SUPPLEMENTARY INFORMATION

Effect of an increased particulate COD load on the aerobic granular sludge process: a full scale study

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Supplementary information S1. Size-distribution of the influent COD, during normal operation and during the test period.

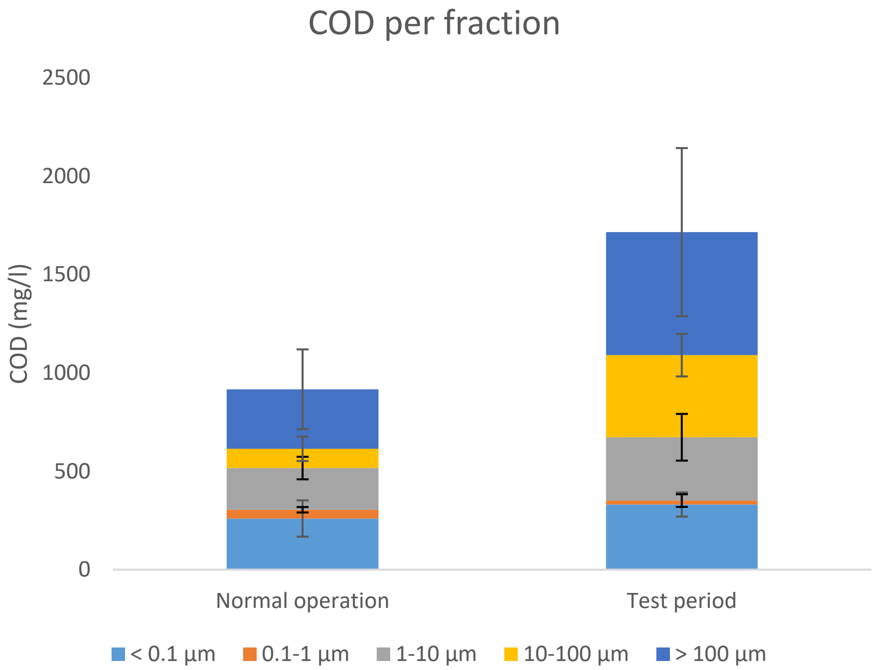

Figure S1. Distribution of the influent COD in different size fractions, during normal operation and during the test period. n=3, in each scenario. Error bars represent standard deviation between the three measurements.

Supplementary information S2. Sludge growth in the reactors during the test period and the months before and after.

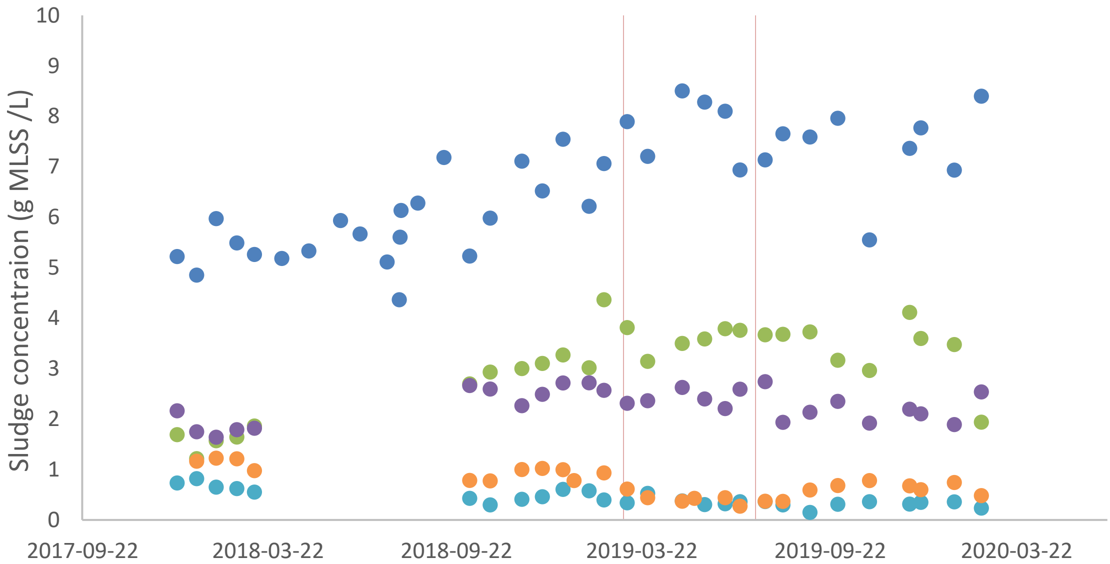

Figure S2. Mixed liquor suspended solids (MLSS) concentration of the reactors at WWTP Epe, and the concentration of different granule size fractions. The values displayed are the average TS concentration from reactors 1, 2 and 3. The granule size distribution data from the period between April and November 2018 was not available, and therefore only the MLSS concentration is plotted on that period. The sludge growth data was measured and provided by Vallei en Veluwe. The vertical red lines delimit the test period.

