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High-rate activated sludge systems combined with dissolved air flotation enable effective organics removal and recovery

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Abstract

High-rate activated sludge (HRAS) systems typically generate diluted sludge which requires further thickening prior to anaerobic digestion (AD), besides the need to add considerable coagulant and flocculant for the solids separation. As an alternative to conventional gravitational settling, a dissolved air flotation (DAF) unit was coupled to a HRAS system or a high-rate contact stabilization (HiCS) system. The HRAS-DAF system allowed up to 78% removal of the influent solids, and the HiCS-DAF 67%. Both were within the range of values typically obtained for HRAS-settler systems, albeit at a lower chemical requirement. The separated sludge had a high concentration of up to 47 g COD L^{-1}, suppressing the need of further thickening before AD. Methanation tests showed a biogas yield of up to 68% on a COD basis. The use of a DAF separation system can thus enable direct organics removal at high sludge concentration and with low chemical needs.

Keywords

Adsorption/Bio-oxidation system; Dissolved air flotation (DAF); High-rate activated sludge (HRAS) system; Sludge thickening; Waste activated sludge (WAS);

1 Introduction

Municipal wastewater is produced in vast quantities, today representing an unused source for water, energy, organics and nutrients (Verstraete et al., 2009). Municipal wastewater is typically treated in a conventional activated sludge (CAS) system. The goal is to create clean water for discharge or reuse by removing organic carbon, nitrogen and phosphorus, with minimal waste sludge formation. To improve the recovery of organics from wastewater, a two-stage adsorption/bio-oxidation system (AB system) can be implemented instead of CAS (Versprille et al., 1985; Boehnke et al., 1997). The primary treatment stage of an AB system is an aerobic A-stage for partial carbon removal. The A-stage operates as a high-rate activated sludge (HRAS) process, with sludge-specific loading rates between 2 and 10 kg biodegradable chemical oxygen demand per kg of volatile suspended solids per day (kg bCOD kg VSS^{-1} d^{-1}). This is followed by a lowly loaded B-stage to ensure nitrogen removal and final polishing (Boehnke et al., 1997). Compared with CAS systems, HRAS systems are operated at a short hydraulic retention time (HRT, 30–60 min) and solids retention time (SRT; < 2 days). This results in a low degree of organics mineralization, so that the majority of the organics are redirected to the sludge via biological flocculation, sorption and storage mechanisms (Boehnke et al., 1997; Jimenez et al., 2015; Meerburg et al., 2015). The produced A-sludge is well suited for organic resource and energy recovery, given its high biodegradability, with a conversion efficiency to CH_4 of 60% to even up to 70% during anaerobic digestion (AD) (Van Haandel and van der Lubbe, 2007; De Vrieze et al., 2013). Theoretically, it is possible to achieve energy-neutral wastewater treatment through the AB system combined with AD of the A-stage sludge (Constantine et al., 2012). Compared with physical-chemical primary treatment, HRAS systems offer the advantage that they can effectively remove soluble organics as well. In full-scale AB plants treating domestic wastewater, the A-stage typically removes around 45–68% of TSS, and 45–74% of COD (de

Graaff et al., 2016; Ge et al., 2017; Trzcinski et al., 2016). However, sludge produced at high loading rates and short SRTs suffers from poor settling characteristics. In case gravitational settling is used, this may result in sub-optimal solid/liquid separation (Modin et al., 2014; Ramalho, 2013). Typically, $FeCl_3$ is added to improve sludge settleability, and achieve phosphorus removal at the same time (Jiang and Graham, 1998; de Graaff et al., 2016). Still, even with the addition of $FeCl_3$, A-stage systems currently only recover between 24–48% of the organic carbon in sewage as sludge (de Graaff et al., 2016). Due to low compaction during the settling process, the concentration of A-sludge is often limited to 0.5–1.5% solids (Cagnetta et al., 2016), and further thickening is required prior to AD, necessitating the addition of polyelectrolytes.

To advance towards an energy-neutral or even energy-positive wastewater treatment, it is necessary to maximize recovery of sewage organics. For this purpose, Meerburg et al. (2015) proposed the use of a high-rate contact stabilization (HiCS) system as an improvement of the HRAS system. This system creates a feast-famine regime in the A-stage, which was shown to improve bio-flocculation, bio-sorption and storage of the organics, increasing the overall recovery of organics into sludge (Meerburg et al., 2015). Alternative technologies include the retention and concentration of A-sludge via the use of a membrane bioreactor type HRAS (HR-MBR), producing a solids-free effluent (Akanyeti et al., 2010; Faust et al., 2014). Similar to other HRAS sludge types, thickening of the HiCS sludge and HR-MBR sludge is however still required before AD, which may lead to further bCOD loss.

This work proposes to improve the separation of A-stage sludge and increase its solids concentration by means of dissolved air flotation (DAF). The DAF process relies on flotation of (sludge) particles via hydrophobic interactions with micro-bubbles produced by supersaturating the liquid with a gas, typically air (Haarhoff, 2008; Wang et al., 2005). To achieve efficient solid/liquid separation in a DAF unit, dosage of coagulants ($FeCl_3$, $AlCl_3$ or

polyelectrolytes), and in some cases also flocculants, is necessary to effectively neutralize negative charges on the sludge particles, so they can form larger aggregates (Liu and Fang, 2003). Treatment of municipal wastewater with DAF in combination with an aerated contact tank has already been tried in limited cases. Such systems can remove up to 81% of the influent TSS and 67% of the COD, using waste activated sludge (WAS) generated in the downstream secondary treatment as inoculum for the contact tank (Ding et al., 2015). DAF has also been used to thicken secondary CAS sludge, and offers multiple benefits compared to a settler, *e.g.*, lower hydraulic retention times, lower volumetric requirements, better solids separation, and increased thickening of the sludge up to 7% solids (Bolto and Gregory, 2007; Bratby and Marais, 1976; Wang et al., 2005). Because of the high solids concentration in the sludge, a DAF system may suppress the need of a sludge thickening step prior to AD and may thus replace the combination of settler and thickener, leading to economic advantages (Ding et al., 2015; Wang et al., 2005).

In this work, it was hypothesized that a HRAS/HiCS system can be successfully used in combination with DAF to improve the solid/liquid separation of A-stage systems to increase the sludge concentration to a level competitive to the combination of gravity settling and thickening. To evaluate the use of HRAS/HiCS-DAF as a primary treatment step (A-stage) in municipal wastewater treatment, the following was assessed in a pilot-scale reactor setup: (1) removal of organic matter, (2) degree of concentration of the DAF sludge, and (3) biogas conversion of the sludge with AD. An assessment on nitrogen removal from the DAF effluent (B-stage) is also given.

2 Materials and Methods

2.1 Pilot-scale high-rate activated sludge system combined with dissolved air flotation setup

The pilot-scale HRAS-DAF consisted of a cylindrical contact tank (A-stage) with a diameter of 1.6 m and a working volume of 2 m^3, connected to a 2 m^3 DAF unit (Nijhuis Water and Technology, The Netherlands) (Figure 1). Municipal wastewater (Table 1) was collected from the wastewater treatment plant (WWTP) of Aartselaar (Belgium), after grit removal and a sand trap. It was filtered in a drum filter to remove large particles (1 mm pore size), and fed to the contact tank at a flow rate of 2 m^3 h^{-1}. The contact tank was continuously mixed and aerated by means of fine-bubble aeration at a dissolved oxygen set point between 1.5 and 2.5 mg L^{-1}. The mixed liquor (ML) was transferred to the DAF unit with a cavity pump. The coagulant was dosed in the ML right before the cavity pump, and the polymer(s) was/were added in the loop of the flocculator pipes that connected the contact tank to the DAF. Pressurized air (7 bar) was added at a flowrate of 0.6 L min^{-1} to a pressurized internal recycle stream of the DAF effluent (4–5 bar). This so-called "white water" was injected into the feed pipe of the DAF unit at various points in the piping system (see below) at atmospheric pressure, which made it supersaturated with air and caused microbubbles to form. The floating sludge was removed from the DAF unit with a skimmer, and collected in a non-aerated mixed return tank with an approximate retention time of 3.5 hours. Part of this sludge was recirculated to the contact tank with a recycle ratio of 0.021–0.027 to achieve a TSS concentration in the contact tank of around 1 g L^{-1}. Sludge recirculation was automatically stopped when the volume in the return tank dropped below 250 L, as the production of return sludge was dependent on the performance of the DAF unit and on daily fluctuations of the wastewater composition. Wasting of sludge was performed when the volume in the return tank rose above 300 L, and was stopped when the volume decreased below 280 L. These

