3 <sup>a</sup>
Total volumetric loading rate (kg tCOD/m <sup>3</sup> /day)	6.4 ± 2.3 <sup>a</sup>	6.5 ± 1.9	5.4 ± 1.3 <sup>b</sup>	3.8 ± 0.6 <sup>ab</sup>	6.6 ± 1.3	5.5 ± 1.4 <sup>b</sup>
<b>Operational Conditions</b>						
MLSS (mg TSS/L)	406 ± 276	421 ± 284 <sup>c</sup>	317 ± 207 <sup>bc</sup>	355 ± 85	673 ± 230 <sup>b</sup>	496 ± 119 <sup>b</sup>
SRT <sub>20</sub> (day)	0.8 ± 0.5 <sup>a</sup>	0.7 ± 0.5 <sup>c</sup>	0.6 ± 0.4 <sup>bc</sup>	0.8 ± 0.3 <sup>ab</sup>	1.8 ± 0.7 <sup>ab</sup>	0.8 ± 0.3
Temperature (°C)	19 ± 2	19 ± 2	20 ± 1	9 ± 1	10 ± 1	12 ± 1
<b>OUR Data</b>						
OUR ratio (-)	1.3 ± 0.5 <sup>a</sup>	1.2 ± 0.5 <sup>c</sup>	1.1 ± 0.3 <sup>bc</sup>	0.7 ± 0.1 <sup>ab</sup>	0.8 ± 0.2 <sup>ab</sup>	0.9 ± 0.2 <sup>a</sup>
OUR Contactor (mg O <sub>2</sub> L <sup>-1</sup> h <sup>-1</sup> )	25 ± 15 <sup>a</sup>	25 ± 15	24 ± 10 <sup>b</sup>	10 ± 2.1 <sup>ab</sup>	10 ± 2.1 <sup>ab</sup>	24 ± 10
OUR Stabilizer (mg O <sub>2</sub> L <sup>-1</sup> h <sup>-1</sup> )	22 ± 13 <sup>a</sup>	22 ± 13	20 ± 11 <sup>b</sup>	15 ± 4 <sup>a</sup>	14 ± 3.5 <sup>a</sup>	29 ± 11 <sup>ab</sup>
<b>Clarifier Operation</b>						
SOR (m/h)	0.8 ± 0.2 <sup>a</sup>	1.1 ± 0.3	0.8 ± 0.3 <sup>b</sup>	1 ± 0.02 <sup>ab</sup>	0.91 ± 0.05	0.93 ± 0.04 <sup>ab</sup>
SLR (kg TSS/m <sup>2</sup> /d)	13 ± 3	13 ± 2	12 ± 2.3	8.7 ± 2.1	11 ± 4.4	10 ± 2.1

658 **Table 2:** Average levels of bioflocculation criteria, effluent quality, and COD mass balances for the  
659 baseline scenario (A) operated with OUR-based wasting control strategy, Scenario B with OUR-based  
660 wasting control strategy with added bioflocculation boundaries, and scenario C with a simplified version  
661 of control relying on direct waste flow control with bioflocculation boundaries. The details of the control  
662 logic are shown in Figure 3. Scenarios with statistically different parameters ( $p < 0.05$ ) share the same letter  
663 in superscript.

Characterization	Scenario					
	A			B1	B2	C
	All	Limited (85%)	Good (15%)	Dynamic OUR-based wasting control with boundaries	Dynamic OUR-based wasting control with boundaries	Wasting flow control with boundaries
<b>Bioflocculation Criteria</b>						
COD capture (%)	12 ± 15 <sup>a</sup>	7 ± 9 <sup>bc</sup>	41 ± 7 <sup>ac</sup>	31 ± 15 <sup>a</sup>	35 ± 9 <sup>a</sup>	22 ± 10 <sup>a</sup>
ESS (mg TSS/L)	58 ± 24 <sup>a</sup>	61 ± 23 <sup>c</sup>	38 ± 18 <sup>abc</sup>	17 ± 7 <sup>ab</sup>	43 ± 7 <sup>a</sup>	38 ± 8 <sup>a</sup>
TSS capture in clarifier (%)	73 ± 22 <sup>ab</sup>	79 ± 16 <sup>abc</sup>	84 ± 11 <sup>abc</sup>	95 ± 4 <sup>ab</sup>	93 ± 2.6 <sup>ab</sup>	92 ± 2 <sup>ab</sup>
<b>Effluent Characterization</b>						
tCOD (mg/L)	170 ± 53 <sup>a</sup>	170 ± 54 <sup>c</sup>	143 ± 42 <sup>bc</sup>	81 ± 14 <sup>ab</sup>	127 ± 31 <sup>a</sup>	118 ± 31 <sup>a</sup>
pCOD (mg/L)	92 ± 43 <sup>a</sup>	92 ± 43 <sup>c</sup>	66 ± 40 <sup>bc</sup>	31 ± 16 <sup>ab</sup>	80 ± 14	63 ± 25 <sup>a</sup>
cCOD (mg/L)	59 ± 19 <sup>a</sup>	59 ± 19	53 ± 17 <sup>b</sup>	11 ± 7 <sup>ab</sup>	8.3 ± 5 <sup>ab</sup>	6 ± 5 <sup>ab</sup>
sCOD (mg/L)	20 ± 16 <sup>a</sup>	20 ± 16	24 ± 14 <sup>b</sup>	38 ± 8 <sup>ab</sup>	39 ± 19	51 ± 12 <sup>ab</sup>
<b>COD mass balance</b>						
COD Effluent (Non-biomass) (% tCOD <sub>in</sub> )	28 ± 13 <sup>a</sup>	29 ± 37 <sup>c</sup>	22 ± 9 <sup>abc</sup>	29 ± 8 <sup>ab</sup>	19 ± 6 <sup>ab</sup>	22 ± 5 <sup>ab</sup>
COD redirection (% tCOD <sub>in</sub> )	46 ± 15 <sup>a</sup>	45 ± 60 <sup>c</sup>	60 ± 10 <sup>abc</sup>	50 ± 16 <sup>ab</sup>	63 ± 10 <sup>ab</sup>	48 ± 8 <sup>ab</sup>
COD oxidation (% tCOD <sub>in</sub> )	26 ± 14 <sup>a</sup>	26 ± 14 <sup>c</sup>	18 ± 10 <sup>abc</sup>	21 ± 15 <sup>ab</sup>	18 ± 10 <sup>ab</sup>	30 ± 11 <sup>ab</sup>
COD Capture efficiency (%)	24 ± 27 <sup>a</sup>	79 ± 15 <sup>c</sup>	69 ± 12 <sup>c</sup>	62 ± 19 <sup>a</sup>	55 ± 8 <sup>a</sup>	45 ± 15 <sup>a</sup>
tCOD removal efficiency (%)	66 ± 20 <sup>a</sup>	65 ± 22 <sup>c</sup>	59 ± 17 <sup>bc</sup>	51 ± 8 <sup>ab</sup>	47 ± 10 <sup>a</sup>	51 ± 12 <sup>a</sup>

