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Title: Comprehensive comparison between chemically enhanced primary treatment and high-rate activated sludge in novel wastewater treatment plant configurations

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Abstract: Novel wastewater treatment plants (WWTPs) are aimed to be more energetically efficient than conventional ones. Their first step includes an organic carbon preconcentration stage with different alternatives, such as chemically enhanced primary treatment (CEPT) or high-rate activated sludge (HRAS), which are compared in this work in terms of energy demand, operational costs and organic micropollutants (OMPs) and viruses removal efficiency. For this purpose, a CEPT pilot-scale plant operated at a hydraulic retention time (HRT) of 30 min and a lab-scale HRAS reactor working at a HRT of 2h and a solid retention time (SRT) of 1 d were operated in continuous mode. A minimum dose of 150 mg/L of ferric chloride (FeCl_3) was needed to achieve a threshold chemical oxygen demand (COD)-to-ammonium ratio below 2 g COD to g of ammonium (N-NH_4^+) (aiming at fulfilling the requirement for a partial nitrification-anammox reactor), reaching high phosphate removal efficiency (>99%). A slightly lower (70 vs 84%) COD recovery as biomass was attained in HRAS reactors, due to the partial oxidation of influent COD (15%). The lower phosphate removal efficiency achieved in the HRAS alternative (13%) was enhanced to a comparable value of that achieved in CEPT by the addition of 30 mg/L of FeCl_3 at the clarifier. The alternative based on CEPT was identified less energetically demanding (0.07 vs 0.13 kWh/m³ of wastewater) but with significantly higher operational costs than the HRAS-based configuration (6.0 vs 3.8 c€/m³ of wastewater). Regarding OMPs, for those presenting $k_{\text{biol}} > 10 \text{ L/gVSS} \cdot \text{d}$ considerably higher removal efficiencies were achieved in HRAS (80-90%) than in CEPT (4 -55%). For the rest of OMPs biotransformation efficiencies were in general higher in HRAS than in CEPT, but in both configurations below 55%. Finally, CEPT was also less efficient than HRAS for viral removal regardless the virus studied. The comprehensive analysis of all results allows to conclude that HRAS followed by FeCl_3 post-treatment appears as a better alternative than CEPT for COD preconcentration in novel WWTPs.

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Santiago de Compostela, 5th of August 2019

Dear Editor,

Please find enclosed, on behalf of all co-authors, the manuscript under the title "**Comprehensive comparison between chemically enhanced primary treatment and high-rate activated sludge in novel wastewater treatment plant configurations**" by Anton Taboada-Santos, Enrique Rivadulla, Lidia Paredes, Marta Carballa, Jesús Romalde and Juan M. Lema to be considered for publication in Water Research, as a full paper. The outputs contained in this manuscript have not been published in any other journal as a whole or in part. The submission has been performed according to the requirements described in the Guide for Authors of the journal. All authors were aware of the ethics in publishing policies of the journal when preparing and submitting the manuscript.

This work aimed at comprehensively evaluating two different alternatives such as chemically enhanced primary treatment and high-rate activated sludge for maximum organic carbon recovery in novel wastewater treatment plant configurations. We think that this work will be a valuable and interesting contribution to the journal since it comprehensively evaluates the differences in energy expenditure, operational costs and organic micropollutants and viruses removal, giving a novel and broad overview of the aspects that should be considered in the design and operation of novel wastewater treatment plants.

Yours sincerely,

The corresponding author on behalf of all authors.

PhD candidate Anton Taboada-Santos

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Highlights:

- High-rate activated sludge and chemically enhanced primary treatment were compared.
- CEPT is less energy demanding than HRAS, but it leads to higher operational costs.
- HRAS achieves higher organic micropollutants removal efficiency than CEPT.
- CEPT was less efficient than HRAS for viral removal regardless the virus studied.
- Holistically, HRAS was found a preferable alternative than CEPT in novel WWTPs.

Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

1 **Comprehensive comparison between chemically enhanced primary**
2 **treatment and high-rate activated sludge in novel wastewater**
3 **treatment plant configurations**

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17

18 **Abstract**

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20 efficient than conventional ones. Their first step includes an organic carbon
21 preconcentration stage with different alternatives, such as chemically enhanced primary
22 treatment (CEPT) or high-rate activated sludge (HRAS), which are compared in this
23 work in terms of energy demand, operational costs and organic micropollutants (OMPs)
24 and viruses removal efficiency. For this purpose, a CEPT pilot-scale plant operated at a
25 hydraulic retention time (HRT) of 30 min and a lab-scale HRAS reactor working at a
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27 minimum dose of 150 mg/L of ferric chloride (FeCl_3) was needed to achieve a threshold
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29 ammonium (N-NH_4^+) (aiming at fulfilling the requirement for a partial nitrification-
30 anammox reactor), reaching high phosphate removal efficiency (>99%). A slightly
31 lower (70 vs 84%) COD recovery as biomass was attained in HRAS reactors, due to the
32 partial oxidation of influent COD (15%). The lower phosphate removal efficiency
33 achieved in the HRAS alternative (13%) was enhanced to a comparable value of that
34 achieved in CEPT by the addition of 30 mg/L of FeCl_3 at the clarifier. The alternative
35 based on CEPT was identified less energetically demanding (0.07 vs 0.13 kWh/m³ of
36 wastewater) but with significantly higher operational costs than the HRAS-based
37 configuration (6.0 vs 3.8 €/m³ of wastewater). Regarding OMPs, for those presenting
38 $k_{\text{biol}} > 10 \text{ L/g}_{\text{VSS}} \cdot \text{d}$ considerably higher removal efficiencies were achieved in HRAS
39 (80-90%) than in CEPT (4 -55%). For the rest of OMPs biotransformation efficiencies
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41 Finally, CEPT was also less efficient than HRAS for viral removal regardless the virus
42 studied. The comprehensive analysis of all results allows to conclude that HRAS

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45 **Keywords:** chemically enhanced primary treatment, energy demand, high-rate activated
46 sludge, operational costs, organic micropollutants, viruses.

47 **1. Introduction**

48 The discovery of Anammox processes opens new possibilities for conceiving more
49 energetically efficient WWTPs (Gikas, 2017; Siegrist et al., 2008), since it offers up to
50 60% reduction in the aeration requirements (Gu et al., 2017; van Loosdrecht and
51 Brdjanovic, 2014; Wan et al., 2016). In these novel strategies, organic carbon is
52 proposed to be recovered in a first stage, followed by a partial nitrification-Anammox
53 (PN-AMX) unit, and subsequently used to produce biogas in anaerobic digesters (AD).
54 For that purpose, different preconcentration alternatives for chemical oxygen demand
55 (COD) recovery such as chemically enhanced primary treatment (CEPT) (Jang et al.,
56 2017; Li et al., 2017) or high-rate activated sludge (HRAS) (Ge et al., 2017; Jimenez et
57 al., 2015) can be applied.

58 However, not only energetical and economic reasons but also environmental and health
59 aspects must be considered in the design of novel WWTPs. New challenges in
60 wastewater policy concerning organic micropollutants (OMPs) have promoted that
61 some countries such as Switzerland, have already established OMPs discharge
62 thresholds in WWTP effluents (GSchVSchweiz. Gewässerschutzverordnung., 2016).
63 Likewise, other countries such as Austria, Germany and The Netherlands are
64 considering some initiatives to enhance the removal of OMPs during wastewater
65 treatment, to achieve an overall elimination of 80% (Benstoem et al., 2017).