Supplementary information S3. Key performance indicators from reactors 1-3 of the WWTP Epe.

Figures S3.1-S3.5 show key performance indicators (KPI) recorded during each SBR cycle of the AGS reactors during a 3-year stretch of time. The data for each of the three reactors is represented in separate graphs.

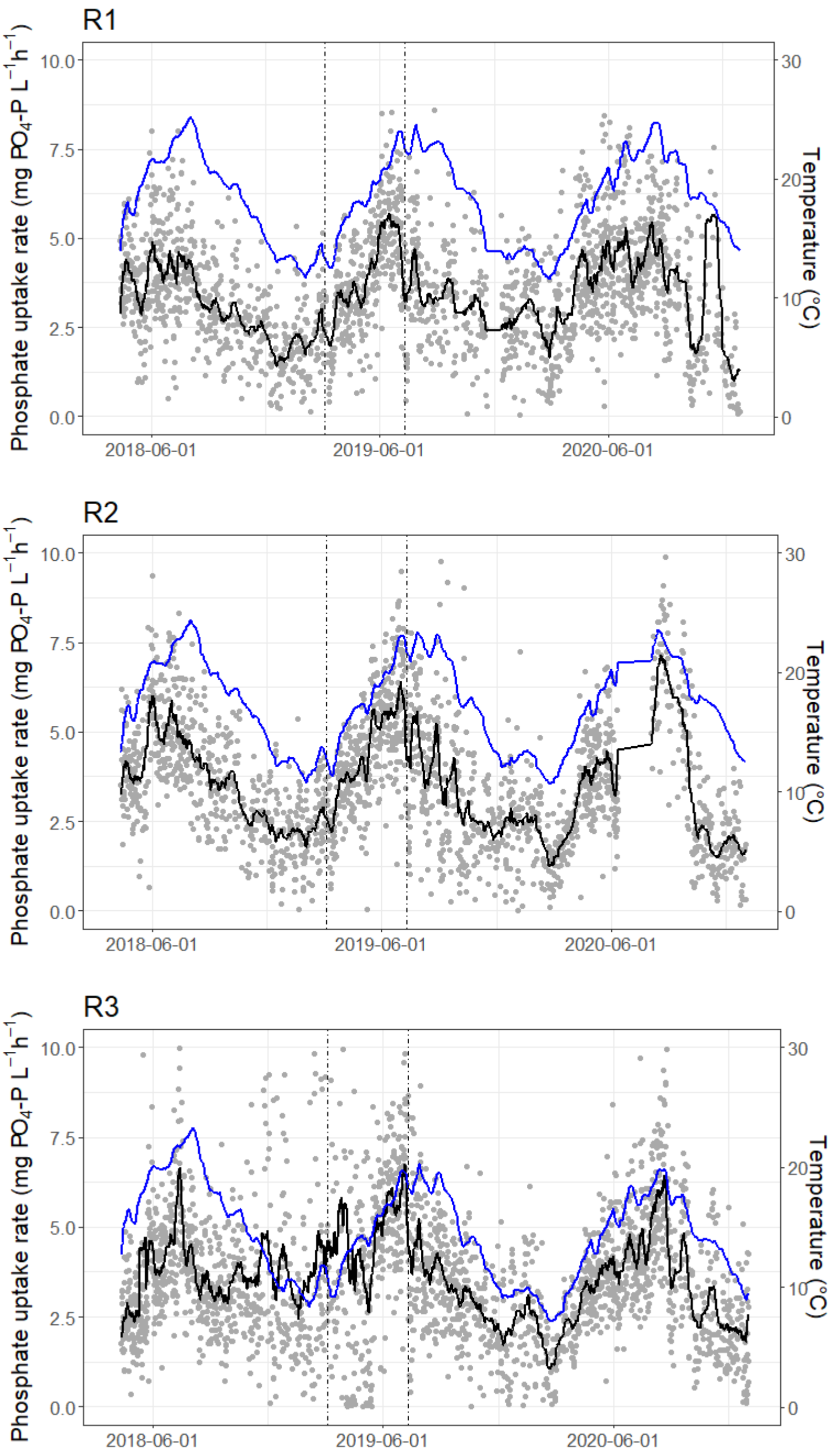

Figure S3.1. PO_3-P uptake rates measured in the reactors at WWTP Epe. Each dot represents the KPI value for a single SBR cycle, and the continuous black line represents the moving average with a sliding window of 7 days. Temperature is plotted in a blue line. The vertical lines indicate the test period, where a higher particulate COD load was fed to the reactors.