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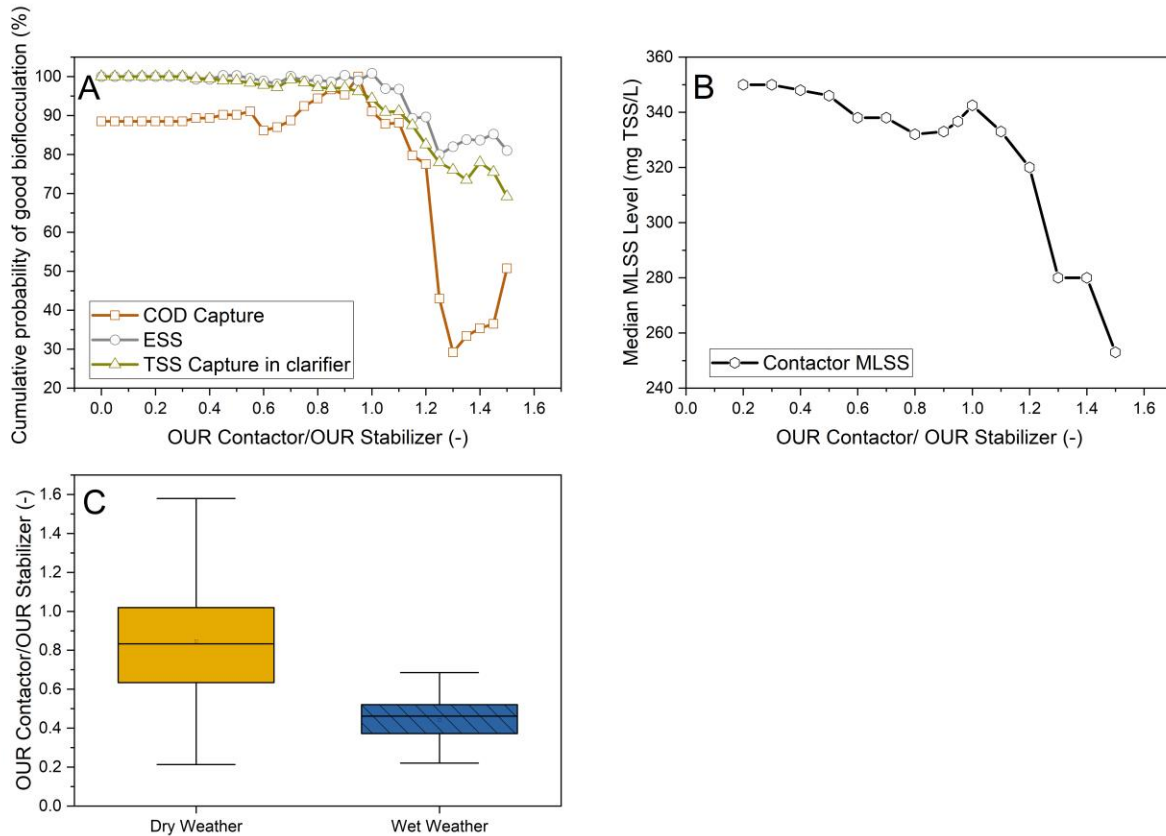
666 **Figure 1:** Average COD capture, effluent suspended solids (ESS) and TSS capture over the clarifier for the  
 667 good and limited bioflocculation data set during scenario A (OUR-based wasting control without  
 668 bioflocculation boundaries). Good and limited bioflocculation was defined by a statistical difference in  
 669 effluent quality ( $p < 0.05$ ) and TSS capture ( $p < 0.1$ ) as carbon capture exceeded 31%.

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674 **Figure 2:** A: Probability (or chance) of achieving good biofloculation criteria of COD capture > 31%,  
 675 ESS < 48 mg TSS/L and TSS capture > 78% in function of minimum OUR ratio between contactor and  
 676 stabilizer (Figure 1). B: Median MLSS levels in relation to a minimum OUR ratio level between contactor  
 677 and stabilizer. C: OUR ratio for dry and wet weather events defined by plant influent flows to the full-scale  
 678 WRRF below and above 1400602 m<sup>3</sup>/d (370 MGD), respectively.