66 Many papers studying the removal of OMPs under aerobic conditions identified factors
67 such as the hydraulic retention time (HRT) and the solid retention time (SRT) as key
68 factors governing their removal efficiency (Clara et al., 2005; Fernandez-Fontaina et al.,
69 2016; Radjenović et al., 2009; Santos et al., 2009). The HRAS technology is
70 characterized by much lower HRT (0.25-4 h) and SRT (0.5-2 d), (Ge et al., 2017;
71 Jimenez et al., 2015) than those applied in conventional activated sludge (CAS) reactors

72 (12-24 hours of HRT and more than 10 days of SRT) (Santos et al., 2009; Suarez et al.,
73 2010), what is expected to lead to significantly lower OMPs removal efficiency.

74 Regarding CEPT, the reported OMPs removal efficiencies are quite controversial;
75 whereas some authors found that CEPT contributes to the removal of some compounds
76 which are precipitated in the flocs formed in the process (Asakura and Matsuto, 2009;
77 Carballa et al., 2005; Suarez et al., 2009) others reported no significant removal for
78 most of them (Taboada-Santos et al., 2019a; Westerhoff et al., 2005). The causes of the
79 discrepancies are still under discussion, but wastewater physico-chemical characteristics
80 such as pH, alkalinity, temperature, fats or dissolved organic matter might have either
81 positive or negative effects on OMPs removal (Luo et al., 2014; Suarez et al., 2009).

82 Another emerging aspect of growing interest is related to the presence of viruses in
83 WWTPs effluents, since conventional WWTPs fail to ensure the complete removal of
84 viral pathogens (Da Silva et al., 2007). Norovirus (NoV) (family Caliciviridae) is
85 considered one of the major etiological agents of acute sporadic and epidemic
86 gastroenteritis worldwide (Atmar and Estes, 2006; Koopmans and Duizer, 2004;
87 Rodríguez-Lázaro et al., 2012). Likewise, viruses such as Sapovirus (family
88 Caliciviridae) have also emerged as important enteric pathogens (Oka et al., 2015).

89 Hepatitis A virus (HAV) (family Picornaviridae) is less common in countries with a high
90 standard of hygiene, although it risks leading to more severe disease outcomes
91 (Hollinger and Emerson, 2007). As pathogens with fecal-oral route of transmission,
92 HAV, NoV and Sav are shed in high numbers in the feces of infected individuals (Atmar
93 and Estes, 2006) and are common in sewage also at high numbers (Sassi et al., 2018).

94 Removing or inactivating viruses in WWTPs is still challenging, despite the
95 developments in secondary and tertiary processes (Schmitz et al., 2016).

96 The goal of this work is to holistically determine the most favourable alternative in two

97 novel configurations of wastewater treatment plant based on chemically enhanced
98 primary treatment and high-rate activated sludge for organic carbon pre-concentration
99 considering energy demand, operational costs and organic micropollutants and viruses
100 removal

101 **2. Materials and methods**

102 **2.1. Wastewater and sludge samples**

103 The wastewater used in this work was collected from a urban WWTP located in
104 Santiago de Compostela (NW of Spain). The WWTP is designed for 184,000 population
105 equivalent with an average wastewater flowrate of approximately 55,000 m³/d.

106 Wastewaters were characterised in terms of pH, total suspended solids (TSS, g TSS/kg)
107 and volatile suspended solids (VSS, g VSS/kg), total chemical oxygen demand (COD_{tot},
108 g O₂/L) and soluble (COD_{sol}, g O₂/L), ammonium (g N-NH₄⁺/L), phosphate (g P-PO₄³⁻
109 /L) according to Standard Methods (APHA, 2005). Inorganic carbon (IC) was
110 determined by a Shimadzu analyser (TOC-5000).

111 **2.2. Chemically enhanced primary treatment**

112 *2.2.1. Jar-test experiments*

113 CEPT tests assays were carried out in a Jar-Test device with vessels of 1 L of liquid
114 volume following the protocol described by Carballa et al. (2005), but in this case
115 without pH neutralization.

116 Two coagulants widely used for coagulation processes such as ferric chloride (FeCl₃)
117 and ferric sulphate (Fe₂(SO₄)₃) (Jang et al., 2017; Liang et al., 2019) were evaluated.

118 The test include an initial 3 min period of rapid stirring (150 rpm) after the addition of
119 the coagulant, followed by 5 min of slow mixing (50 rpm) for emulsion breaking and
120 floc formation, and finally 30 minutes period without mixing for floc separation, after
121 which 500 mL of supernatant were collected for the characterisation. The influence of

122 coagulants dose (0-300 mg/L) on pH and on the removal of TSS, VSS, COD_{tot}, COD_{sol},
123 IC, N-NH₄⁺ and P-PO₄³⁻ was studied at 25°C.

124 2.2.2. *Continuous pilot plant*

125 The pilot plant used, described by Suarez et al. (2009), consists of three main sections;
126 (i) a 4.4 L coagulation tank with a fixed-speed stirrer; (ii) a 15 L flocculation tank
127 provided with a speed-regulated stirrer; and (iii) a 35-L lamellar settler with 10 stainless
128 steel (AISI-304) plates. The operational conditions were selected from the results
129 obtained in batch experiments to optimise the coagulant selection and dose. The
130 wastewater flowrate was fixed at 70 L/min to achieve a HRT of 30 min in the lamellar
131 settler section, with continuous addition of FeCl₃ (selected from the Jar-test results).
132 After 90 min of steady operation of the pilot plant (corresponding to 3 HRT), the
133 effluent was sampled and characterized in terms of pH, COD_{tot}, COD_{sol}, TSS, VSS, N-
134 NH₄⁺, P-PO₄³⁻, IC, OMPs and viruses concentrations.

135 **2.3. High-rate activated sludge reactor**

136 A 2-L continuous stirred-tank reactor coupled to a 1-L settler was inoculated with
137 biomass collected from a heterotrophic activated sludge reactor from a WWTP in
138 Madrid, Spain, working at a SRT of 2.5-3 d. It was operated at a HRT of 2 h (plus 1 h in
139 the settling tank). Target SRT was 1 d, this being controlled by wasting sludge from the
140 bottom of the settler (10 times per day). Dissolved oxygen (DO) concentration was
141 maintained between 3 and 3.5 mg O₂/L, without neither temperature nor pH control (Ge
142 et al., 2017). After inoculation, the reactor was operated for 80 days (15 days of biomass
143 adaptation + 65 days of steady-state operation). Biomass target concentration in the
144 reactor was 2 g VSS/L, although it ranged between 2 and 3 g VSS/L due to some
145 operational difficulties to control it. Reactor walls were scratched every day to remove
146 biofilm formation which could eventually increase the effective SRT.

147 Influent and effluent of the reactor was sampled every day and characterized in terms of
148 pH, COD_{tot}, COD_{sol}, TSS, VSS, N-NH₄⁺, P-PO₄³⁻ and IC. Four sampling campaigns for
149 OMPs measurement in the influent and effluent were carried out on days 18, 33, 48 and
150 63 of operation. Two sampling campaigns for viruses measurement were carried out, on
151 days 18 and 48. On day 18 the effluent of the HRAS reactor was collected and treated
152 with FeCl₃ (0-30 mg/L) in Jar-test devices as explained in section 2.4.1 to assess the
153 influence on phosphate, OMPs and viruses removal efficiency.