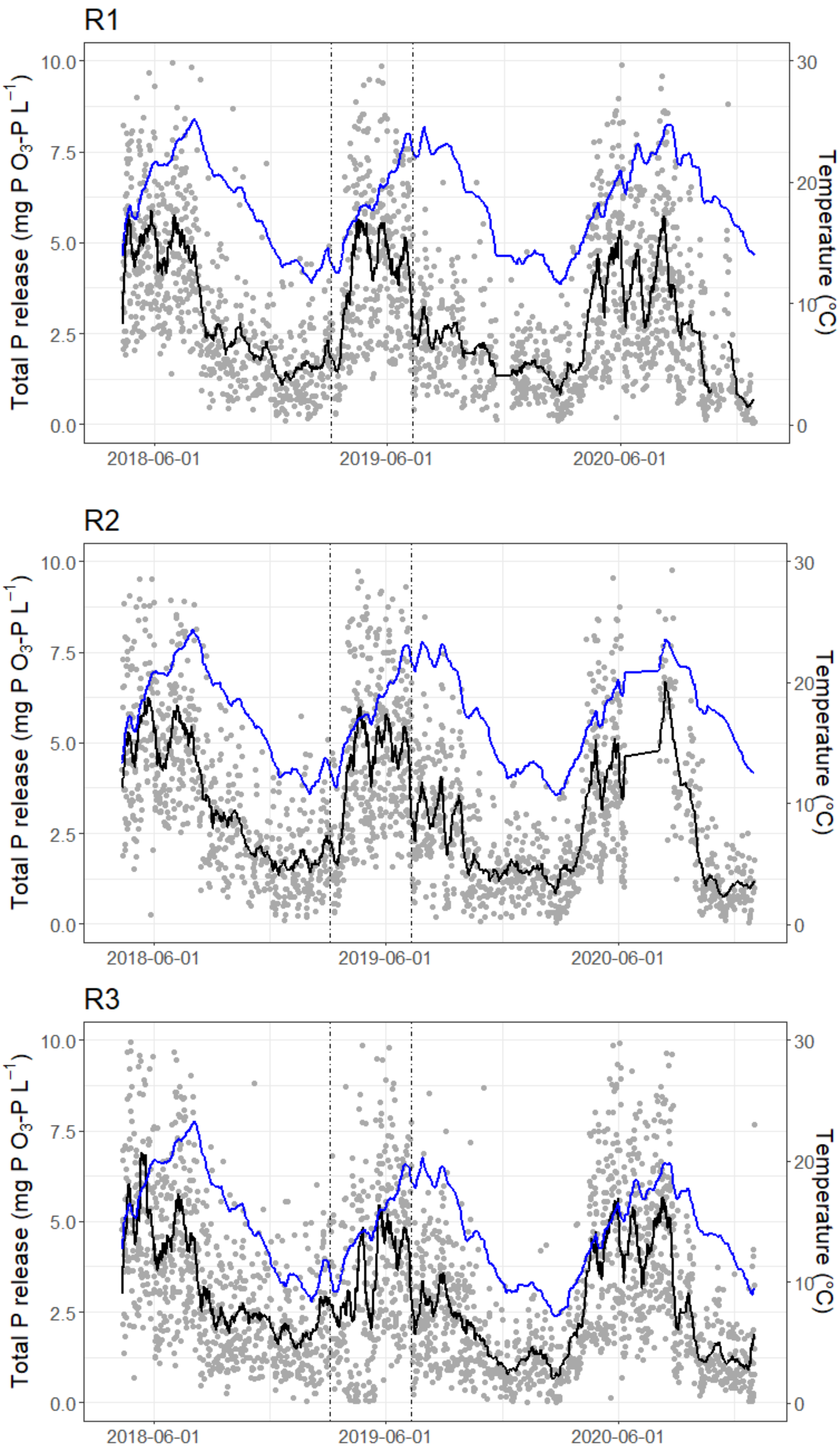

Figure S3.2. Total PO_3-P release measured in the reactors at WWTP Epe. Each dot represents the KPI value for a single SBR cycle, and the continuous black line represents the moving average with a sliding window of 7 days. Temperature is plotted in a blue line. The vertical lines indicate the test period, where a higher particulate COD load was fed to the reactors.