112 settings were chosen to approach a SRT of 1 day, to be well below the maximum of 2 days
113 defined for HRAS systems (Meerburg et al., 2015).
114 The test period lasted 164 days, and can be divided in two different phases, based on different
115 strategies used: single polymer (treatment 1, 104 days) and dual polymer (treatment 2, 65
116 days). During treatment 1, 40% $FeCl_3$ was added at a final concentration of 50 mg L^{-1} to assist
117 coagulation of the sludge, as preliminary batch tests had shown that this concentration led to a
118 good coagulation performance (data not shown). The optimal iron dosage was likely a
119 combined result of iron required for coagulation as well as for phosphorus removal, since Fe^{3+}
120 precipitated as $FePO_4$ is not available for coagulation of sludge particles anymore. An anionic
121 polyacrylamide based (PAM) polymer (A135.HP, Kemira: linear copolymers of acrylamide
122 and acrylic acid, molecular weight 11.000.000 – 12.000.000 g mol^{-1}, charge density +/- 40
123 mol%) was dosed at a concentration of 3 mg L^{-1} to assist formation of sludge aggregates. In
124 the first 64 days, the anionic polymer was dosed in the piping system 1.5 m before entering
125 the DAF unit. The pressurized white water was injected into the DAF at the point of entry of
126 the ML. On day 65, in order to improve the sludge flocculation, the configuration was
127 changed (Figure 1). After dosing the coagulant in the ML, the ML was pumped through a pipe
128 length of 4 m (inner diameter 56 mm with Reynolds Number 19000), after which roughly half
129 of the pressurized white water was injected in the pipe to increase floc buoyancy.
130 Subsequently, the ML was pumped through a 12 m plug-flow flocculator (PFF, a serpentine
131 of pipes with different inner diameters: 33 mm in the first 1 m to favor polymer mixing, and
132 44 mm inner diameter in the remaining 11 m to apply further turbulence and mixing;
133 Reynolds numbers were 32000 and 24000, respectively) before entering the DAF unit. The
134 anionic polymer was added directly in the PFF after 0.5 m. The remaining white water was
135 added to the DAF unit at the point of entry of the ML.
136 Treatment 2 lasted for 65 days and the same coagulant was dosed (40% $FeCl_3$ at 50 mg L^{-1}).

The addition of two polymers occurred directly in the PFF, with a cationic organic PAM polymer (C492 Kemira: linear copolymers of acrylamide and the cationic ester acryloyloxyethyltrimethyl-ammonium chloride, molecular weight 5.000.000 – 7.000.000 g mol^{-1}, charge density 10 mol%) dosed at 2 mg L^{-1}, 0.5 m after the first white water injection point, and an anionic PAM polymer (A135.HP, Kemira) dosed at 0.5 mg L^{-1}, 0.5 m further. Both polymers were added from 0.1% stock solutions that were replaced every 1 to 3 days. As such, the effect of dual polymer dosage could be investigated, as literature reports that dual cationic-anionic polymer dosage may form more stable floc aggregates compared with those formed when a single polymer is dosed (Petzold et al., 2003) (see also paragraph 3.1.2). As the effectiveness of a polymer for flocculation depends on the characteristics of the wastewater matrix and sludge flocs, the choice for the specific polymer types used in this study was made in agreement with the supplier, based on jar flocculation tests (Kemira; personal communication). Before each treatment and on day 65 of treatment 1, the contact tank was inoculated with secondary WAS (8.46 ± 1.46 g TSS L^{-1}), from the Aartselaar WWTP. The WAS was pumped in the contact tank until the ML reached a concentration of 1 g TSS L^{-1}. Liquid samples of domestic wastewater, ML, effluent and DAF sludge were collected 3 times per week and analyzed for total COD (tCOD), soluble COD (sCOD), TSS and VSS. Total ammonia nitrogen (TAN) and total Kjeldahl nitrogen (TKN) were analyzed every two weeks.

2.2 Pilot-scale high-rate contact stabilization system combined with dissolved air flotation setup

The same pilot setup described in section 2.1 was used for 46 days in the HiCS-DAF configuration. In this configuration, the contact tank was not aerated, while the sludge return tank was aerated by means of fine-bubble aeration at a working volume between 260 L and 270 L (as explained in section 2.1) and a dissolved oxygen set point between 2.0 and 3.0 mg

L^{-1} (Figure 1). As such, the advantage or disadvantage of a HiCS system over an HRAS system could be investigated. On the first day of the HiCS-DAF treatment, the return tank was inoculated with 300 L of secondary WAS (8.46 ± 1.46 g TSS L^{-1}), from the Aartselaar WWTP. Before entering the DAF unit, the ML was dosed with coagulant and flocculants using the same dual polymer approach as in treatment 2 of the HRAS-DAF configuration. However, the dosages were lower: 40% $FeCl_3$ was dosed at 30 mg L^{-1} to apply a similar $Fe/COD_{influent}$ ratio as in treatment 1 (Table 1), cationic polymer was dosed at 1.5 mg L^{-1}, and anionic polymer at 0.5 mg L^{-1}. This was done in accordance with batch flocculation tests to determine the optimal polymer dosage for the HiCS sludge (data not shown). Other test conditions were kept identical over the treatment periods, although natural variation in wastewater composition and temperature did occur.

2.3 Biomethane potential tests

The BMP tests were carried out to estimate the anaerobic biodegradability of the A-sludge obtained from the pilot-scale HRAS-DAF and HiCS-DAF systems. The tests were performed in triplicate under mesophilic conditions (35 °C) in serum flasks with a working volume of 80 mL. Inoculum or substrate were replaced alternatingly by tap water in the control tests. The substrate to inoculum ratio was maintained at 0.5 g COD g^{-1} $VS_{inoculum}$ with the inoculum diluted with tap water to 10 g VS L^{-1}. For HRAS-DAF, BMP tests were performed with sludge generated after 14 days and 76 days from the start of treatment 1, and after 53 days from the start of treatment 2. Finally, for the HiCS-DAF, two BMP tests were performed with sludge generated after 22 and 38 days of operation. Co-digestion of A-sludge generated during HiCS-DAF (day 9) with secondary WAS (8.46 ± 1.46 g TSS L^{-1}) from a full-scale bio-P installation treating the same wastewater influent as the pilot, in a ratio of 70% A-sludge and 30% WAS, TSS based. Controls, with only A-sludge or WAS, were also added. The inoculum biomass for these tests was obtained from a full-scale anaerobic digester treating

187 municipal wastewater sludge (Aquafin, Leuven, Belgium) at 13, 27, 24 and 27 g VS L^{-1} for
188 the tests during HRAS-DAF treatments 1, 2, HiCS-DAF and co-digestion of A-sludge and
189 WAS, respectively. Flasks were sealed with a rubber stopper and an aluminum sealer, and
190 then connected to glass columns in which biogas production was measured by means of water
191 displacement. Biogas volumetric production was checked every day during the first week and
192 two times per week (Monday and Thursday) afterwards. Biogas composition was evaluated at
193 the end of the experiment, once the biogas production reached the steady state and was
194 constant for three sampling points. Methane yield was expressed as the COD of methane
195 (CH_4) produced per gram of COD of substrate. Values are reported at standard temperature
196 (237 K) and pressure (101325 Pa) (STP) conditions.