154 Sludge was also sampled every day and characterized in terms of COD_{tot}, TSS and VSS.
155 The fraction of COD oxidized to CO₂ was calculated from the COD balance as
156 considering the COD fed to the reactor, the COD recovered in the sludge and the COD
157 in the effluent.

158 **2.4. Energetic evaluation**

159 As calculation basis, a typical medium-strength urban wastewater with a COD_{tot} of 500
160 mg/L and an ammonium concentration of 25 mg N-NH₄⁺/L was considered (Metcalf &
161 Eddy, 2003; Wan et al., 2016). In the biogas line, a methane heat combustion of 11
162 kWh/m³ (N) CH₄ (Perry, 1984) and electrical efficiency (η) of 0.35 in the co-generation
163 motor (Mills et al., 2014) were assumed. The energy consumption considered in the
164 different technologies studied in this work is gathered in Table 1.

165 In order to determine the expected methane production in each configuration,
166 biomethane potential (BMP) tests of the different sludges (sludge from the continuous
167 operation of the CEPT pilot plant and from the purge of the HRAS reactor) were carried
168 out in an AMPTS II equipment (Bioprocess Control), The tests were conducted in 2 L
169 bottles (1.9 L of working volume) in triplicate and with an ISR (inoculum to substrate
170 ratio in terms of VSS) of 2, following the protocol described by Taboada-Santos et al.
171 (2019c).. The inoculum was anaerobic flocculant biomass (15-20 g VS/L) from a

172 mesophilic sewage sludge anaerobic digester. Anaerobic biodegradability (AB) was
 173 expressed as the percentage of the initial COD of the substrate converted to methane. At
 174 the end of the test, bottles were opened and pH and volatile fatty acids (VFAs)
 175 concentration were measured to confirm that no acidification had occurred.
 176 **Table 1.** Literature data considered for the WWTP energetic evaluation.

Technology	Energy demand
Wastewater pumping	0.03 kWh/m ³ wastewater (Longo et al., 2016)
Chemically enhanced primary treatment	0.03 kWh/m ³ wastewater (Longo et al., 2017)
High-rate activated sludge reactor	0.07 kWh/m ³ wastewater (Smith et al., 2014)
Sludge recirculation	0.03 kWh/m ³ wastewater (Longo et al., 2016)
Sludge thickening	0.02 kWh/m ³ wastewater (Longo et al., 2016)
Chemicals addition (tertiary treatment)	0.01 kWh/m ³ wastewater (Longo et al., 2016)
Partial nitrification-anammox reactor	*0.20-0.25 kWh/m ³ wastewater (Schaubroeck et al., 2015)
Sludge dewatering	0.03 kWh/m ³ wastewater (Longo et al., 2016)

* Calculated as 60% of those of a conventional activated sludge reactor (0.42 kWh/m³ of wastewater according to Gikas (2017)).

178 2.5. Economic evaluation

179 A FeCl₃ and electricity costs of 220 €/ton and 0.12 €/kWh, respectively, were
 180 considered for the evaluation (De Feo et al., 2008; STOWA, 2010) and a hygienization
 181 cost for composting of digested sludge of 80 €/ton TS was assumed (Management
 182 Company, 2019). Eq. 1-3 were applied to assess digested sludge production.

$$VS_{rec} = COD_{inf} \cdot COD_{rec} \cdot \left(\frac{VS}{COD} \right)_{sl} \quad [1]$$

$$TS_{rec} = VS_{rec} \cdot \left(\frac{TS}{VS} \right)_{sl} \quad [2]$$

$$TS_{dig} = TS_{rec} - VS_{rec} \cdot AB_{sludge} \quad [3]$$

183 Where:

184 VS_{rec} : Volatile solids recovery in each preconcentration technology (kg VS/m³

185 wastewater treated).

186 TS_{rec} : Total solids recovery in each preconcentration technology (kg TS/m³ wastewater
187 treated).

188 COD_{inf} : Total influent COD (kg COD/ m³ wastewater treated).

189 COD_{rec} : Total influent COD recovery (fraction of COD of the influent recovered as
190 sludge).

191 $(VS/COD)_{sl}$: Volatile solids-to-total COD sludge ratio (kg VS/kg COD).

192 $(TS/VS)_{sl}$: Total solids-to-volatile solids sludge ratio (kg TS/kg VS).

193 TS_{dig} : Digested total solids production (kg TSS of digested sludge /m³ wastewater
194 treated).

195 TS_{dig} : Digested total solids production (kg TSS of digested sludge /m³ wastewater
196 treated).

197 TS_{prod} : TS production in each preconcentration technology (kg TSS in sludge /m³
198 wastewater treated).

199 VS_{prod} : VS production in each preconcentration technology (kg VS in sludge/m³
200 wastewater treated).

201 AB_{sludge} : anaerobic biodegradability, i.e. fraction of COD of sludge converted to CH₄ in
202 the BMP tests (the same value was considered for VS degradation).

203 **2.6. Organic micropollutants**

204 18 compounds commonly used in daily life were considered in this study: three musk
205 fragrances: galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI); three anti-
206 inflammatories: ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF); four anti-
207 biotics: sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY) and
208 roxithromycin (ROX); four neurodrugs: fluoxetine (FLX), carbamazepine (CBZ),
209 diazepam (DZP) and citalopram (CTL); one endocrine disrupting compound, triclosan
210 (TCS); and three hormones: estrone (E1), 17 β -estradiol (E2) and 17 α -ethinylestradiol

211 (EE2). To ensure their presence in wastewater, OMPs were spiked at the influent stream,
212 according to in the range of the values reported by Luo et al. (2014) and Verlicchi et al.
213 (2012) (20 ppb for musk fragrances, 1 ppb for hormones and 10 ppb for the others).
214 The liquid phase of the samples was pre-filtered (AP4004705, Millipore) and filtered by
215 0.45 mm (HAWP04700, Millipore) before performing the solid phase extraction (SPE)
216 with 200 mg OASIS HLB cartridges (Waters, Milford, MA, USA). Quantification of
217 musk fragrances (HHCB, AHTN, ADBI), anti-inflammatories (IBP, NPX, DCF) and
218 TCS was accomplished using a gas chromatograph (Varian CP-3900) coupled with an
219 ion trap spectrometer (Varian CG-2100). Antibiotics (ERY, ROX, SMX, TMP),
220 neurodrugs (FLX, CBZ, DZP, CTL) and hormones (E1, E2, EE2) were quantified using
221 an Agilent G1312A LC with a binary pump and automatic injector HTC-PAI (CTC
222 Analytics) connected to a mass spectrometer API 4000 triple quadrupole (Applied
223 Biosystems). The volume concentrated was 250 mL and the final volume of extract was
224 3 mL, leading to an enrichment factor of $83 L_{\text{supernatant}}/L_{\text{extract}}$. The procedure is
225 explained in detail by Gonzalez-Gil et al. (2016)
226 The solid phase was frozen and lyophilized to afterwards perform ultrasonic solvent
227 extraction. Three sequential extractions with methanol and two with acetone were
228 performed on the freeze-dried samples (0.5 g). In each extraction, samples were
229 sonicated for 15 min and centrifuged at 1500 rpm for 5 min. The resulting supernatants
230 were combined, filtered through glass wool and the resulting volume was evaporated to
231 1 mL (TurboVap LV, Biotage) flowing nitrogen (200 kPa, 30 °C) and resuspended in
232 100 mL of Milli-Q water prior to SPE. Finally, SPE and OMPs quantification were
233 performed as previously described for the liquid phase. The enrichment factor was 500
234 $g_{\text{sludge}}/L_{\text{extract}}$.