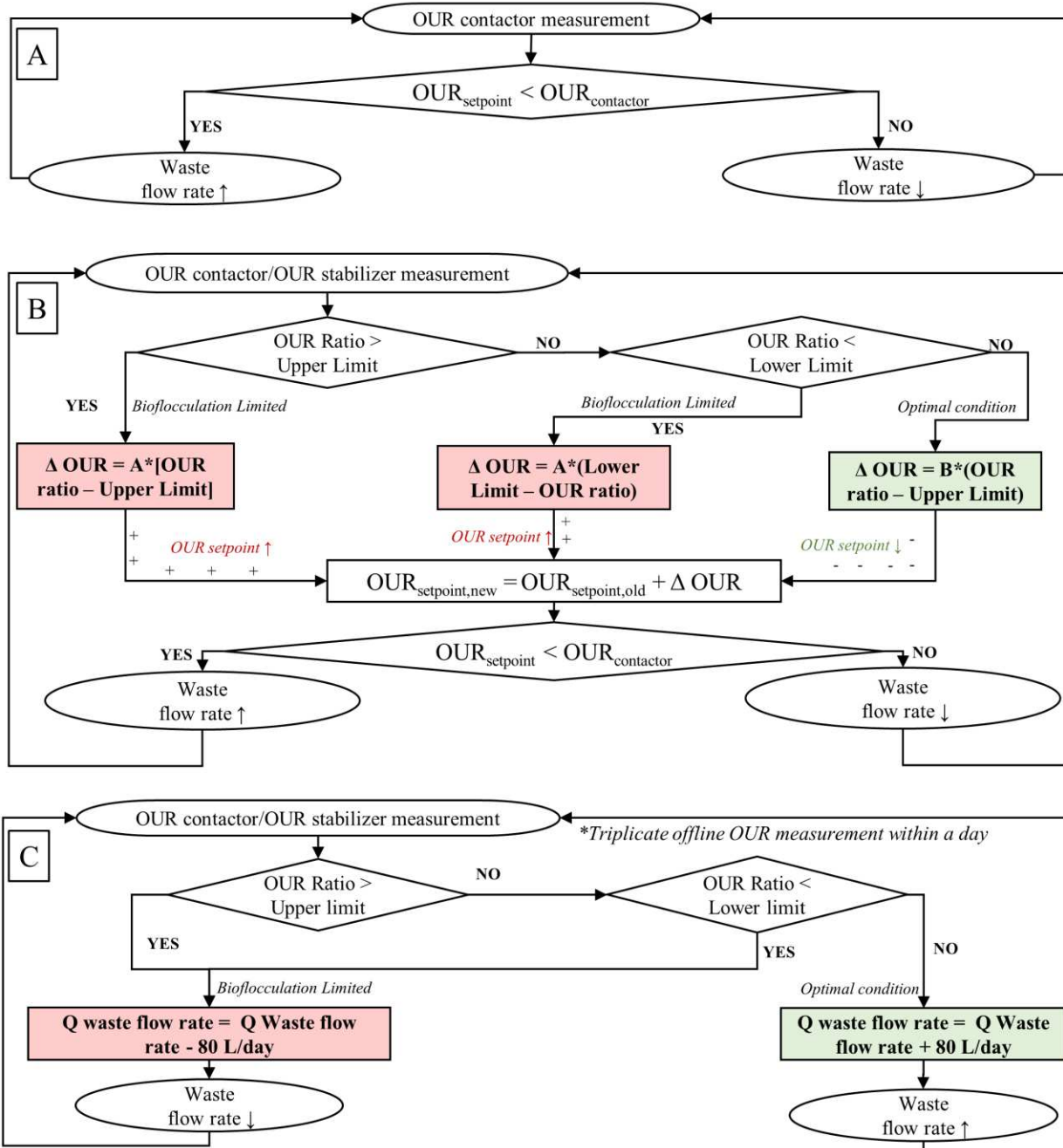
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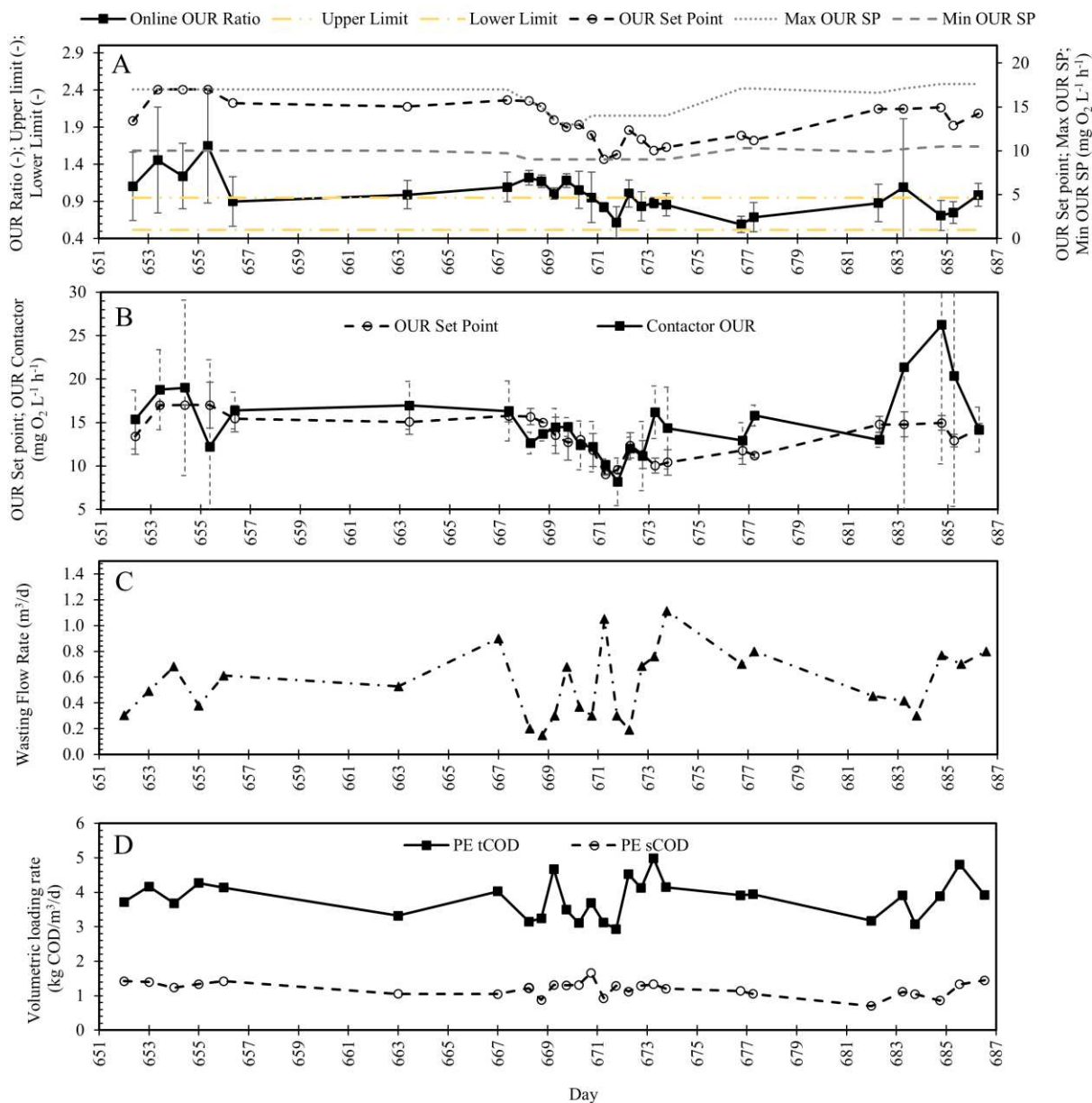
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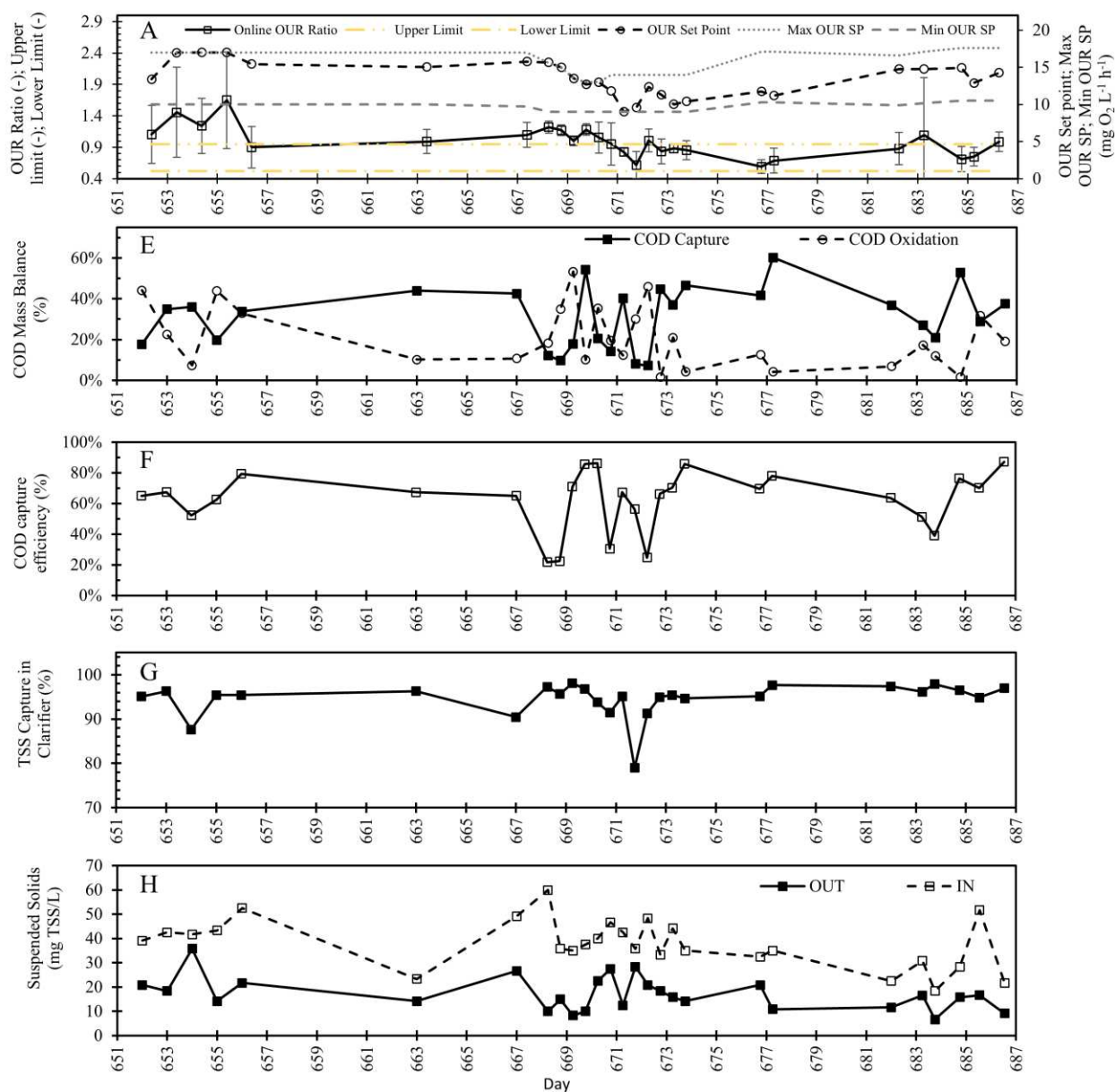
685 **Figure 3:** Scenario A was operated with OUR-based wasting control without biofloculation boundaries  
 686 (A) ( Van Winkel, 2019; Van Winckel et al., in review). Biofloculation boundaries were added into OUR-  
 687 based wasting control (B) and wasting flow control (C). Scenario C applied a stepwise change in wasting  
 688 setting rather than continuous changes as applied in scenario B. In addition, control decision were made  
 689 within 20 minutes for scenario A and B, and on daily basis for scenario C.