197 *2.4 Post-treatment of dissolved air flotation effluent for nitrogen removal*

198 Four identical batch setups were used to evaluate nitrification as post-treatment after the
199 HiCS-DAF A-stage. Each setup consisted of a 1 L Schott bottle containing a 0.5 L mixed
200 liquor with a final concentration of 0.72 ± 0.05 g VSS L^{-1} freshly acquired activated sludge
201 from a municipal WWTP (Ghent, Belgium). Two out of four mixed liquors were synthetic
202 with 70 mg NH_4^+-N L^{-1}, 0.5 g $NaHCO_3$ L^{-1}, 0.2 g $MgSO_4 \cdot 7H_2O$ L^{-1}, 0.1 g $CaCl_2 \cdot 2H_2O$ L^{-1},
203 0.005 g KH_2PO_4-P L^{-1} and 1 mL L^{-1} of trace element solution (Third et al., 2001). For the
204 remaining two setups, DAF effluent was mixed with the biomass, with an ammonium
205 concentration of 19 and 23 mg N L^{-1}. The mixtures were continuously stirred and aerated at
206 28 °C, while the pH was controlled between 7.2 and 7.4 using 0.02 M solutions of HCl or
207 NaOH. Liquid samples were taken over time (at 0 h, 1.2 h, 2.7 h, 4.0 h, 5.1 h and 6.3 h) for
208 TAN, nitrite and nitrate analyses. Anoxic batch activity tests were performed with anammox
209 sludge originating from a DEMON facility (Nieuwveer Breda, The Netherlands) to
210 investigate the feasibility of anammox as a post-treatment for DAF effluent. Serum flasks of
211 120 mL were used, containing 80 mL of mixed liquor with a final biomass concentration of

212 0.33 ± 0.01 g VSS L^{-1}. Conditions imposed on the anammox biomass to test for different
213 sources of inhibition are given in supplementary information (SI). The synthetic medium was
214 a pH 7.2 corrected medium of 25 mM HEPES containing 70 mg NH_4^+-N L^{-1}, 70 mg NO_2^--N
215 L^{-1}, 0.5 g $NaHCO_3$ L^{-1}, 0.2 g MgSO4x7H_2O L^{-1}, 0.1 g $CaCl_2$x2H_2O L^{-1}, 0.005 g KH_2PO_4-P L^{-1}
216 and 1 mL L^{-1} of trace element solution (Third et al., 2001). To the DAF effluent, 40 mg NO_2^-
217 -N L^{-1} was added to enable the anammox process. Rubber stoppers sealed off the flasks, after
218 which they were flushed with N_2 gas. Tests were performed in triplicate on a temperature
219 controlled shaker (120 rpm and 28°C). Liquid samples were taken over time (at 0 h, 1.1 h, 1.6
220 h, 2.6 h and 3.5 h) for TAN and nitrite analysis.

221 *2.5 Analytical techniques*

222 The tCOD, sCOD, TSS, VSS, TAN, and TKN analyses were performed according to
223 Standard Methods (Greenberg et al., 1992). Biogas composition was analyzed with a Compact
224 GC (Global Analyser Solutions, The Netherlands) equipped with a Porabond precolumn and a
225 Molsieve 5A column. Concentrations of CH_4, CO_2 and H_2 were determined using a thermal
226 conductivity detector with a lower detection limit of 1 ppmv for each gas. Nitrite and nitrate
227 concentrations were determined on a 930 Compact Ion Chromatograph (Metrohm,
228 Switzerland), equipped with a conductivity detector. During the anammox batch tests, liquid
229 samples for ammonium and nitrite determination were always immediately analyzed
230 spectrophotometrically with the Berthelot and Montgomery reaction (Bucur, B., et al. (2006);
231 Montgomery and Dymock, (1961)), including a triplicate standard curve for each analysis.
232 Measurements were obtained using a Tecan infinite plate reader (Tecan, Switzerland).

233 *2.6 Statistical analysis*

234 Statistical analysis of the operational data was carried out using the software SigmaPlot 13
235 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com). The normality and

equal variances (homoscedasticity) were evaluated by means of the Shapiro-Wilk and Brown-Forsythe tests, respectively. A parametric t-test was used to compare the reactors when normality and homoscedasticity could be confirmed, otherwise a non-parametric Mann-Whitney U Test was used.

3 Results and Discussion

A HRAS system (or HiCS system) coupled with a DAF unit, was evaluated as an alternative to a HRAS-settler combination for primary treatment of municipal wastewater (A-stage). The main goals were to (1) increase removal efficiency of the wastewater organics, (2) increase sludge concentration so that gravity-thickening is no longer required, and (3) increase the conversion of sludge chemical energy to methane through AD, to advance towards energy-neutral wastewater treatment. Table 1 lists the characteristics of the influent wastewater and mixed liquor for the three subsequent treatments.

3.1 Performance of high-rate activated sludge systems combined with dissolved air flotation-pilots

3.1.1 High-rate activated sludge system combined with dissolved air flotation: impact of mixing regime of coagulant/flocculant prior to DAF separation

For wastewater treatment systems such as a HRAS system where a positively charged coagulant is used (i.e., Fe^{3+}), the suspended particles generally have a positively charged surface and thus, an anionic PAM polymer should give best flocculation performances (Bolto and Gregory, 2007). Thus, during treatment 1, $FeCl_3$ and an anionic PAM polymer were used to coagulate and flocculate the HRAS sludge flocs into floc-aggregates. An optimal mixing regime is essential to uniformly distribute coagulant and flocculant in solution, and to create shear-induced collisions between polymer molecules and particles to form floc-aggregates

suitable to DAF separation (Bolto and Gregory, 2007; Wang et al., 2005). The first experimental period (days 1 - 64), where the plug-flow flocculator (PFF) was not used, did not yield satisfactory organics removal. Only 51 ± 17% of the TSS, 53 ± 20% of the VSS and 50 ± 13% of the tCOD in the influent could be removed (Figure 2), leaving a substantial fraction of organics in the effluent. It was hypothesized that the short distance between the polymer addition and the DAF unit did not allow sufficient mixing in the pipes, and thus resulted in insufficient adsorption of the polymer onto the flocs and floc-aggregating action of the polymer, resulting in the formation of small and unstable floc-aggregates with poor floatability. From day 65, a PFF unit was added which improved floc formation. Part of the pressurized white water was added to the PFF before polymer dosage to increase the degree of turbulence of the flow in the PFF, allowing a high energy mixing which is crucial for high molecular weight polymers such as the A135HP used in this study, and allowed more collision of flocs. The addition of white water in the PFF also allowed the formation of floc-aggregates with enmeshed microbubbles, which likely further enhanced flotation capacity. Moreover, the 12 m length of the PFF system increased the contact time between polymer and sludge, allowing better polymer dispersion and improved floc-aggregates formation. As a result, the organics removal efficiency in the last 40 days of treatment 1 was increased to 78 ± 12% of the TSS, 82 ± 10% of the VSS and 63 ± 14% of the total COD (Figure 2), which lies within the values typically obtained for conventional HRAS-settler systems (de Graaff et al., 2016; Ge et al., 2017; Trzcinski et al., 2016). After further optimization, replacing gravity-settling with DAF separation in HRAS systems thus has the potential to improve the removal of organics from domestic wastewater albeit at considerably smaller land footprint.