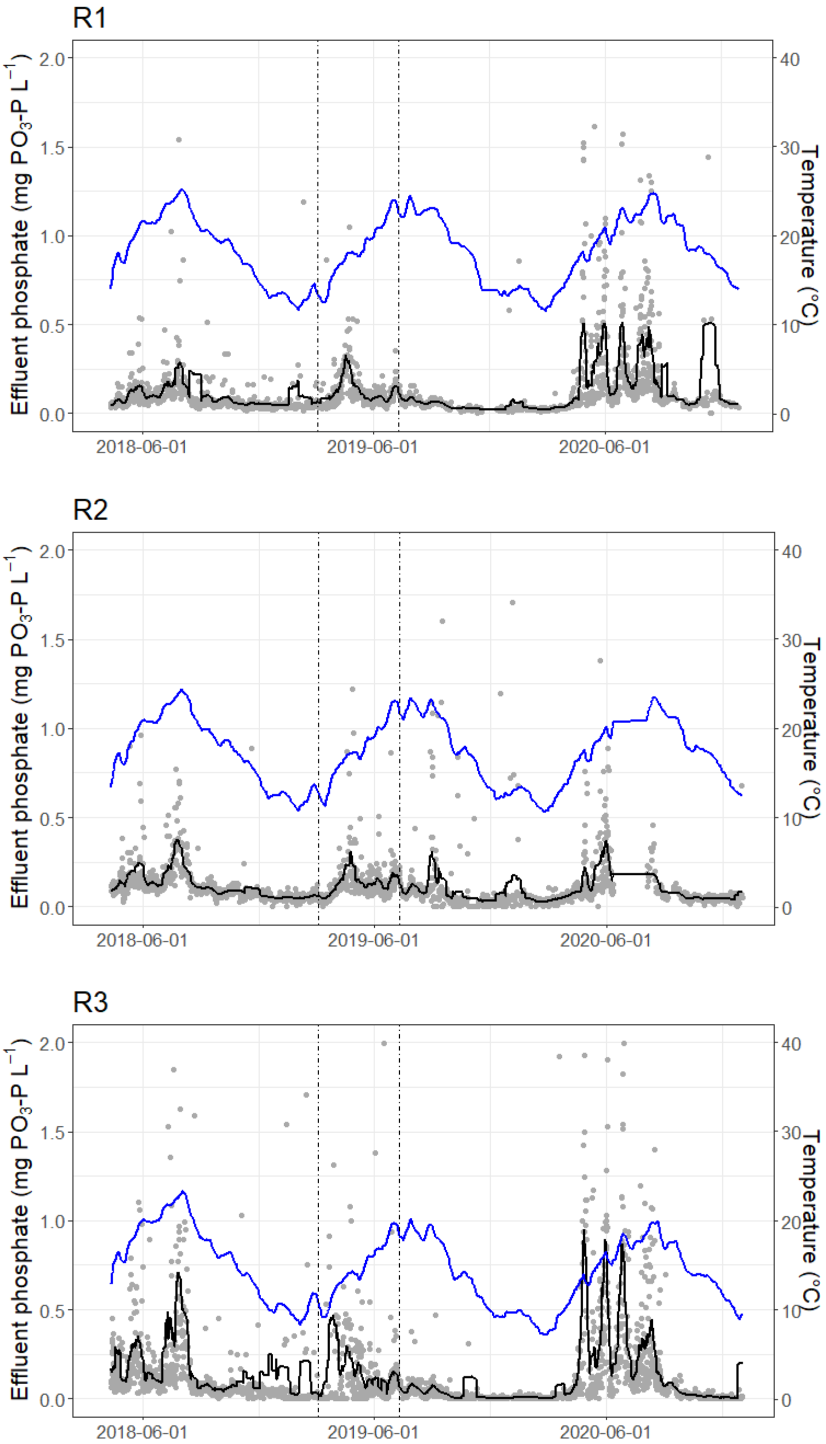

Figure S3.3. Effluent PO_3-P concentration measured in the reactors at WWTP Epe. Each dot represents the KPI value for a single SBR cycle, and the continuous black line represents the moving average with a sliding window of 7 days. Temperature is plotted in a blue line. The vertical lines indicate the test period, where a higher particulate COD load was fed to the reactors.