691

692 **Figure 4:** OUR ratio and OUR setpoint (A); OUR setpoint and contactor OUR (B); wasting flow rate (C)  
 693 and the change of volumetric loading rate (D) as a result of applying the OUR wasting control logic with  
 694 bioflocculation boundaries (see panel A), referred to as scenario B1 in Table 1 and 2. Figure 3B shows  
 695 more details on control logic.

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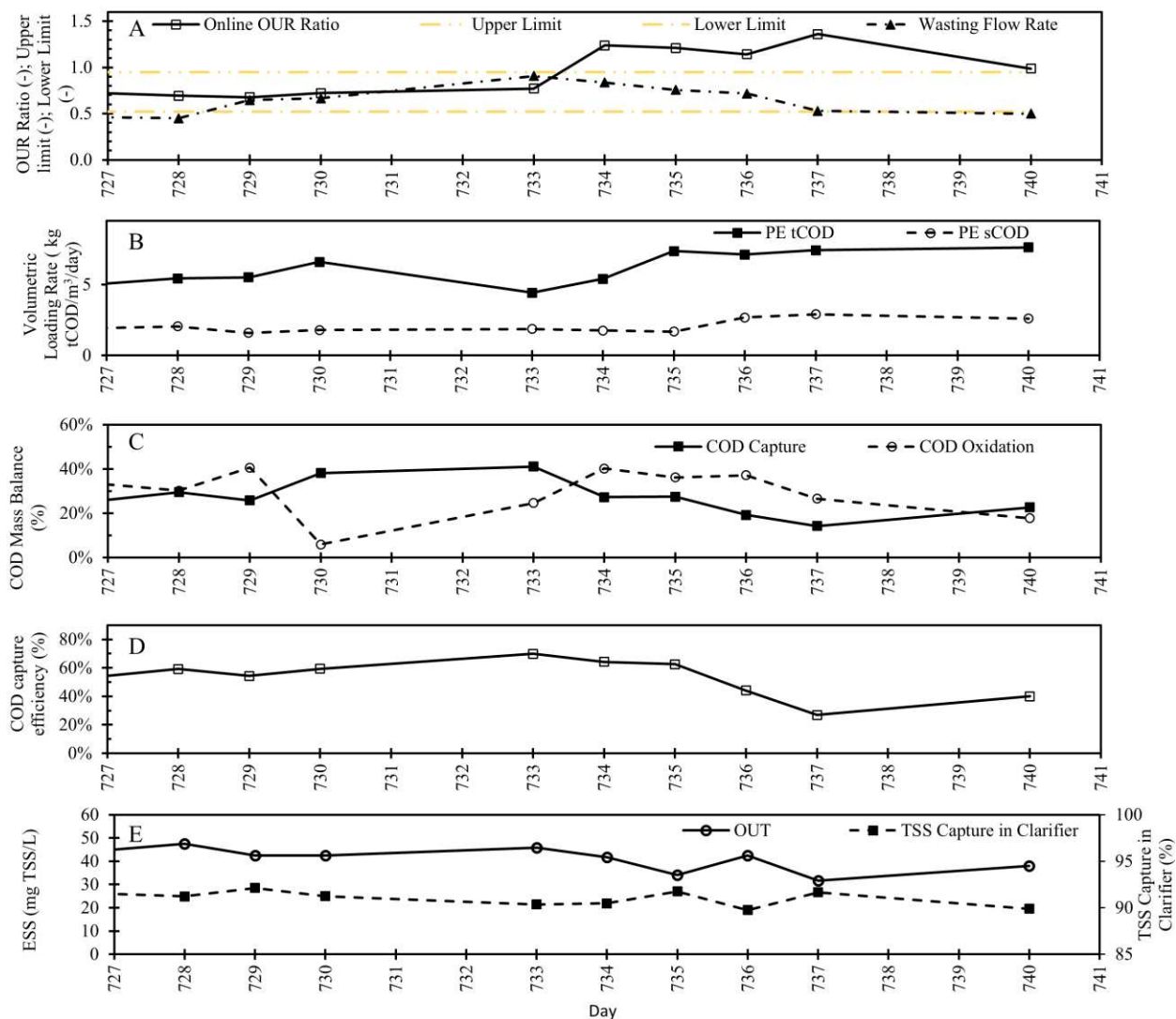
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699 **Figure 4 (continued):** OUR ratio and OUR setpoint (A); carbon oxidation and capture (E); capture  
 700 efficiency (F); TSS capture in clarifier (G), and effluent suspended solids (ESS) (H) as a result of applying  
 701 the OUR wasting control logic with bioflocculation boundaries, referred to as scenario B1 in Table 1 and  
 702 2. Figure 3B shows more detail on control logic.

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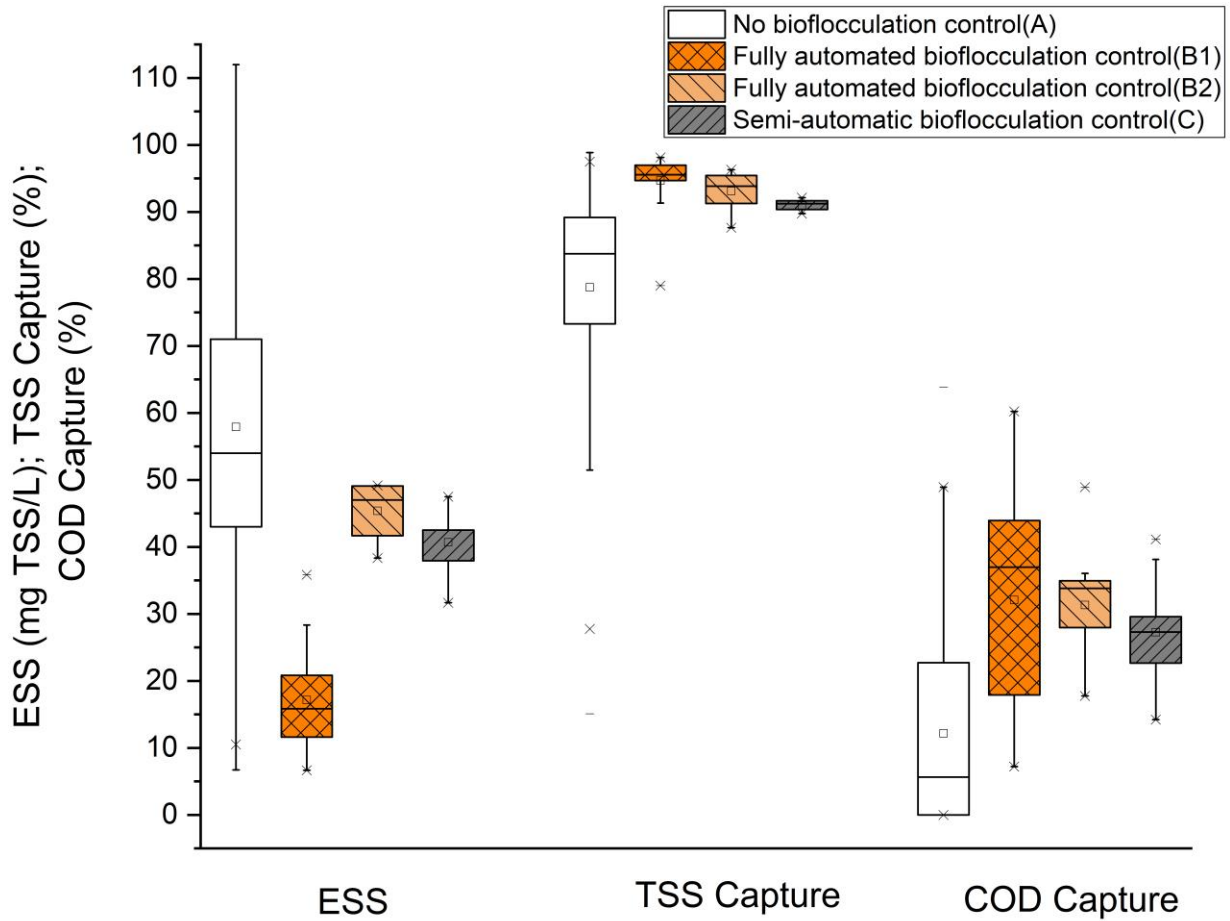