3.1.2 High-rate activated sludge system combined with dissolved air flotation: impact of a dual polymer system

The solid/liquid separation in DAF systems occurs by means of hydrophobic interactions between floc-aggregates and air microbubbles. Therefore, increasing aggregation and adhesion is the key for an effective solids separation and to increase organics removal efficiency (Bolto and Gregory, 2007). In the second treatment of the HRAS-DAF system, optimization of hydrophobic interactions between microbubbles and floc-aggregates was investigated with the so-called dual polymer system, where a combination of a cationic PAM polymer (C492) and an anionic PAM polymer (A135HP) are used to enhance flocculation (Yu et al. 1996; Fan et al., 2000; Petzold et al., 2003). These polymers adsorb on the sludge flocs with different mechanisms: cationic polymers interact electrostatically with negative sites on the sludge flocs while anionic polymers adsorb on negatively-charged surfaces through ion bridging, interacting with cations and positive patches (e.g., from the coagulation phase and the cationic polymer) that function as bridges between the polymer and the sludge floc surface (Bolto and Gregory, 2007). The dual polymer system combines electrostatic patch (formation of positive and negative charge patches due to the cationic polymer) and ion bridging (anionic polymer), which enhances charge neutralization (Bolto and Gregory, 2007; Petzold et al., 2003). Polymer conformation has also been reported to play a predominant role in flocculation. The use of a linear anionic polymer, alike the A130HP used in this study, can provide better interparticle ion bridging when adsorbed on particles with pre-adsorbed polymer of cationic charge rather than when dosed alone (Yu et al. 1996). Removal efficiencies during this second treatment were 70 ± 12% for the TSS, 71 ± 11% for the VSS and 57 ± 10% for the total COD (Figure 2). The removal efficiency appeared to be lower compared to the first treatment, although the difference was not significant with 95% confidence ($p = 0.303$ for the TSS; $p = 0.065$ for the VSS; $p = 0.128$ for the tCOD). The

influent COD and ML concentration were 2.25 and 1.44 times lower, respectively, compared with the previous treatment period, due to seasonal variation of the wastewater (Table 1). The effluent of the HRAS-DAF had a similar organics concentration during treatment 1 and treatment 2 (Figure 3) ($p = 0.422$ for the TSS; $p = 0.462$ for the VSS; $p = 0.063$ for the COD). Hence, irrespective of the initial concentration of the wastewater, the effluent of both treatments reached a similar quality. This suggests that the system performed better during treatment 2, although immediate conclusions are difficult to draw given the lower influent strength compared to treatment 1. The system would likely achieve higher removal efficiencies when treating medium- or high-strength wastewater, as opposed to the low-strength wastewater in this study. Anionic polymers, when used in a single polymer dosing scheme (*i.e.,* treatment 1) may lead to the generation of sludge flocs more susceptible to daily variations in wastewater concentration, since lower influent suspended solids can lead to polymer overdosing when the dosage rate is not dynamically controlled, and thus to the formation of heavy flocs not suitable for flotation. In contrast, the improved charge neutralization resulting from the use of a dual polymer system, can potentially ensure a higher stability of the flocs formed, irrespective of the seasonal variations of the wastewater (Bolto and Gregory, 2007; Petzold et al., 2003). Moreover, the combination of cationic and anionic polymers may reduce the overall polymer dosing (Yu et al. 1996; Fan et al., 2000) as shown in the current study (Table 1), which would make polymer overdosing less likely and improve the resistance of the system against perturbations in influent solids load. This was the case in the current study as lower overall dosage in treatment 2 did not seem to lead to significantly lower removal efficiencies and effluent quality, although it is difficult to quantify the true effect of the dual polymer dosage, given the lower influent strength in treatment 2 (Figure 2 and Figure 3).

3.1.3 High-rate contact stabilization system combined with dissolved air flotation

During the following treatment, the configuration of the pilot-scale was changed to a HiCS-DAF. This change consisted of aerating the return flow in the return tank, and leaving the contact tank unaerated (Figure 1). It was hypothesized that a HiCS-DAF system could improve the removal efficiencies due to improved overall sludge capture, and thus likely improve sludge recovery as CH_4, due to the improved bioflocculation that occurs in contact stabilization systems, compared with HRAS systems (Meerburg et al., 2015). Removal efficiencies during this treatment were similar to the values obtained during treatment 2 of the HRAS-DAF configuration ($p = 0.440$ for the TSS; $p = 0.350$ for the VSS; $p = 0.621$ for the COD) (Figure 2). The influent tCOD concentrations during this treatment were lower compared to treatment 1 of the HRAS-DAF but similar to treatment 2 (Table 1). Effluent concentrations during this treatment were also similar to the values obtained during HRAS-DAF treatment 2 (Figure 3) ($p = 0.582$ for the TSS; $p = 0.539$ for the VSS; $p = 0.044$ for the COD). The amounts of coagulant and polymer dosed during this treatment were however lower: the coagulant dosage was 40% lower than for HRAS-DAF treatment 1 and 2, and total polymer dosage was 33% and 20% lower than in HRAS-DAF treatment 1 and 2, respectively (Table 1). The presence of an aerated stabilization tank likely enhanced bioflocculation and floc formation, similar to what has been observed in other studies (Meerburg et al., 2015; Rahman et al., 2016). In combination with the use of a dual polymer system, this likely resulted in a lower requirement of chemicals to obtain similar separation efficiency.

3.2 Sludge concentration: dissolved air flotation as a combination of a settler and a thickener

Coupling a HRAS or HiCS treatment system to a DAF led to the formation of a more concentrated sludge compared to sludge generated with classic HRAS systems, where a settler

is used for sludge separation (Figure 4). In case of the HRAS-DAF with single polymer dosage, the sludge had a concentration of 47 ± 10 g COD L^{-1}, which is 4 – 9 times higher than that of conventional HRAS systems (Figure 4). The sludge concentrations achieved here are similar to those reported for sludge gravity thickened before AD, at 40–50 g L^{-1} (Tchobanoglous et al., 2003). The sludge concentrations achieved during treatment 2 of the HRAS-DAF configuration and during HiCS-DAF were lower, at 23 ± 6 g COD L^{-1} and 26 ± 5 g COD L^{-1}, respectively (Figure 4). These sludge concentrations are still higher than those achieved with a conventional HRAS-settler system (Figure 4). In a HRAS treatment system, by replacing a settler by a DAF unit, WWTPs could, thus potentially reduce energy and capital expenditures required for sludge thickening, and this has been investigated by means of an economic assessment (see below). Furthermore, a HRAS-DAF system would have a lower area footprint compared to a primary settler system. A similar advantage in sludge concentration exists when comparing the HRAS-DAF or HiCS-DAF systems to a HR-MBR. Akanyeti et al. (2010) and Faust et al. (2014) reported a sludge concentration of 3.8 g COD L^{-1} and 11.4 g COD L^{-1} by applying a SRT of 1 day and a HRT of 1.2 and 0.7 days, respectively, using a HR-MBR setup.

The reason why the sludge generated during the last two treatments was less concentrated compared to treatment 1 is likely due to the lower wastewater strength in this period (Figure 1). The ratio between sludge COD and influent COD was similar between the different treatments, at 122 ± 43 and 137 ± 50 for HRAS-DAF during treatment 1 and 2, respectively, and at 142 ± 51 for the HiCS-DAF. It might therefore prove to be difficult to further increase the sludge concentration in this system when treating low-concentrated wastewater. The lower influent strength during treatment 2 and the HiCS/DAF treatment also led to a lower F/M ratio (as seen in Table 1). This may explain the differences in tCOD/VSS ratio of the sludge, which decreased from 1.84 ± 0.29 in treatment 1 to 1.43 ± 0.33 in treatment 2, and 1.59 ± 0.97 in the

HiCS-DAF treatment. Possibly, the lower F/M ratio during the latter two treatments resulted in a higher incidence of endogenous decay and, thus, a slight shift in sludge characteristics towards secondary sludge, which has a lower COD/VSS ratio than primary sludge (Tchobanoglous et al., 2003

3.3 Conversion efficiencies to methane

Conversion efficiencies to CH_4 for sludge generated during HRAS-DAF treatment 1 (single polymer) were 68 ± 1% and 58 ± 1% for the sludge harvested after 14 and 76 days of operation, respectively, which is comparable with values reported for AD of A-sludge from conventional HRAS-settler systems (up to 70%) and primary sludge (about 60%) (De Vrieze et al., 2013; De Vrieze at al., 2016). Conversion efficiencies to CH_4 during subsequent treatments were lower, at 40 ± 2% during HRAS-DAF treatment 2 (dual polymer) and 42 ± 3% for sludge generated in HiCS-DAF (dual polymer), which may have been a result of the lower tCOD/VSS ratio during treatment 2 and the HiCS-DAF treatment (see above).