235 Total OMPs concentration was considered in the analysis of influent (Eq.4), whereas for
236 effluent only soluble concentration was measured due to its very low TSS concentration.

$$C_{j,\text{total}} = C_{j,\text{dissolved}} + \text{TSS} \cdot C_{j,\text{solid}} \quad [4]$$

237 where, $C_{j,\text{total}}$ is the total concentration of compound j ($\mu\text{g/L}$), $C_{j,\text{dissolved}}$ is the soluble
238 concentration of compound j ($\mu\text{g/L}$), $C_{j,\text{solid}}$ is the concentration in the solid phase ($\mu\text{g/g}$)
239 and TSS the suspended solids content (g/L) of the stream.

240 Mass balances of OMPs in the biological unit were calculated by eq. 5, after achieving
241 steady-state conditions, assuming a continuous stirred tank reactor (CSTR) model.

$$F_{\text{biot}} = F_{\text{inf}} - F_{\text{eff}} - F_{\text{sorb}} \quad [5]$$

242 where F_{inf} , F_{eff} , F_{sorb} represent mass flows (in $\mu\text{g/d}$) corresponding to the influent,
243 effluent and sorbed onto solids. Biotransformation kinetic constants (k_{biol} , in L/gVSS
244 day) were obtained considering pseudo-first order kinetics (Eq. 6).

$$F_{\text{biot}} = k_{\text{biol}} \cdot \text{VSS} \cdot C_{\text{eff}} \cdot V \quad [6]$$

245 where C_{eff} is the OMP concentration in the effluent ($\mu\text{g/L}$), VSS is the biomass
246 concentration in the reactor (g VSS/L) and V is the reactor volume (L).

247 **2.7. Viruses**

248 Viral particles were concentrated using the skimmed milk flocculation (SMF) method
249 described by Fernandez-Cassi et al. (2018). Briefly, wastewater (5 L) was
250 preconditioned to a pH of 3.5, and 50 mL of a pre-flocculated skim milk solution at
251 pH 3.5 and a conductivity superior to 1.5 mS/cm^2 was added to each sample. After 8 h
252 of stirring, flocks were centrifuged at $8,000 \times g$ for 40 min, and the pellet was
253 suspended in 15 mL of phosphate buffer [v/v] ($0.2 \text{ M Na}_2\text{HPO}_4$ and $0.2 \text{ M NaH}_2\text{PO}_4$).
254 The viral concentrates were kept at $-80 \text{ }^\circ\text{C}$ until further use.

255 The viral RNA from each sample was extracted in duplicate using the commercial kit
256 NucleoSpin® RNA Virus kit (Macherey-Nagel, Düren, Germany) according to the

257 manufacturer's protocols. Following the ISO 15216-1:2017 standard method (ISO,
258 2017), mengovirus clone (vMC0) and external controls were employed to determine the
259 extraction and amplification efficiencies respectively, as previously described (Varela et
260 al., (2018). According to this standard procedure, samples with <5% extraction
261 efficiency or <25% RT-qPCR efficiency were re-extracted and tested again.
262 RT-qPCR for HAV, NoV (GI and GII) and SaV was performed on an Mx3005p QPCR
263 System (Stratagene; USA) thermocycler. Platinum® Quantitative RT-PCR
264 Thermoscript™ One-step System kit (Invitrogen; France). Primers and probes used for
265 virus detection and quantification were described elsewhere (Costafreda et al., 2006; Da
266 Silva et al., 2007; Svraka et al., 2007; Kageyama et al., 2003; Loisy et al., 2005; Oka et
267 al., 2006; Varela et al., 2018). Amplification conditions were reverse transcription at
268 55 °C for 30 min, denaturation at 95 °C for 5 min, followed by 45 cycles of
269 amplification with a denaturation at 95 °C for 15 s, annealing at 60 °C (50°C for SaV)
270 for 1 min and extension at 65 °C for 1 min.
271 Viral RNA was tested undiluted and at ten-fold dilution to reduce the effect of potential
272 RT-PCR inhibitors. Negative controls containing no nucleic acid as well as positive
273 controls were introduced in each run. Quantification was estimated by standard curves
274 constructed with serial dilutions of RNA in the case of HAV and RNA transcripts for
275 NoV GI, GII and SaV plotting the number of genome copies against the Ct. Results
276 were expressed as number of RNA viral genome copies per litre of wastewater sample.

277 **3. Results and discussion**

278 **3.1. Wastewater physico-chemical characterization**

279 The physico-chemical characteristics of wastewater, in the typical range for urban
280 WWTPs influents (Metcalf & Eddy, 2003), are shown in Table 2. The important
281 fluctuations of the measured parameters are mainly due to the dilution caused by

282 rainfalls, since the city has not a separated sewer system (Carballa et al., 2005).

283 **Table 2.** Wastewater physico-chemical characterization.

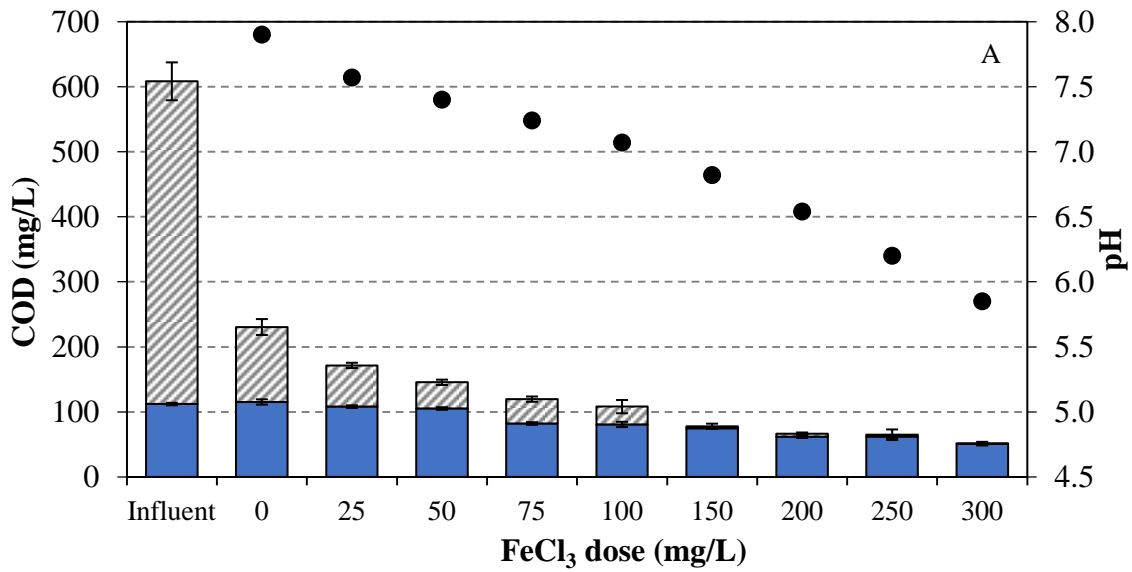
Parameter	Value
pH	7.2-7.8
COD _{tot} (mg/L)	375-750
COD _{sol} (mg/L)	80-150
TSS (mg/L)	330-780
VSS (mg/L)	300-540
N-NH ₄ ⁺ (mg/L)	30-60
P-PO ₄ ³⁻ (mg/L)	1.9-2.6
IC (mg /L)	30-65