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707 **Figure 5:** Wasting flowrate manipulated by OUR ratio (A); volumetric sCOD and tCOD loading (B);  
708 carbon oxidation and carbon capture (C); capture efficiency (D); effluent suspended solids (ESS), and TSS  
709 capture in clarifier (E) as a result of applying the flow waste control logic with bioflocculation boundaries,  
710 referred to as scenario C in Table 1 and 2. Figure 3B shows more detail on control logic.

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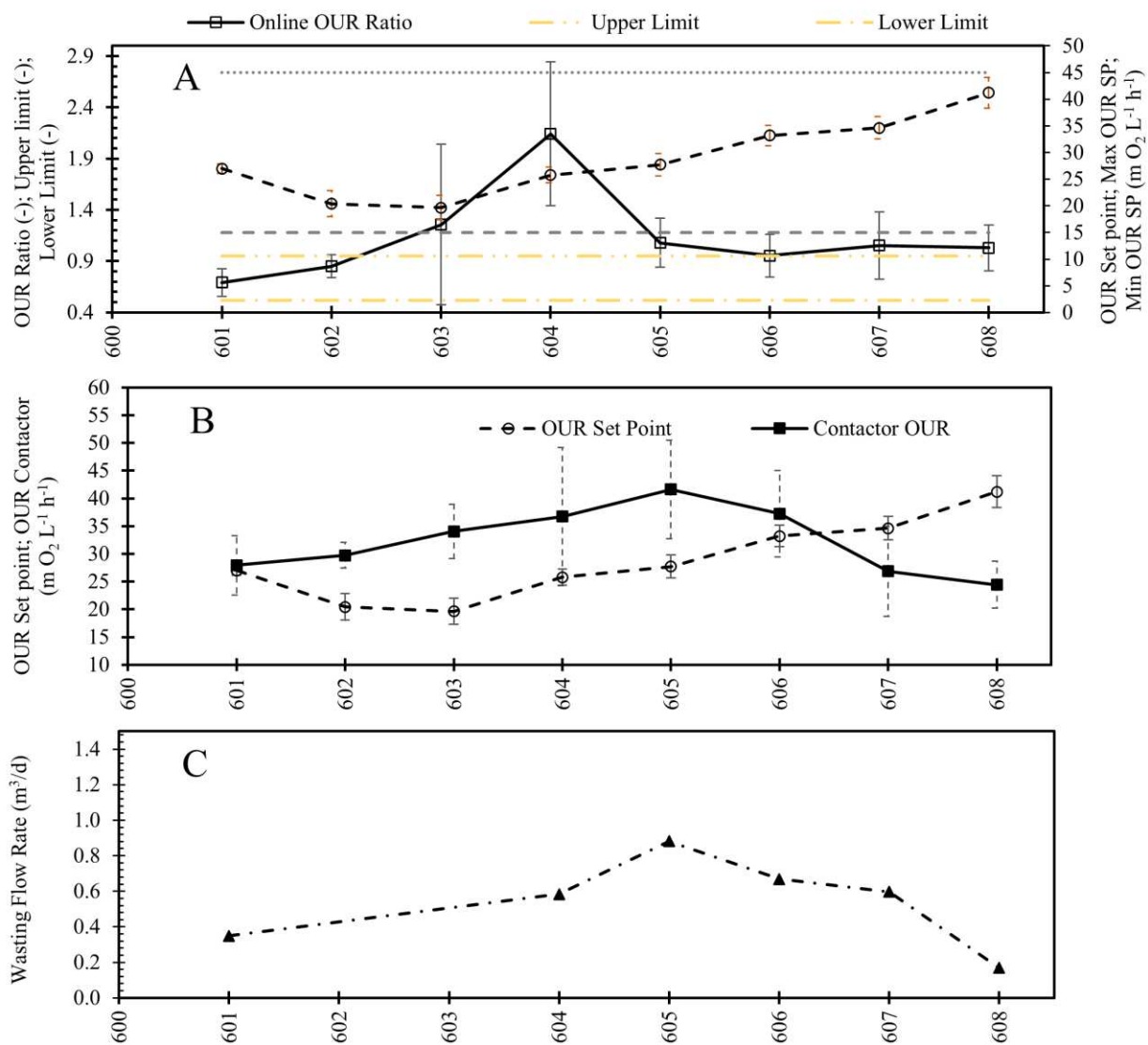
714 **Figure 6:** Summaries of all the bioflocculation criteria, including ESS, TSS capture, and COD capture for  
715 all control scenarios without bioflocculation boundaries (scenario A) and with bioflocculation boundaries  
716 (scenario B1, B2, and C). Figure 3 shows the details of the control logic and Table 1 and 2 show wastewater  
717 and operational conditions.