The influent COD concentration was considerably higher during treatment 1 of the HRAS-DAF compared with subsequent treatments (Table 1). Possibly, the lower incoming wastewater COD resulted in a proportionally higher loss of biodegradable COD in the sewer system, and an increased relative presence of recalcitrant organics in the sludge. The lower reactor loading rate could also have resulted in an overdose of coagulant or flocculant(s) in the system. For instance, whereas the dosage rate of $FeCl_3$ remained constant between treatment 1 and treatment 2, the weight ratio between the Fe dosed and the tCOD of the influent rose from 0.04 to 0.10 (Table 1). Fe is an important cofactor and coenzyme for methanogens and methanogenic activity is thus strongly dependent on the presence and availability of Fe in the substrate (Zandvoort et al., 2006). Several studies showed that increased concentrations of Fe resulted in an increase of CH_4 production and, thus, a high dosage of Fe^{3+} should not cause a lower CH_4 yield (Hoban et al., 1979; Cagnetta et al., 2016).

However, Fe^{3+} overdose could lead to depletion of phosphorus (P) or trace elements substantial for the methanogenic activity (Feng et al., 2009; Jiang and Graham, 1998; Zandvoort et al., 2006). In this study, co-digestion of A-sludge generated during the HiCS-DAF treatment with secondary WAS (70% A-sludge and 30% WAS, TSS based) was performed to test the hypothesis that the A-sludge was depleted in P or trace elements, which could then be provided by the WAS. The CH_4 yield for the A-sludge in co-digestion with WAS was 280 ± 42 mg COD-CH_4 $g^{-1}COD_{fed}$, while in case of the A-sludge digested solely the yield was 365 ± 12 mg COD-CH_4 $g^{-1}COD_{fed}$, indicating that depletion of P or trace elements was most likely not the cause of the lower conversion efficiencies obtained during the second treatment.

The polymers used in the present study were polyacrylamide (PAM) based, and PAM is widely used at WWTP level to enhance sludge dewatering and thickening prior to AD (Dai et al., 2014). Although PAM are generally accepted as non-toxic and inert to deterioration, contradictory results are reported about PAM biodegradation under aerobic and anaerobic conditions. El-Mamouni et al. (2002) showed that PAM are highly recalcitrant to microbial degradation, due to its high molecular weight. However, Dai et al. (2014) demonstrated that after hydrolysis and digestion of easy biodegradable organics, PAM carbon backbone can be broken down in different smaller molecules, and consumed as a carbon source for CH_4 production. Thus, degradation of PAM polymers did not seem to hamper anaerobic digestion. The main difference between HRAS-DAF treatment 1 and the following treatments is that in treatment 1 only an anionic polymer was used to assist flocculation, while during treatment 2 and the HiCS-DAF treatment, a dual polymer system (cationic and anionic polymers) was used. There are no studies in literature assessing different behavior of sludge types dewatered using anionic or cationic PAM, which are both mainly copolymers of acrylamide, or a combination of these, towards anaerobic degradation and digestion. In a dual polymer system,

cationic and anionic polymers interact with sludge particles and with each another forming more stable floc-aggregates compared with those formed when a single polymer is dosed (Petzold et al., 2003). This increased stability might have resulted in a reduced hydrolysis of the floc-aggregates which were then more difficult to hydrolyze and not accessible to hydrolytic-acidogenic bacteria. This might be the cause of the lower yields obtained in the last two treatments compared with treatment 1. Further optimization studies on the HRAS/HiCS-DAF systems should focus on different types and dosage of coagulants (e.g., $AlCl_3$ or Poly-aluminum chloride) and polymer(s), optimization of the storage concentration to avoid possible polymer degradation, and implementation of an automated dosing control system based on the daily variation of the solids load. Additional investigation of the effect of PAM on the hydrolysis of sludge floc-aggregates during AD of sludge could likely ameliorate the system.

3.4 Post-treatment of dissolved air flotation effluent for nitrogen removal

As the DAF effluent remains rich in ammonia, post-treatment is necessary to ensure a dischargeable effluent quality. Several possibilities arise to remove nitrogen in the so called B-stage: nitrification-denitrification, nitritation-denitritation or partial nitritation and anammox (PN/A). Nitrifying and anammox bacteria are generally more sensitive to environmental stress factors than denitrifying organisms (Cao et al., 2017). Therefore, if nitrification or anammox treatment of DAF effluent is feasible, so will denitrification be. Nitrification is the microbial oxidation of ammonium (AO) to nitrate and consists of two steps. The first step is catalyzed by bacteria (AOB) or archaea (AOA) and transforms ammonium to nitrite. Subsequently, nitrite oxidizing bacteria (NOB) convert nitrite to nitrate. Nitrification of DAF effluent was tested in duplicate using batch activity tests and was compared to nitrification in synthetic medium. Nitrite accumulated as ammonium was oxidized during the experiment, meaning that the maximum activity of the AOB/AOA was

454 higher than that of the NOB. For this, the maximum specific activity of NOB was represented
455 as the nitrate production rate, while for the AOB/AOA activity, the nitrite accumulation rate
456 was added to the nitrate production rate. Comparable maximum AOB/AOA and NOB activity
457 of about 150–170 and 120–130 mg N g^{-1} VSS d^{-1}, respectively, were observed in synthetic
458 medium and DAF effluent (further information in SI). This finding proves that there is no
459 acute inhibiting effect of DAF effluent on nitrifying activity, thus rendering post-treatment of
460 DAF effluent with nitrification-denitrification feasible.
461 PN/A is a process that is expanding in use, with a growing number of full-scale applications
462 (Vlaeminck et al., 2012). In this process, half of the ammonium is oxidized to nitrite by AOB
463 or AOA, followed by the oxidation of the remaining ammonium using the produced nitrite as
464 electron acceptor by anammox bacteria, generating nitrogen gas as major final product. The
465 reasons for the growing interest in PN/A is that there is no need for carbon addition, sludge
466 production is about 80 % lower and almost 60 % less oxygen is required compared to
467 nitrification-denitrification (Cao et al., 2017; Morales et al., 2015). In order to test the effect
468 of DAF effluent on the anammox activity, anoxic batch activity tests were performed under
469 different conditions (further information in SI). About 60–65 % of the maximum anammox
470 activity was retained when treating DAF effluent compared to the ideal conditions in synthetic
471 medium (Figure 5). This observed inhibition could not be attributed to particulate matter,
472 phosphorous limitation or inorganic carbon limitation. The observed inhibition is in contrast
473 with an earlier study investigating the treatment of pre-treated sewage with anammox, where
474 no inhibition was observed when comparing to synthetic medium (Saha et al., 2015).
475 Nonetheless, high biomass specific anammox activity of 255–275 mg NH_4^+-N g^{-1} VSS d^{-1}
476 was obtained, rendering the use of anammox for post-treatment of DAF effluent feasible. The
477 NO_2-/NH_4^+ ratio in the experiments treating DAF effluent were slightly higher than the ratio
478 in synthetic medium (Figure 5). This might be due to the occurrence of denitrification on

some biodegradable COD passing the DAF installation. Thus, nitrification was not inhibited when exposed to DAF effluent, as opposed to the anammox activity, which was inhibited by about 35–40 %. Nonetheless, both processes achieve sufficiently high conversion rates rendering them feasible as a post-treatment unit after a DAF installation.

4 Conclusions

Coupling a DAF unit with a HRAS system allowed removal of 78% of the influent TSS when an anionic polymer was dosed. The DAF acted as a combination of settling and gravitational thickening, generating A-sludge with a concentration of up to 47 g COD L^{-1}. 68% of the sludge COD was converted to methane during AD. Application of a dual polymer system resulted in lower removal efficiencies, sludge concentration and AD yields, albeit at a similar quality of the effluent. This was likely due to the lower concentration of the wastewater organics (more than 2-fold lower) during these treatment periods.

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Appendix: Supplementary information

Supplementary information to this article can be found online.

References

1. Agrawal, S., Seuntjens, D., De Cocker, P., Lackner, S., Vlaeminck, S., 2018. Success of mainstream partial nitritation/anammox demands integration of engineering, microbiome and modeling insights. Curr. Opin. Biotechnol. 50, 214–221.

2. Akanyeti, I., Temmink, H., Remy, M., Zwijnenburg, A., 2010. Feasibility of bioflocculation in a high-loaded membrane bioreactor for improved energy recovery from sewage. Water Sci. Technol. 61, 1433–1439.