284

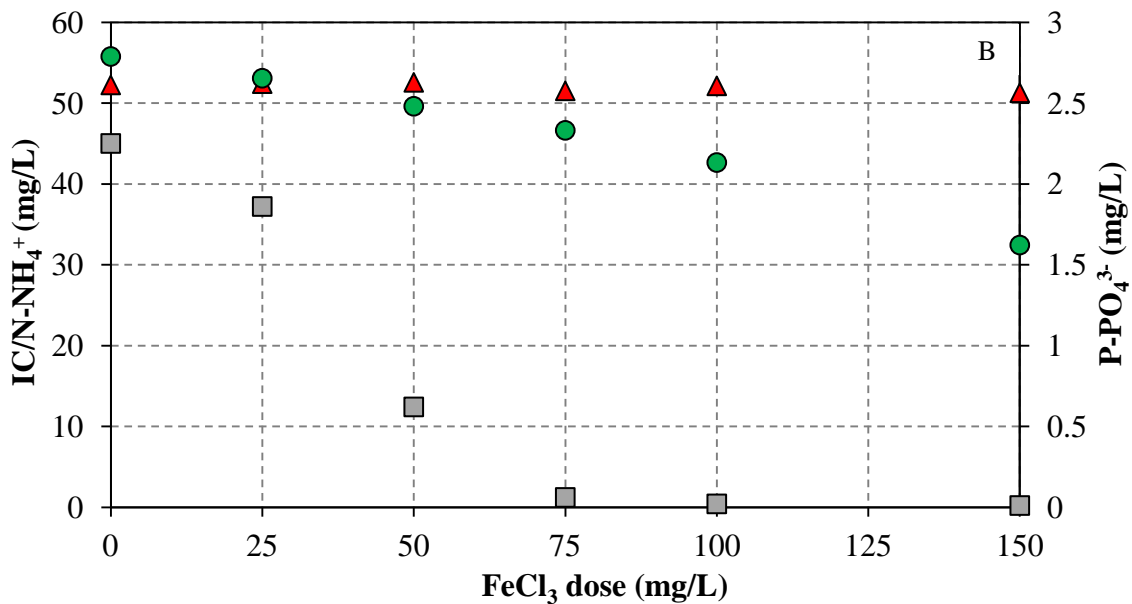
285 **3.2. Performance of chemically enhanced primary treatment: Jar-test and pilot** 286 **plant operation**

287 The selected coagulants (FeCl₃ and Fe₂(SO₄)₃) were tried and compared in Jar-Test, and
288 FeCl₃ was selected for the experiments since it led to a higher removal efficiency under
289 the same coagulant dose (a detailed discussion is included in Section S1 in the
290 Supplementary Information). Figure 1A shows the influence of FeCl₃ dose on effluent
291 pH and on COD_{sol} and COD_{part} (calculated as COD_{tot} minus COD_{sol}) removal efficiency.
292 COD_{part} was completely removed with a dose of 150 mg/L, similar concentration to
293 those used by other authors in urban WWTPs (Mbamba et al., 2019). Under these
294 conditions a COD_{tot} removal efficiency of 88% was achieved, and higher doses barely
295 enhanced COD_{tot}. A noticeable pH dropped from 7.8 to 6.8 was observed under these
296 conditions, which was even higher with higher FeCl₃ doses.

297 Figure 1B displays the influence of FeCl₃ on phosphate, inorganic carbon and
298 ammonium concentration. Whereas phosphate removal efficiency was above 99% with
299 doses from 100 mg/L of FeCl₃, in accordance with Diamantis et al. (2013), reaching a
300 phosphate discharge limit of 0.2 mg P-PO₄³⁻/L (Mbamba et al., 2019). IC showed a total
301 concentration decrease of 22 mg/L.



302



303

304 **Figure 1.** Influence of ferric chloride dose on (A) pH decrease (●) and on the removal
 305 of soluble COD (■) and particulate COD (▨) and (B) on the removal of phosphate (■),
 306 ammonium (▲) and inorganic carbon (●). * 0 mg /L refers to settling without coagulant
 307 addition.

308 The results from the Jar-tests experiments were validated in a continuous pilot plant
 309 under different wastewater characteristics but maintaining a ferric chloride dose of 125-
 310 150 mg/L, achieving very comparable results to those of the batch tests (detailed
 311 information of the results is gathered in Section S2 in the Supplementary Information).
 312 Under these conditions, a COD_{tot} to N-NH₄⁺ ratio close to 1 was achieved, this being

313 suitable for a PN-AMX unit according to Jin et al. (2012), who reported system failure
 314 with a COD_{tot} to N-NH_4^+ ratio above 2 due to the growth of heterotrophic denitrifiers
 315 which compete for nitrite. However, a minimum alkalinity-to-ammonium ratio of 1-1.25
 316 g IC to g NH_4^+ -N was reported as threshold to prevent the PN-AMX unit from
 317 acidification (Pedrouso et al., 2017) and subsequent inhibition due to the formation of
 318 free nitrous acid (Jin et al., 2012), so depending on the alkalinity and ammonium
 319 concentration in wastewater an external alkalinity dose could be required, substantially
 320 increasing the treatment costs.

321 **3.3. Performance of high-rate activated sludge reactor**

322 The results of the operation of the HRAS reactor are summarised in Table 3. COD
 323 removal efficiency was approximately 90%. However, COD recovery as sludge was
 324 slightly lower than in the CEPT- based configuration (71%), due to the partial oxidation
 325 of wastewater COD (averaged at 17% , which might be reduced working at lower HRT
 326 and/or SRT to maximize COD capture (Ge et al., 2017; Jimenez et al., 2015).

327 **Table 3.** Summary of the operation of the HRAS reactor.

Parameter	Value
pH	7.4 ± 0.3
SRT (d)	0.9 ± 0.1
VSS (mg VSS/L)	2.3 ± 0.4
$\text{COD}_{\text{removal}}$ (%)	87 ± 4
$\text{COD}_{\text{recovery}}$ (%)	71 ± 5
$\text{COD}_{\text{oxidation}}$ (%)	16 ± 10
N-NH_4^+ removal (%)	19 ± 4
P-PO_4^{3-} removal (%)	13 ± 4
$\text{IC}_{\text{removal}}$ (mg IC/L)	5 ± 1