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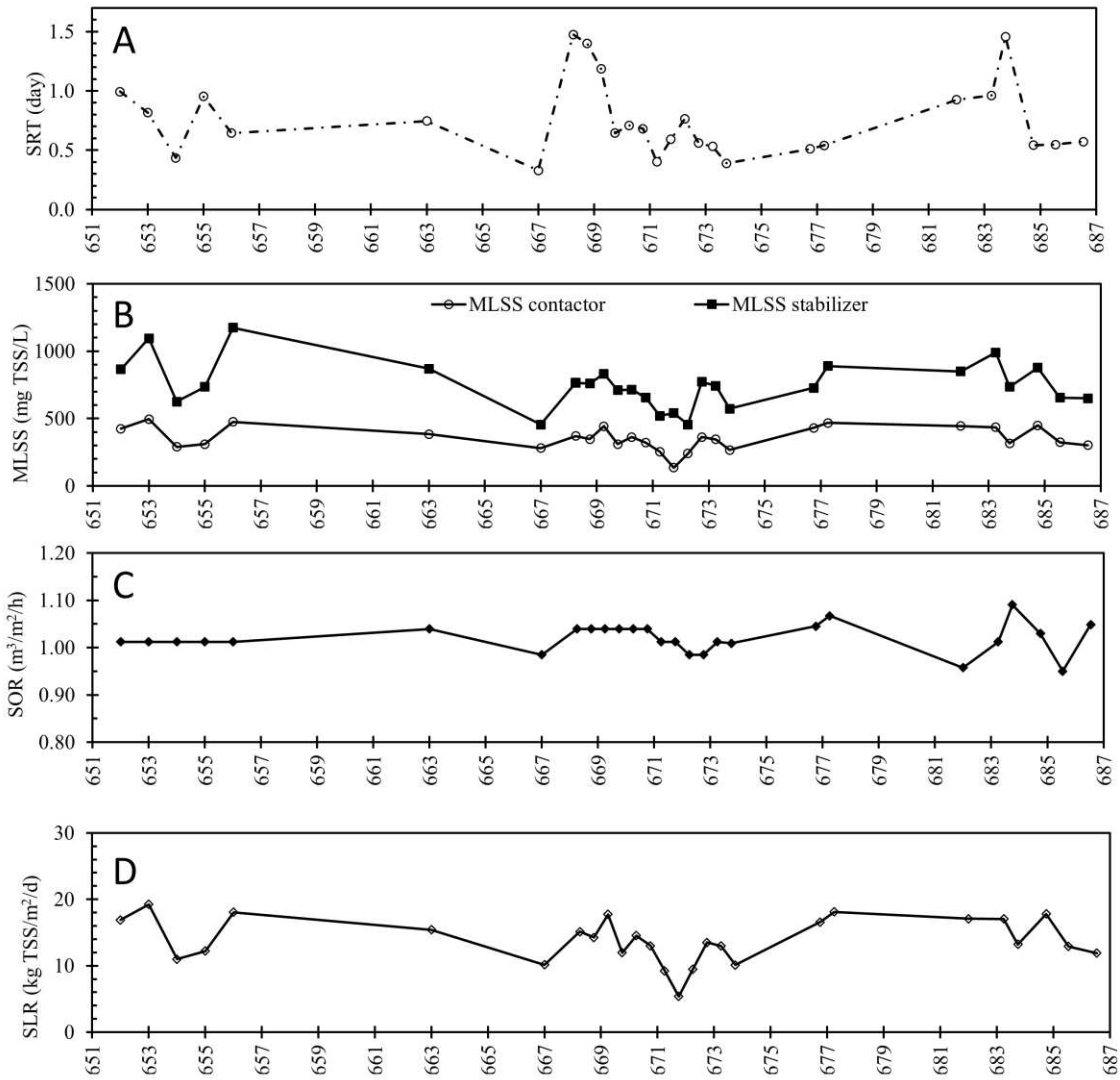
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723  
 724 **Figure S1:** A proof of principle of scenario B2 with dynamic OUR-based wasting control with  
 725 biofloculation boundaries (Figure 3B) includes: OUR setpoint driven by OUR ratio (A); OUR setpoint  
 726 and contactor OUR (B); and wasting flow rate (C).