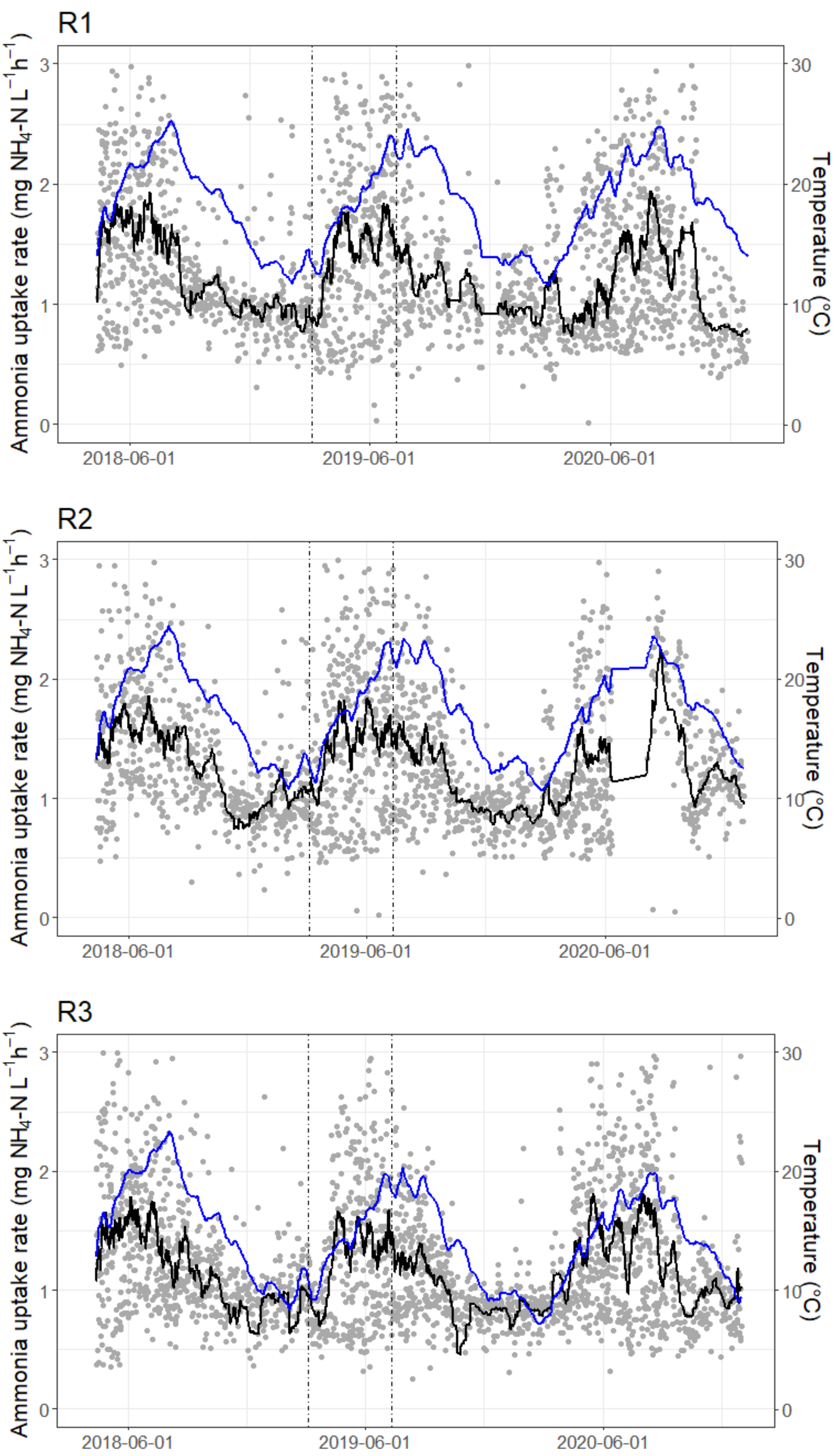

Figure S3.4. NH_4-N removal rates measured in the reactors at WWTP Epe. Each dot represents the KPI value for a single SBR cycle, and the continuous black line represents the moving average with a sliding window of 7 days. Temperature is plotted in a blue line. The vertical lines indicate the test period, where a higher particulate COD load was fed to the reactors.