3. Boehnke, B., Diering, B., Zuckut, S.W., 1997. Cost-effective wastewater treatment process for removal of organics and nutrients (part I). Water Eng. Manag. 144, 30–35.

4. Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Res. 41, 2301–2324.

5. Bratby, J., Marais, G., 1976. A Guide for the design of dissolved-air (pressure) flotation systems for activated sludge processes. Water SA, 2, 87–100.

6. Cagnetta, C., Coma, M., Vlaeminck, S.E., Rabaey K., 2016. Production of carboxylates from high rate activated sludge through fermentation. Biores. Technol. 217, 165–172.

7. Cao, Y., van Loosdrecht, M. C., & Daigger, G. T., 2017. Mainstream partial nitritation-anammox in municipal wastewater treatment: status, bottlenecks, and further studies. Appl. Microbiol. Biotechnol. 101, 1365-1383.

520 8. Constantine, T. Houweling, D., Kraemer, J., Hill, C.H.M., 2012. "Doing the two-step" -
521 reduced energy consumption sparks renewed interest in multistage biological treatment.
522 Proceedings of the 2012 Water Environment Federation's Annual Technical Exhibition
523 and Conference, 5771–5783.

524 9. Dai, X., Luo, F., Yi, J., He, Q., Dong, B., 2014. Biodegradation of polyacrylamide by
525 anaerobic digestion under mesophilic condition and its performance in actual dewatered
526 sludge system. Biores. Technol. 153, 55–61.

527 10. de Graaff, M.S., van den Brand, T.P.H., Roest, K., Zandvoort, M.H., Duin, O., van
528 Loosdrecht, M.C.M., 2016. Full-scale highly-loaded wastewater treatment processes (A-
529 stage) to increase energy production from wastewater. Environmental Eng. Sci. 33, 571–
530 577.

531 11. De Vrieze, J., De Lathouwer, L., Verstraete, W., Boon, N., 2013. High-rate iron-rich
532 activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste. Water
533 Res. 47, 3732–3741.

534 12. De Vrieze, J., Smet, D., Klok, J., Colsen, J., Angenent, L.T., Vlaeminck, S.E., 2016.
535 Thermophilic sludge digestion improves energy balance and nutrient recovery potential in
536 full-scale municipal wastewater treatment plants. Biores. Technol. 218, 1237–1245.

537 13. Ding, H.-B., Doyle, M., Erdogan, A., Wikramanayake, R., Gallagher, P., 2015. Innovative
538 use of dissolved air flotation with biosorption as primary treatment to approach energy
539 neutrality in WWTPs. Water Pract. Technol. 10, 133.

540 14. El-Mamouni, R., Frigon, J.-C., Hawari, J., Marroni, D., Guiot, S.R., 2002. Combining
541 photolysis and bioprocesses for mineralization of high molecular weight polyacrylamides.
542 Biodegrad. 13, 221–227.

543 15. Fan, A., Turro, N.J., Somasundaran, P., 2000. A study of dual polymer flocculation.
544 Colloids and Surfaces A: Physicochem. Eng. Asp. 162, 141–148.

545 16. Faust, L., Temmink, H., Zwijnenburg, A., Kemperman, A.J.B., Rijnaarts, H.H.M., 2014.
546 High loaded MBRs for organic matter recovery from sewage: Effect of solids retention
547 time on bioflocculation and on the role of extracellular polymers. Water Res. 56, 258–266.

548 17. Feng, L., Chen, Y., Zheng, X., 2009. Enhancement of waste activated sludge protein
549 conversion and volatile fatty acids accumulation during waste activated sludge anaerobic
550 fermentation by carbohydrate substrate addition: the effect of pH. Environ. Sci. Technol.
551 43, 4373–4380.

552 18. Ge, H., Batstone, D.J., Mouiche, M., Hu, S., Keller, J., 2017. Nutrient removal and energy
553 recovery from high-rate activated sludge processes – impact of sludge age. Biores.
554 Technol. 245, 1155–1161.

555 19. Greenberg, A.E., Clesceri, L.S., Eaton, A. D. (Eds), 1992. Standard methods for the
556 examination of water and wastewater, American Public Health Association Publications,
557 Washington, D.C., U.S.A.

558 20. Haarhoff, J., 2008. Dissolved air flotation: progress and prospects for drinking water
559 treatment. J. Water Supply Res. Technol. 57, 555–567.

560 21. Han, M., Vlaeminck, S.E., Al-Omari, A., Wett, B., Bott, C., Murthy, S., De Clippeleir, H.,
561 2016. Uncoupling the solids retention times of flocs and granules in mainstream
562 deammonification: A screen as effective out-selection tool for nitrite oxidizing bacteria.
563 Biores. Technol. 221, 195-204.

564 22. Hoban, D.J., Van Den Berg, L., 1979. Effect of Iron on Conversion of Acetic Acid to
565 Methane During Methanogenic Fermentations. J. Appl. Bacteriol. 47, 153–159.

566 23. Jiang, J.-Q., Graham, N.J.D., 1998. Pre-polymerised inorganic coagulants and phosphorus
567 removal by coagulation - a review. Water SA, 24, 237–244.

568 24. Jimenez, J., Miller, M., Bott, C., Murthy, S., De Clippeleir, H., Wett, B., 2015. High-rate
569 activated sludge system for carbon management – evaluation of crucial process
570 mechanisms and design parameters. Water Res. 87, 476–482.

571 25. Laureni, M., Falås, P., Robin, O., Wick, A., Weissbrodt, D.G., Nielsen, J.L., Ternes, T.A.,
572 Morgenroth, E., Joss, A., 2016. Mainstream partial nitritation and anammox: Long-term
573 process stability and effluent quality at low temperatures. Water Res. 101, 628–639.

574 26. Liu, Y., Fang, H.H.P., 2003. Influences of extracellular polymeric substances (EPS) on
575 flocculation, settling, and dewatering of activated sludge. Crit. Rev. Environ. Sci. Technol.
576 33, 237–273.

577 27. Meerburg, F.A., Boon, N., Van Winckel, T., Vercamer, J.A.R., Nopens, I., Vlaeminck,
578 S.E., 2015. Toward energy-neutral wastewater treatment: a high-rate contact stabilization
579 process to maximally recover sewage organics. Biores. Technol. 179, 373–381.

580 28. Modin, O., Gustavsson, D.J., Tumlin, D.J., Mattsson, A., Jansen, J.I.C., Wilén, B.-M.,
581 2014. Back to basics: high-loaded activated sludge. In: Activated sludge - 100 years and
582 counting. IWA.

583 29. Morales, N., del Río, Á. V., Vázquez-Padín, J. R., Méndez, R., Mosquera-Corral, A., &
584 Campos, J. L., 2015. Integration of the Anammox process to the rejection water and main
585 stream lines of WWTPs. Chemosphere 140, 99-105.

30. Petzold, G., Mende, M., Lunkwitz, K., Schwarz, S., Buchhammer, H.-M., 2003. Higher efficiency in the flocculation of clay suspensions by using combinations of oppositely charged polyelectrolytes. Coll. Surf. A Physicochem. Eng. Asp. 218, 47–57.

31. Rahman, A., Meerburg, F.A., Ravadagundhi, S., Wett, B., Jimenez, J., Bott, C., Al-Omari, A., Riffat, R., Murthy, S., De Clippeleir, H., 2016. Bioflocculation management through high-rate contact-stabilization: A promising technology to recover organic carbon from low-strength wastewater. Water Res. 104, 485–496.

32. Ramalho, R., 2012. Introduction to Wastewater Treatment Processes. Elsevier.

33. Saha, S., Badhe, N., Seuntjens, D., Vlaeminck, S. E., Biswas, R., Nandy T., 2015. Effective carbon and nutrient treatment solutions for mixed domestic-industrial wastewater in India. Water Sci. Technol. 72, 651-657.

34. Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater engineering, treatment and reuse - Metcalf & Eddy. McGraw-Hill, New York.

35. Third, K.A., Olav Sliekers, A., Kuenen, J.G., Jetten, M.S.M., 2001. The CANON System (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. Systemat. Appl. Microbiol. 24, 588–596.