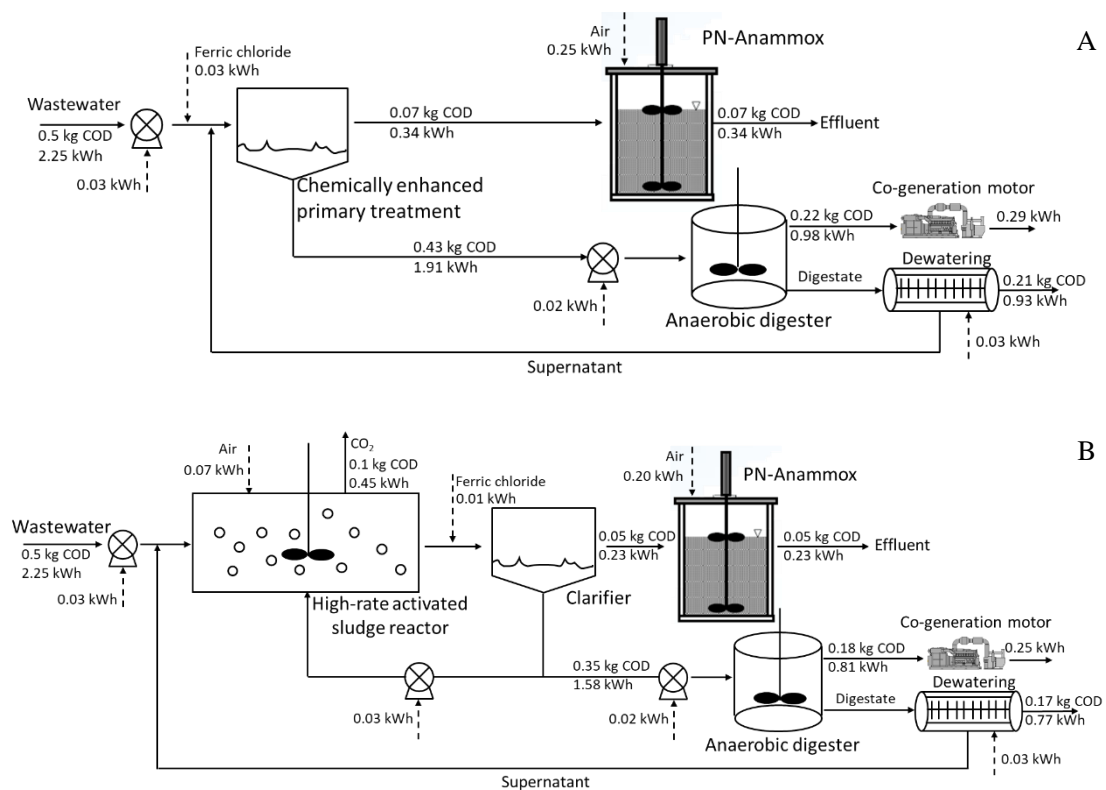
328
 329 Ammonium and phosphate removal efficiency resulted on 19% and 13%, respectively,
 330 and attributed to biomass growth. These results are in the range of those found
 331 elsewhere (Ge et al., 2017; Laurenzi et al., 2016), and also in accordance with Chan et al.

332 (2017), who found that a minimum SRT of 3.6-4 days is needed to obtain a proper
333 phosphorus removal. An average decrease of approximately 5 mg IC/L was observed,
334 much lower than in the CEPT- based configuration. Therefore, the effluent of the HRAS
335 reactor would satisfy the requirements for a PN-AMX unit (Jin et al., 2012; Pedrouso et
336 al., 2017) not only in terms of both IC-to-ammonium ratio but also on COD-to-
337 ammonium ratio. However, the effluent does not fulfil phosphate discharge limit of 0.2
338 mg P-PO₄³⁻/L (Mbamba et al., 2019) so a further removal through chemical
339 precipitation in the clarifier was studied, according to the recommendations of Longo et
340 al. (2017). By the addition of 10, 20 and 30 mg/L of FeCl₃, phosphate concentration in
341 the effluent decreased to 1.1 mg P-PO₄³⁻/L, 0.5 mg P-PO₄³⁻/L and 0.2 mg P-PO₄³⁻/L,
342 respectively. Thus, 30 mg/L was selected as the required FeCl₃ dose to carry out the
343 economic evaluation and to assess the OMPs and viruses removal efficiency.

344 **3.4. Energetic evaluation**

345 Figure 2 represents the energy flow in the WWTP based on: CEPT (A) and HRAS (B).
346 The HRAS-based configuration presents a higher energy demand for COD removal (0.1
347 kWh/m³ wastewater) due to aeration (0.07 kWh/m³ wastewater), and sludge
348 recirculation (0.03 kWh/m³ wastewater). Moreover, considering that a partial
349 ammonium removal is achieved in this unit, the energy demand of the PN-AMX unit
350 resulted on 0.20 kWh/m³ wastewater, whereas the energy consumption in the PN-AMX
351 unit of the CEPT-based configuration reached a higher value of 0.25 kWh/m³
352 wastewater. For the HRAS-based configuration, additional 0.01 kWh/m³ wastewater are
353 required to dose the coagulant to enhance phosphate removal efficiency. Finally, in both
354 alternatives 0.05 kWh/m³ of wastewater are needed for i) sludge thickening before AD
355 (0.02 kWh/m³ of wastewater) and ii) further sludge dewatering (0.03 kWh/m³ of
356 wastewater). Therefore, considering all the energy inputs, both configurations present a

357 comparable energy demand; CEPT approximately 0.36 kWh/m^3 wastewater (Figure 2A)
 358 and HRAS 0.39 kWh/m^3 wastewater (Figure 2B).



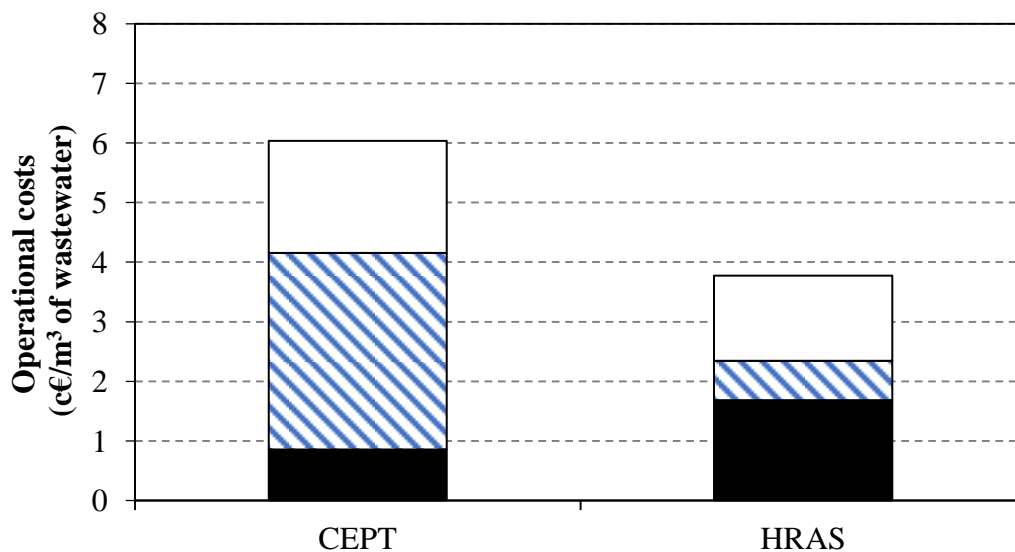
359 **Figure 2.** WWTP configurations studied in this work based on: A) CEPT and B) HRAS.

360

361 The results of the BMP test showed very comparable AB, 51% and 52% for CEPT and
 362 HRAS sludges, respectively, (results of the BMP test are discussed in detail in Section
 363 S3 in the Supporting Information). Since higher COD recovery from wastewater is
 364 obtained in the CEPT-based configuration, higher methane production is achieved (0.29
 365 kWh/m^3 of wastewater) in comparison with the HRAS-based one (0.25 kWh/m^3 of
 366 wastewater). The overall WWTP balance shows that the CEPT-based WWTP presents a
 367 lower energy demand (0.07 kWh/m^3 of wastewater) than the HRAS-based one (0.13
 368 kWh/m^3 of wastewater).