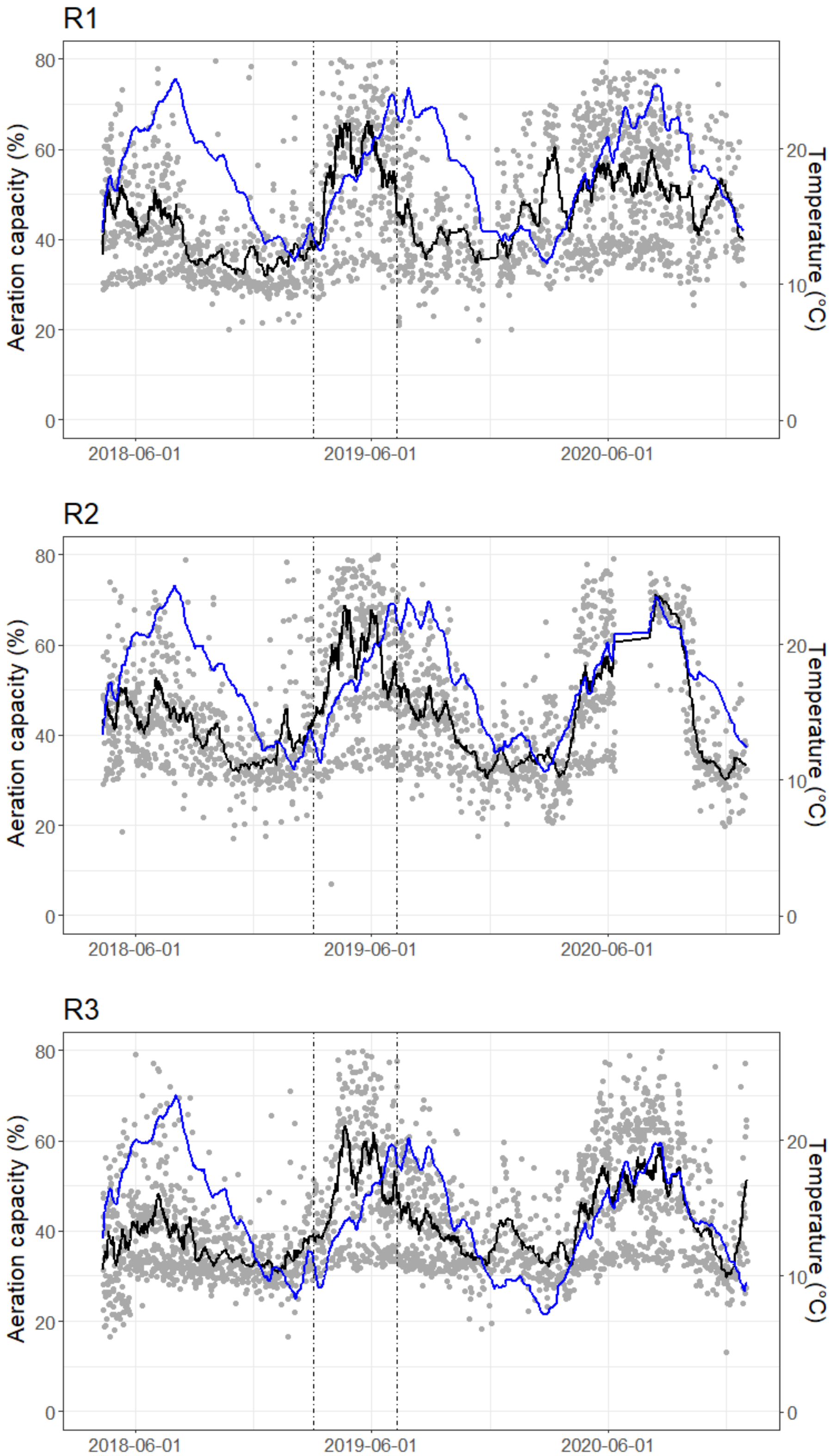

Figure S3.5. Aeration capacity used in the reactors at WWTP Epe. Each dot represents the KPI value for a single SBR cycle, and the continuous black line represents the moving average with a sliding window of 7 days. Temperature is plotted in a blue line. The vertical lines indicate the test period, where a higher particulate COD load was fed to the reactors.

Supplementary information S4. Calculation of total hydrolytic activity during feeding.