36. Trzcinski, A. P., Ganda, L., Kunacheva, C., Zhang, D.Q., Lin, L.L., Tao, G., Lee, Y., Ng, W.J., 2016. "Characterization and biodegradability of sludge from a high rate A-stage contact tank and B-stage membrane bioreactor of pilot-scale AB system treating municipal wastewaters." Water Sci. Technol. 74, 1716–1725.

607 37. Van Haandel, A.C., van der Lubbe, J., 2007. Handbook biological wastewater treatment -
608 Design and optimisation of activated sludge systems, 1st edition. Uitgeverij Quist,
609 Leidschendam, The Netherlands.

610 38. Versprille, A.I., Zuurveen, B., Stein, T., 1985. The A-B process: a novel two stage
611 wastewater treatment system. Water Sci. Technol. 17, 235–246.

612 39. Verstraete, W., Van de Caveye, P., Diamantis, V., 2009. Maximum use of resources
613 present in domestic "used water". Biores. Technol. 100, 5537–5545.

614 40. Vlaeminck, S.E., De Clippeleir, H., Verstraete, W., 2012. Microbial resource management
615 of one-stage partial nitritation/anammox. Microbial Technol. 5, 433–448.

616 41. Wang, L.K., Hung, Y.-T., Shammas, N.K., 2005. Physicochemical Treatment Processes.
617 Handbook of Environmental Engineering, vol. 3. Humana Press.

618 42. Yu, X., Somasundaran, P., 1996. Role of Polymer Conformation in Interparticle-Bridging
619 Dominated Flocculation. J. Coll. Interface Sci. 177, 283–287.

620 43. Zandvoort, M.H., van Hullebusch, E.D., Fermoso, F.G., Lens, P.N.L., 2006. Trace metals
621 in anaerobic granular sludge reactors: bioavailability and dosing strategies. Eng. Life Sci.
622 6, 293–301.

623 **Figure and Table Captions**

624 **Figure 1.** Pilot-scale A-stage-DAF setup in HRAS-DAF and HiCS-DAF configurations.
625 Acronyms stand for: an.pol: anionic polymer. cat.pol: cationic polymer.

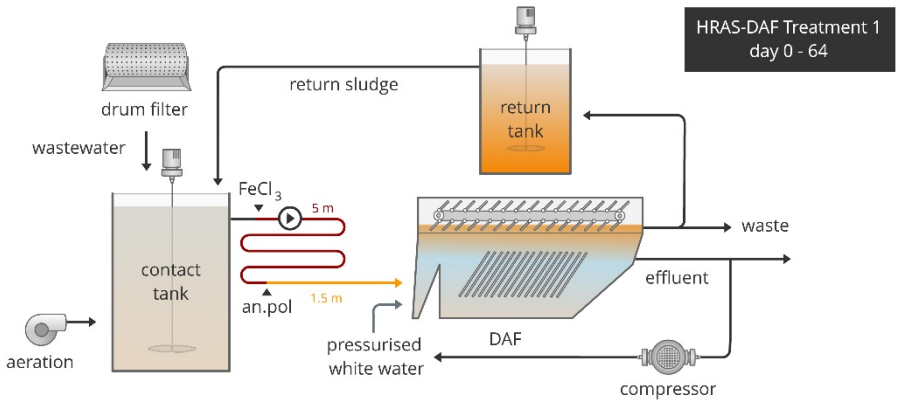
626 **Figure 2.** Removal efficiency of TSS, VSS and total COD (tCOD) over the three treatments.

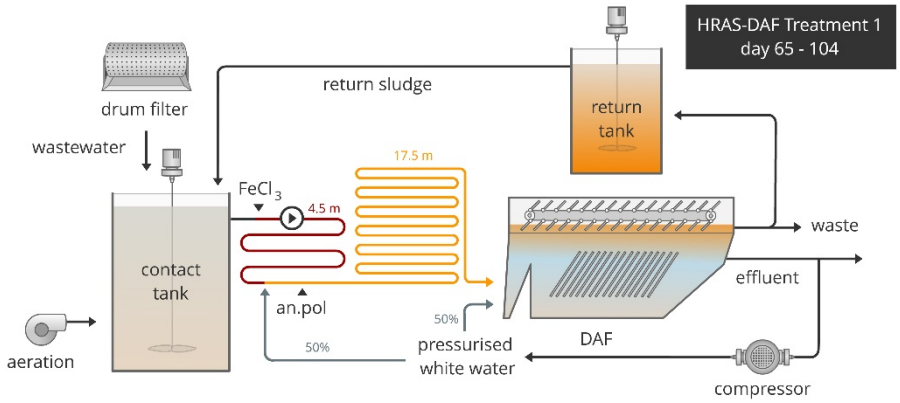
627 **Figure 3.** Influent and effluent concentrations of TSS, VSS and tCOD over the three
628 treatments.

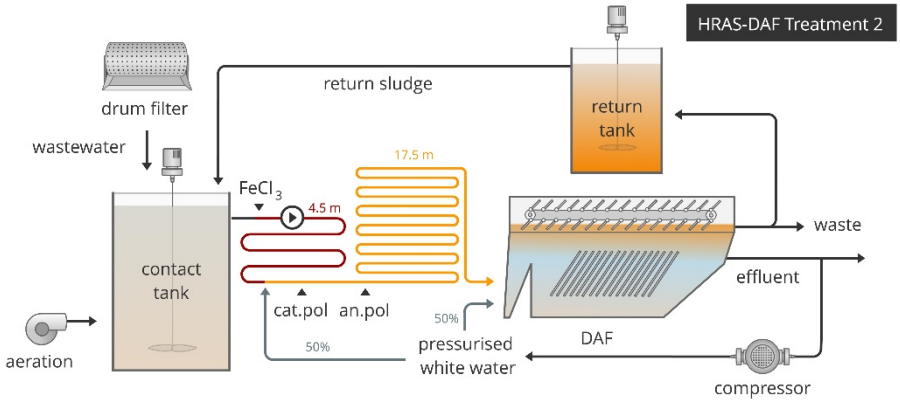
629 **Figure 4.** TSS, VSS and tCOD concentrations for the sludge generated during HRAS-DAF
630 and HiCS-DAF treatments. Values reported for HRAS-settler* and HRAS-settler** are for A-
631 sludge collected from Nieuwveer WWTP (Breda, NL) in November 2013 and October 2015,
632 respectively (Cagnetta et al., 2016).

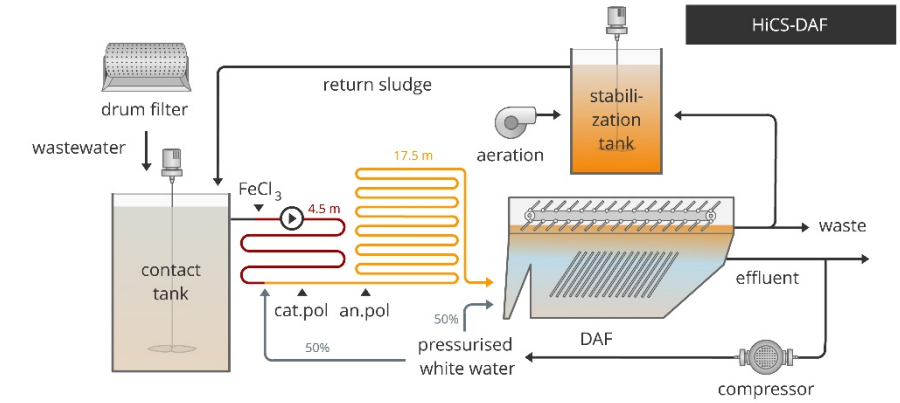
633 **Figure 5.** Biomass specific ammonium, nitrite removal rate and NO_2^-/NH_4^+ removal ratio of
634 anammox biomass in (from left to right) synthetic medium (ideal conditions), DAF effluent,
635 filtered DAF effluent, filtered DAF effluent with 5 mg P L^{-1} and filtered DAF effluent with 5
636 mg P L^{-1} and 0.5 g $NaHCO_3$ L^{-1}.