369 **3.5. Economic evaluation**

370 The operational costs include electricity, chemicals and sludge management. As
 371 explained in the previous section, the CEPT-based WWTP needs up to 0.8 c€/m³ of
 372 wastewater while the HRAS-based WWTP consumes 1.7 c€/m³ of wastewater (Figure
 373 3). Besides, a 20% higher digested solids production was achieved in the CEPT
 374 alternative (0.23 kg TSS/m³ of wastewater) than in the HRAS configuration (0.18 kg
 375 TSS/m³ of wastewater), due to the higher COD recovery attained in this alternative and
 376 also to the higher presence of inorganics in CEPT sludge (detailed information for the
 377 solids production is gathered in Section S4 in the Supplementary Information), this
 378 meaning 1.9 and 1.4 c€/m³ of wastewater for CEPT and HRAS-based WWTPs,
 379 respectively (Figure 3). Finally, the coagulant represents in the CEPT and HRAS
 380 configurations 3.3 and 0.7 c€/m³ of wastewater, respectively.



381
 382 **Figure 3.** WWTP operational costs due to energy (■), coagulant (▨) and sludge
 383 management (□).

384
 385 Considering jointly all costs, the alternative based on CEPT leads to significantly higher
 386 operational costs (6.0 c€/m³ of wastewater) in comparison with the HRAS-based
 387 configuration (3.8 c€/m³ of wastewater). Moreover, it must be highlighted that for the
 388 CEPT-based configuration the most optimistic situation was evaluated, considering that

389 these operational costs would be higher if an external alkalinity supply was needed.

390 **3.6. Fate of organic micropollutants**

391 *3.6.1. Chemically enhanced primary treatment*

392 Even though it seems to be a consensus in the literature that most OMPs are poorly
393 removed during CEPT, some authors reported that the different compositions of
394 wastewater can play a major role in OMPs elimination. For example, a high fat content
395 in wastewater was reported to improve the removal of hydrophobic compounds (log
396 solid-liquid equilibrium constant (K_D)>3.5, Table 4). In contrast, the presence of COD_{sol} ,
397 might inhibit the OMPs removal efficiency due to the preference of the coagulant for
398 COD_{sol} (Choi et al., 2008; Vieno et al., 2006).

399 **Table 4.** Relative amount of OMPs sorbed onto CEPT and HRAS sludges (K_D).

OMP	K_D (L/kg TSS)		
	Influent	CEPT	HRAS
ADBI	3,856 ± 845	2,461 ± 411	4,574 ± 832
HHCB	5,927 ± 2,168	3,412 ± 679	6,853 ± 1,945
AHTN	8,857 ± 2,148	5286 ± 1,066	9,969 ± 2,557
TCS	10,439 ± 2,170	5,918 ± 225	8,748 ± 1,635
IBP	8 ± 8	15 ± 2	16 ± 16
NPX	9 ± 9	0	18 ± 12
DCF	13 ± 3	7 ± 5	21 ± 2
ERY	25 ± 10	87 ± 18	40 ± 11
ROX	54 ± 13	200 ± 25	69 ± 12
SMX	35 ± 11	45 ± 15	52 ± 20
TMP	156 ± 47	108 ± 18	188 ± 28
FLX	1,355 ± 174	1,518 ± 219	1,750 ± 575
CTL	446 ± 67	547 ± 132	667 ± 53
CBZ	50 ± 15	101 ± 21	76 ± 15
DZP	91 ± 17	141 ± 22	166 ± 14
E1	399 ± 49	322 ± 22	346 ± 150
E2	359 ± 53	265 ± 23	599 ± 19
EE2	529 ± 44	407 ± 26	464 ± 6

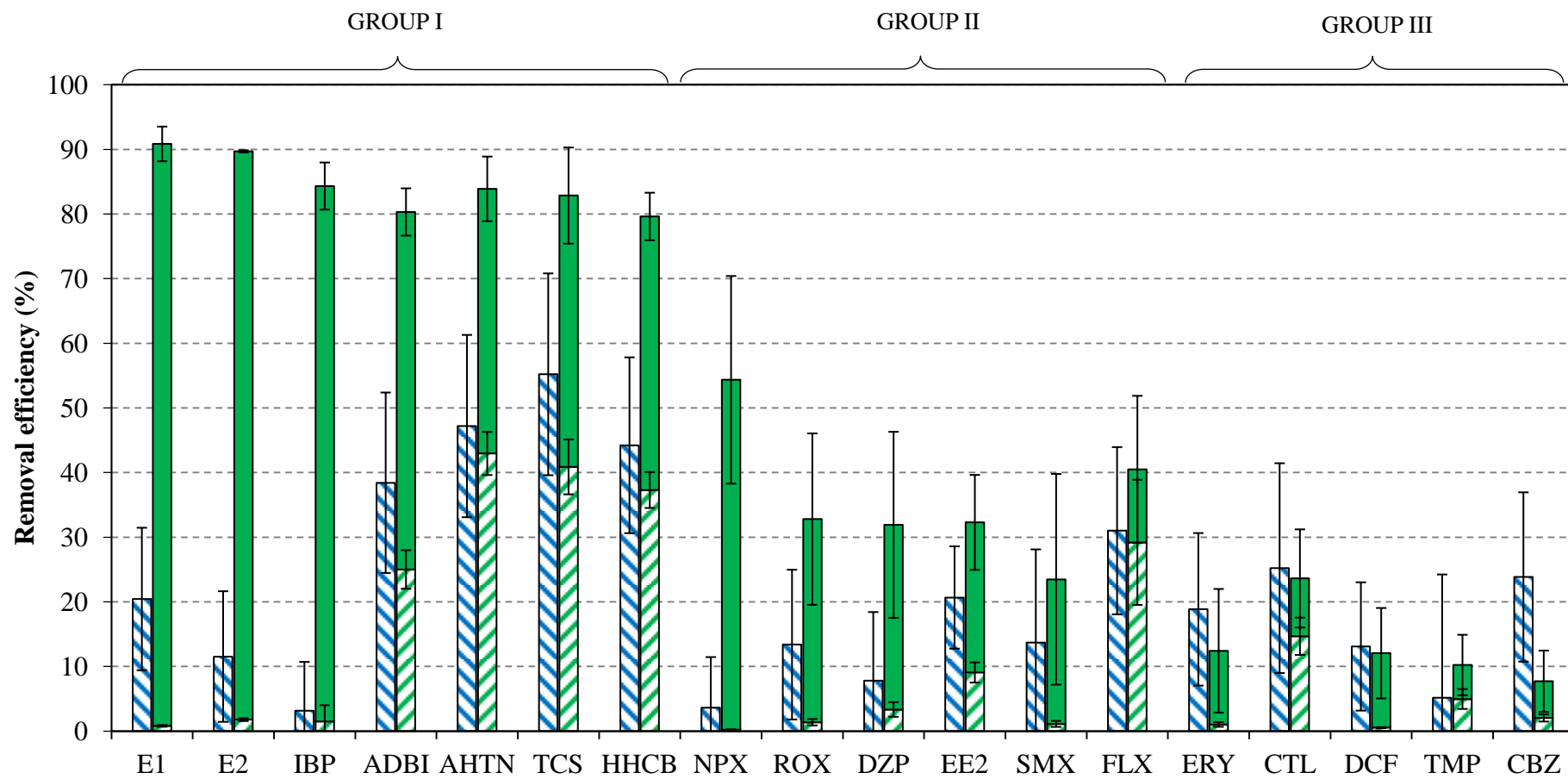
400

401 A medium-low removal efficiency (38%-55%) of hydrophobic OMPs (ADBI, AHTN,

402 HHCB and TCS) was obtained (Figure 4). This removal is attributed to the
403 corresponding to the sorbed fraction of these OMPs into TSS, in accordance with
404 Suarez et al. (2009).