The total hydrolytic activity during feeding is calculated by multiplying the specific hydrolytic activities measured in the enzyme assays by the weight of the settled sludge layer that is passed by the influent during plug-flow feeding. The volume of sludge "filled" with influent during feeding is considered to be the same as the feed batch volume. The total settled sludge bed volume is larger than the batch volume (Table S4.1), i.e. the volume fed per SBR cycle is smaller than the total settled sludge volume, which leads to the assumption that only large granules (at the bottom of the reactor) will be in contact with the influent during the plug-flow feeding . Hence, only large granule hydrolytic activity of the mass of sludge filled with influent is used to calculate the anaerobic hydrolysis during feeding. That is, the influent fed granule volume (=batch volume) divided by the SVI_5.

Table S4.1. Reactor sludge composition and SBR cycle characteristics, during normal plant operation.

TS (g/L)	6.4
VS (g/L)	4.9
SVI5 (mL/g VS)	45
Reactor vol (m^3)	4500
Sludge weight (kg VS)	22078
Settled sludge bed volume (m^3)	994
Q (m^3/d)	5066
Batch volume/SBR cycle (m^3)	422
Granule mass in contact with influent (kg)	9381
Specific protease activity large granules	26.77 mg protein g VS^{-1} h^{-1}
Specific protease activity mixed sludge	87.63 mg protein g VS^{-1} h^{-1}
Specific lipase activity large granules	8.13 mg lipid g VS^{-1} h^{-1}
Specific lipase activity mixed sludge	68.52 mg lipid g VS^{-1} h^{-1}

The bed volume filled with influent is divided in 10 segments, the activity of which is accounted for only once they are filled with influent, during a 2 hour plug-flow feeding. In table S4.2, the time during which each of the segments is active is displayed. Zero order kinetics is assumed for simplicity (substrate concentration>K_s) It also shows the total activity contributed by that segment during the feeding time (its specific activity * the granule mass in that segment * time active).

Table S4.2. Hydrolytic activity contributed by each segment during feeding, during normal plant operation.

	Active (h)	Protease activity contributed (g protein)	Lipase activity contributed (g lipid)
segment 1	2	50233	15256
segment 2	1.8	45210	13730
segment 3	1.6	40187	12205

segment 4	1.4	35163	10679
segment 5	1.2	30140	9154
segment 6	1	25117	7628
segment 7	0.8	20093	6102
segment 8	0.6	15070	4577
segment 9	0.4	10047	3051
segment 10	0.2	5023	1526

Table S4.3. Comparison of the lipid and protein content in the influent (during normal operation and test period), and hydrolytic activity during feeding during normal plant operation.

	Protein	**Lipid**
Total activity in 2 h feeding (g substrate hydrolysed)	276282	83908
Influent substrate concentration hydrolysed in 2h feeding (mg/L)	654	199
Influent substrate concentration normal operation	76	45
Influent substrate concentration test period	135	290

As shown in Table S4.3, the sludge has enough hydrolytic capacity to (theoretically) hydrolyse all the influent proteins within the anaerobic feeding phase, both during normal operation and during the test period. However, the increased influent lipid concentration during the test period is higher than the sludge can hydrolyse during the feeding phase. Thus, the aerobic hydrolysis is estimated to assess whether the aerobic phase would allow complete lipid hydrolysis. During aeration, all the reactor sludge is in contact with the influent due to fully mixed conditions. Therefore, the specific activity of the mixed sludge is used for the calculations. Even if the assays only quantified anaerobic hydrolytic activity, the results were extended to aerobic conditions. This assumption was based on previous studies that proved hydrolysis rates to be unaffected by short-term (one SBR cycle) changes of redox conditions, when the hydrolytic activity was cell or biofilm associated [1, 2]. Considering the hydrolytic activity of the sludge in normal operation (68.52 mg lipid g VS^{-1} h^{-1}), and the sludge mass in the reactor (22078 kg VS) the hydrolytic activity during aeration would be 1370974 g lipid/h, or if divided by the batch volume (422 m^3), 305 mg/L of influent lipids per hour of aeration. Therefore, the reactor has the capacity to hydrolyse the lipids remaining after the anaerobic feeding phase within the first hour of aeration.

Supplementary information S5. Comparison of estimated and observed sludge production.