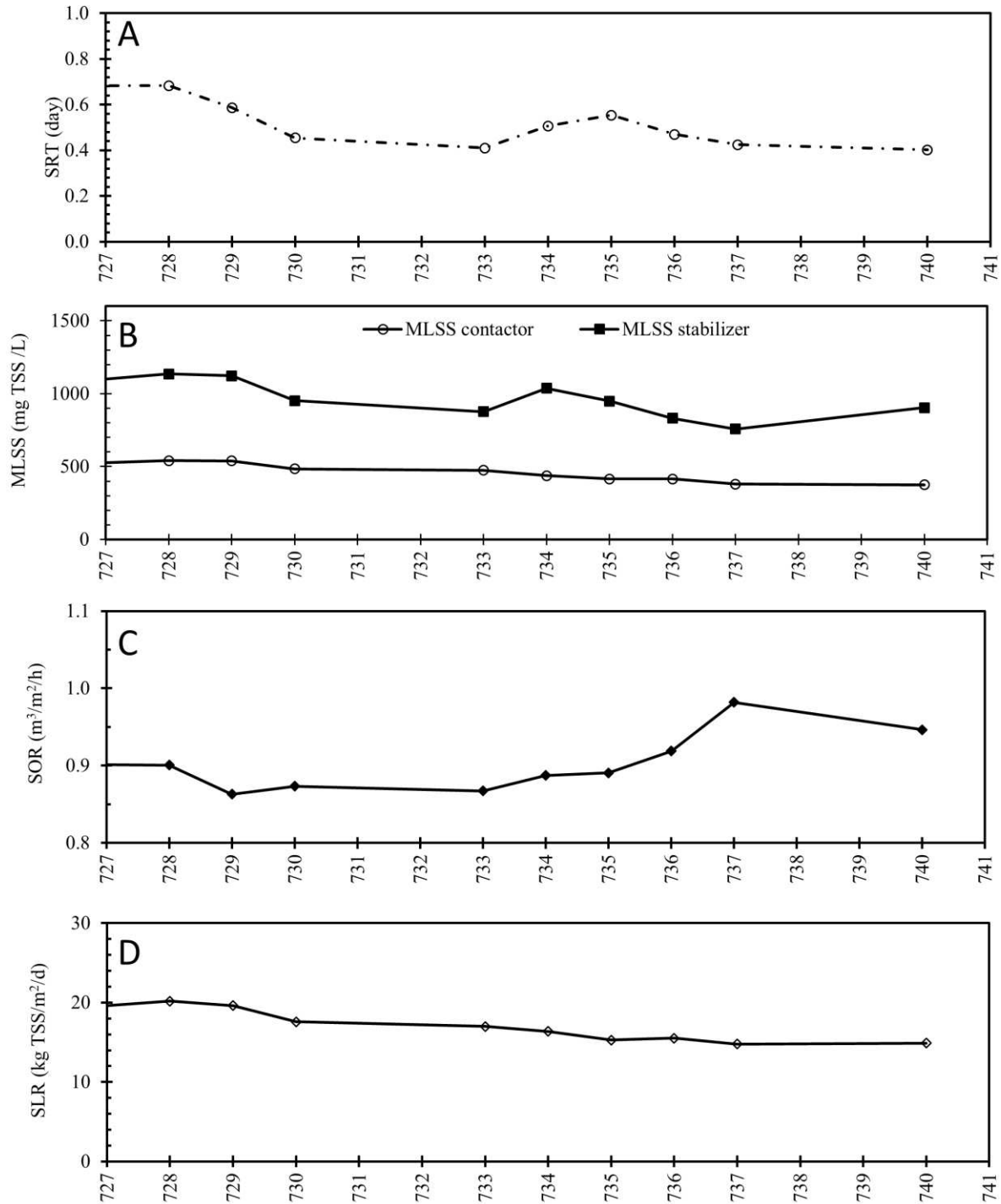


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728 **Figure S2:** Operational conditions of scenario B1 consist of sludge retention time corrected at 20<sup>0</sup>C (SRT);

729 contactor and stabilizer TSS (B); surface overflow rate (SOR) (C); and sludge loading rate (SLR) (D).

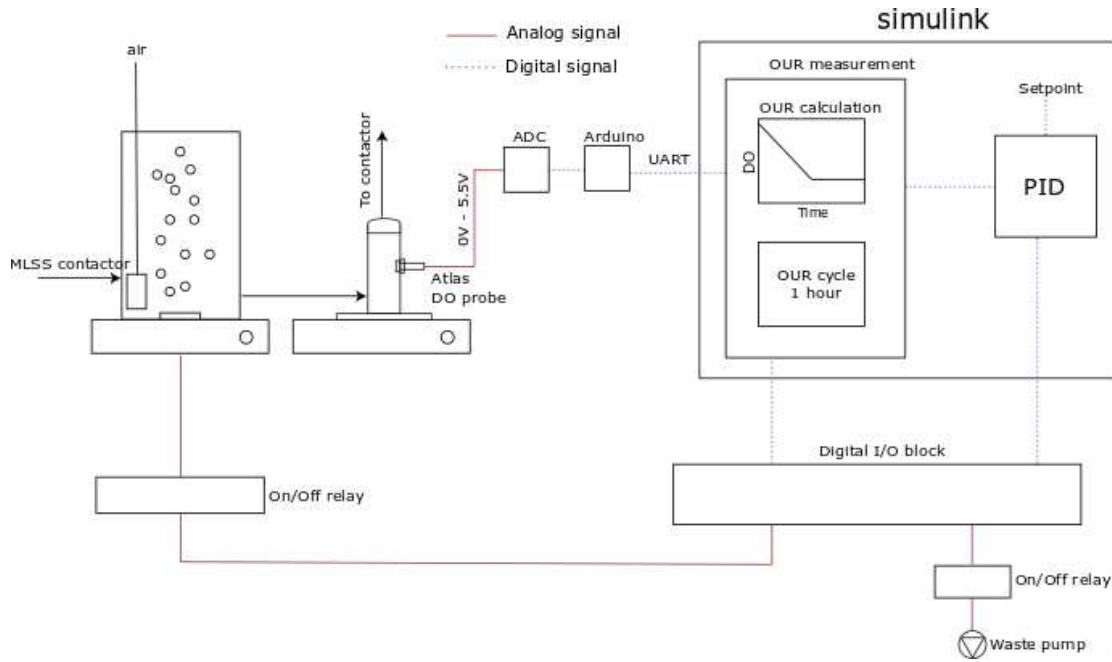




730

731 **Figure S3:** Operational conditions of scenario C consist of sludge retention time corrected at 20<sup>0</sup>C (SRT);

732 contactor and stabilizer TSS (B); surface overflow rate (SOR) (C); and sludge loading rate (SLR) (D).



733

**Figure S4.** Simplified experimental setup for the automated on-line measurement of OUR. Solid lines indicate analog signals, while dotted lines represent digital signals (Figure is adapted from Van Winkel et al. 2019).

734

$$\%COD_{redirection} = \frac{M_{COD_{particulate\ wasted}} + M_{COD_{particulate\ effluent}}}{M_{COD_{influent}}} \quad (1)$$

$$\%COD_{capture} = \frac{M_{COD_{particulate\ wasted}}}{M_{COD_{influent}}} \quad (2)$$

$$\%COD_{particulate\ effluent} = \frac{M_{COD_{particulate\ effluent}}}{M_{COD_{influent}}} \quad (3)$$

$$\%COD_{non\ biomass\ effluent} = \frac{M_{COD_{soluble\ effluent}} + M_{COD_{colloidal\ effluent}}}{M_{COD_{influent}}} \quad (4)$$

$$\%COD_{oxidation} = 100 - \%COD_{redirection} - \%COD_{non\ biomass\ effluent} \quad (5)$$

735

736 **Supplemental information S5:** Calculation details of COD mass balances over high-rate system.

737 The approach was similar to Rahman et al. (2016).