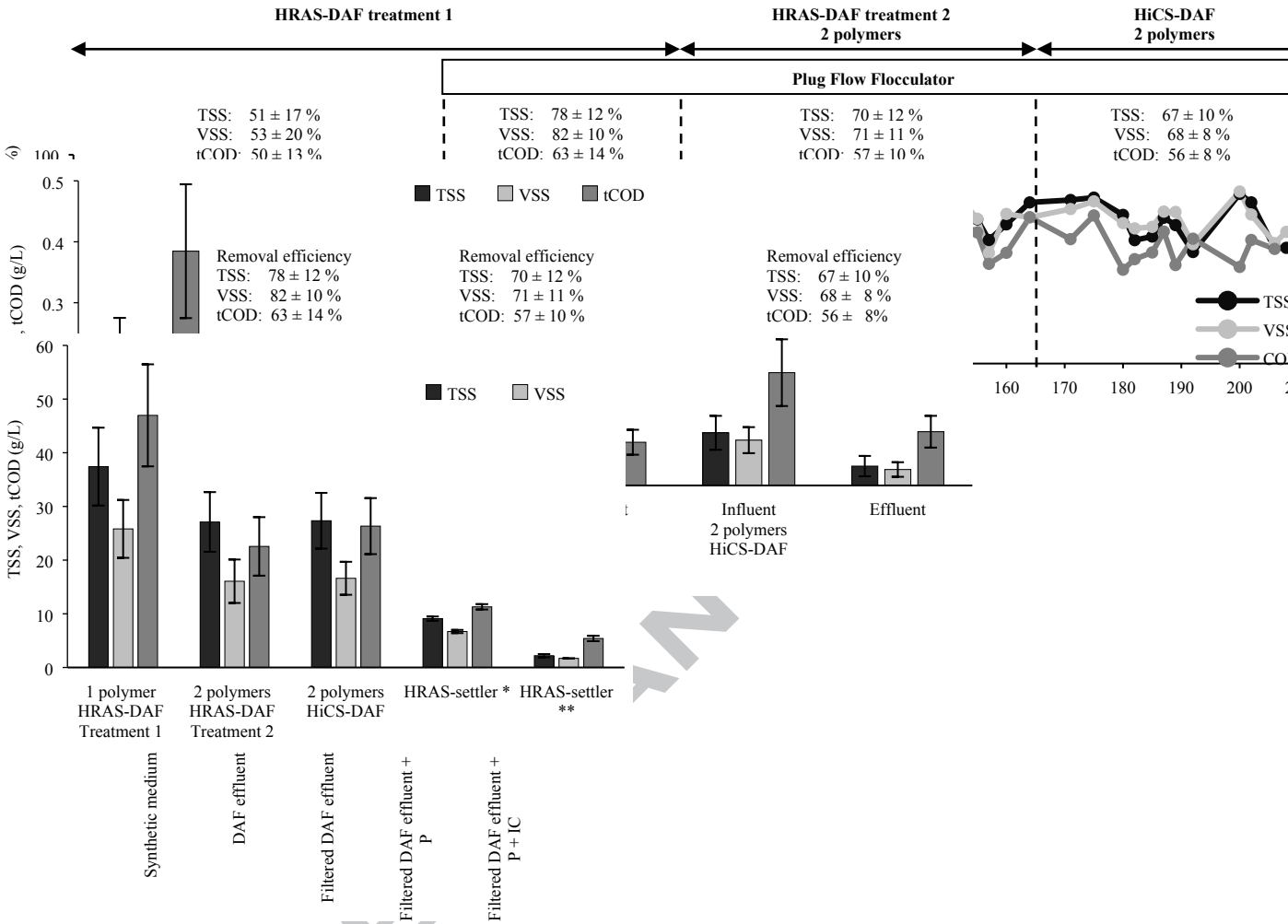
637 **Table 1.** Characteristics of domestic wastewater and mixed liquor. Standard deviations are
638 calculated over time during the different treatments.











	HRAS-DAF treatment 1	HRAS-DAF treatment 2	HiCS-DAF
Number of samples	26	20	18
Municipal wastewater (influent)			
Total suspended solids, TSS (g L^{-1})	0.19 ± 0.09	0.08 ± 0.02	0.09 ± 0.03
Volatile suspended solids, VSS (g L^{-1})	0.15 ± 0.07	0.07 ± 0.02	0.07 ± 0.02
Volatile suspended solids, VSS (%)	79	85	86
Total chemical oxygen demand, tCOD (g L^{-1})	0.38 ± 0.12	0.17 ± 0.04	0.19 ± 0.05

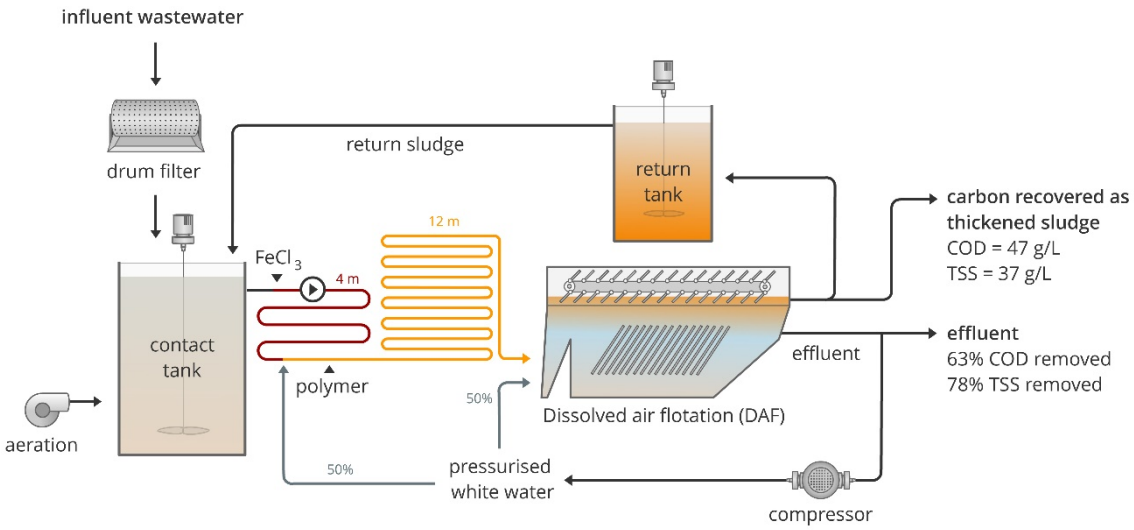
Soluble chemical oxygen demand, sCOD (g L^{-1})	0.10 ± 0.03	0.05 ± 0.01	0.06 ± 0.01
Soluble chemical oxygen demand, sCOD (%)	26	31	31
Total Kjeldahl nitrogen, TKN (mg N L^{-1})	52 ± 11	37 ± 11	33 ± 9
Total ammonia nitrogen, TAN (mg N L^{-1})	32 ± 10	16 ± 4	21 ± 3
Phosphate, P (mg L^{-1})	1.9 ± 0.5	1.3 ± 0.7	1.3 ± 0.5
Mixed liquor			
Total suspended solids, TSS (g L^{-1})	1.14 ± 0.40	0.73 ± 0.25	1.02 ± 0.46
Volatile suspended solids, VSS (g L^{-1})	0.66 ± 0.20	0.43 ± 0.15	0.48 ± 0.18
Volatile suspended solids, VSS (%)	58	60	48
Food to Microorganisms ratio, F/M (kg COD kg^{-1} MLVSS d^{-1})	14.1 ± 5.9	9.0 ± 4.1	9.2 ± 4.3
$Fe/COD_{influent}$ (g Fe g^{-1} COD)	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Dosage of coagulant and polymer			
40% $FeCl_3$ (mg L^{-1})	50	50	30
Cationic polymer (C492, Kemira) (mg L^{-1})	-	2	1.5
Anionic polymer (A130.HP, Kemira) (mg L^{-1})	3	0.5	0.5

Highlights

- A high-rate activated sludge system was coupled to a dissolved air flotation unit.
- Removal of up to 78% TSS and 63% COD from domestic wastewater.

653 • The HRAS-DAF sludge did not need further thickening before anaerobic digestion.

654



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