Sludge production was estimated from the COD and TN load to the reactors, and compared to the observed sludge production in order to explore the fate of the pCOD added in the test period. Equation 1 estimates the production of heterotrophic and nitrifying biomass, based on influent substrate (COD) and nitrogen [3]:

$$P_{X,VSS} = \frac{QY(S_i - S)}{1 + k_d SRT}(1 + f_d k_d SRT) + \frac{QY_n(NO_x)}{1 + k_{dn} SRT} + QnbVSS \quad [1]$$

where:

$P_{X,VSS}$ = Net waste sludge produced per day [kg VSS d^{-1}]

Q = Influent flow [m^3 d^{-1}]

Y = Biomass yield [kg VSS kg^{-1} COD]

Y_n = Nitrifying biomass yield [kg VSS kg^{-1} COD]

S_i = influent substrate concentration [kg COD m^{-3}]

S = effluent substrate concentration [kg COD m^{-3}]

NO_X = concentration of NH4-N in the influent that is nitrified [kg N m^{-3}]

k_d = endogenous decay coefficient [d^{-1}]

k_{dn} = endogenous decay coefficient for nitrifying organisms [d^{-1}]

SRT= theoretical average solids retention time [d]

f_d = fraction of biomass that remains as cell debris

nbVSS= non-biodegradable influent suspended solids [kg VSS m^{-3}]

Sludge production was estimated considering biodegradable COD (bCOD) as substrate. Biodegradable COD was estimated from BOD, applying a factor of 1.6 (bCOD = 1.6 x BOD_5) [3]. Since the non-biodegradable influent suspended solids (nbVSS) concentration was not known, sludge production was predicted based only on heterotrophic and nitrifying biomass growth, therefore excluding the last component of Eq. 1 (Q x nbVSS). nbVSS was then estimated from the difference between the prediction and the observed sludge production. The values used in the calculation are listed in Table S5.1.

Table S5.1. values used in the prediction of sludge growth based on influent composition.

	Normal operation	**Test period**	**Reference**
Q [m^3 d^{-1}]	4696	5229	This study
tCOD [g COD m^{-3}]	840	1456	This study
BOD_5 [g BOD m^{-3}]	341	537	This study
$BOD_{5,eff}$ [g COD m^{-3}]	1,5	2,4	This study
bCOD [g COD m^{-3}]	546	859	Calculated, this study
$bCOD_{eff}$ [g COD m^{-3}]	2.4	3.8	Calculated, this study
TN [g N m^{-3}]	77	101	This study

NO_X [g N m^{-3}]	62	81	Calculated, this study
k_d [d^{-1}]	0.12		[3]
k_{dn}[d^{-1}]	0.08		[3]
f_d	0.15		[3]
SRT [d]	25		[4]
Y [kg VSS kg^{-1} COD]	0.4		[3]
Y_N [kg VSS kg^{-1} N]	0.12		[3]

The concentration of NH_4-N in the influent that is nitrified (NO_X) was assumed to be 80% of the TN. Based on the calculation above, the predicted sludge production is 381 kg VSS d^{-1} during normal operation, and 665 kg VSS d^{-1} in the test period. The actual sludge production, considering both the increase in reactor VSS concentration and spill production, was 1,146 kg VSS d^{-1} during normal operation and 1,495 kg VSS d^{-1} during the test period. From the difference between the prediction and the observed sludge production, an influent nbVSS concentration of around 160 g VSS m^{-3} was calculated for both situations.

It must be noted that several assumptions are made in the prediction of sludge production. For instance, the theoretical average SRT of our study was not measured, and it might not be the same for the normal operation and the pilot period, which would influence the prediction. Moreover, the uncertainty of influent composition measurements decreases the accuracy of the values used in the balance. For example, instead of using different influent flows during normal operation and test period, an average flow of 4.808 m^3 d^{-1} could be considered for both situations, since the difference in flow was not significant between periods. In that case, the calculated nbVSS would be 157 g VSS m^{-3} during normal operation and 184 g VSS m^{-3} during the test period. This would be more in agreement with the higher nbCOD (tCOD-bCOD) during the pilot. This example illustrates that the limitations associated to full-scale monitoring do not allow to accurately estimate the fraction of pCOD consumed in the reactor during the higher load period. However, using typical growth and decay values it is possible to make a prediction of the sludge production that reasonably matches the observations. The prediction shows that at least some of the additional particulates from the slaughterhouse influent have to be degraded to explain the observed sludge production. With a higher nbVSS concentration, a considerably higher sludge production would be expected.

References

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