405 For hydrophilic compounds, including anti-inflammatories, antibiotics, neurodrugs and
406 hormones, the removal efficiencies were below 31%, in accordance with other authors
407 (Bodzek and Dudziak, 2006; Suarez et al., 2009; Westerhoff et al., 2005), but in
408 disagreement with others who suggested that OMPs with pKa close to 7 such as IBP,
409 SMX or NPX are well removed in CEPT process by precipitating when adding metal
410 salts to wastewater (Carballa et al., 2005; Luo et al., 2014; Suarez et al., 2009)
411 .

412



413

414 **Figure 4.** OMPs removal efficiencies in CEPT due to sorption (▨); and in HRAS due to sorption (▧) and biotransformation (■).

415 3.6.2. High-rate activated sludge

416 Figure 4 compares the removal efficiency of OMPs achieved in the HRAS reactor with
 417 that found in the CEPT process. OMPs were classified into three groups according to
 418 their behaviour in this biological unit.

419 It can be observed that those OMPs presenting k_{biol} values >10 L/kg $v_{\text{SS}} \cdot \text{d}$ (Table 5)
 420 such as E1, E2, IBP, ADBI, AHTN, HHCB and TCS (Group I) are much better
 421 removed in the HRAS ($>80\%$) unit than in the CEPT one. For musk fragrances and TCS,
 422 not only biotransformation but also sorption onto sludge significantly contributed to
 423 their removal, explained by their high K_{D} values also in this biological sludge (Table 4).
 424 Even though the applied HRT in this work is much lower than in CAS reactors and that
 425 the k_{biol} for this group of OMPs are in general lower than those reported by other
 426 authors under nitrifying conditions (Table 5), they are well removed under these
 427 heterotrophic conditions.

428 **Table 5.** Kinetic pseudo first-order degradation constants (k_{biol}) measured in the HRAS.

OMP	k_{biol} (L/g $v_{\text{SS}} \cdot \text{d}$)		
	HRAS	Alvarino et al. (2014)	Suarez et al. (2010)
E1	57 ± 20	43	170
E2	46 ± 10	40	170
IBP	29 ± 9	24	20
ADBI	16 ± 4	63	75
AHTN	15 ± 5	38	115
TCS	13 ± 5	-	-
HHCB	11 ± 4	41	170
NPX	7 ± 3	9	9
ROX	3.3 ± 2.1	9	9
DZP	2.6 ± 1.6	0.4	<0.4
EE2	1.7 ± 0.9	7	20
SMX	1.5 ± 0.8	9	0.3
FLX	1.3 ± 1.1	10	9
ERY	0.9 ± 0.8	4	6
CTL	0.7 ± 0.6	-	3
DCF	0.5 ± 0.4	2	1.2
TMP	0.4 ± 0.3	0.6	0.15
CBZ	0.4 ± 0.3	0.2	<0.06

429 Group II includes OMPs such as NPX, ROX, DZP, EE2, SMX, and FLX, presenting
430 kinetic constants in the range 1-10 L/g_{VSS}·d and biotransformation efficiencies between
431 20 and 55% (Figure 4). These removal efficiencies were in general lower than those
432 found in CAS reactors and attributed to two reasons; i) the lower k_{biol} values found in
433 this work in comparison with those reported in CAS reactors for OMPs such as ROX,
434 EE2 and FLX (Table 5), proving the major role of nitrifiers on the biotransformation of
435 most of the OMPs (Fernandez-Fontaina et al., 2016; Men et al., 2017) and ii) the low
436 HRT applied in this reactor that kinetically limits the biotransformation of OMPs such
437 as NPX or DZP, which present comparable or higher k_{biol} values under heterotrophic
438 than under nitrifying conditions.

439 Finally, in Group III are included OMPs such as ERY, CTL, DCF, TMP and CBZ,
440 which present k_{biol} values lower than 1 L/g_{VSS}·d (Table 5) and subsequent
441 biotransformation efficiencies below 25% (Figure 4). In the case of ERY and CTL, the
442 k_{biol} values found in this work are again much lower than those reported in CAS systems
443 (Table 5). Contrary, DCF, TMP and CBZ are not removed neither in HRAS nor CAS
444 reactors.

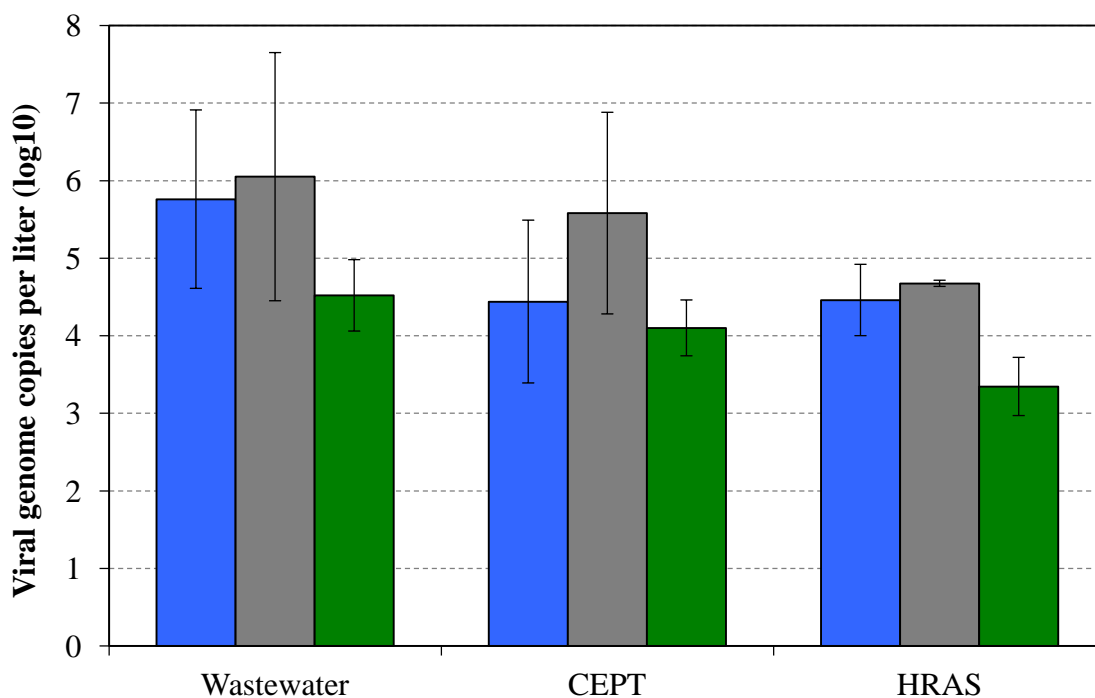
445 Moreover, unnoticeable reduction of OMPs concentration was found (<15%) after
446 treating the effluents of the HRAS reactors with 30 mg/L of FeCl₃ (data not shown),
447 being these variations attributed to analytical deviations, proving that the addition of
448 chemicals in secondary clarifiers was neither effective for the removal of OMPs.

449 The comparative against the CEPT shows that whereas for those OMPs classified in
450 Group I considerably higher biotransformation efficiency was achieved in the HRAS
451 reactor, for those of Group II and III the elimination efficiencies were quite comparable.
452 Although the results of this work indicate that HRAS is a better technology than CEPT
453 in terms of OMPs removal, further research is needed to holistically assess the fate of

454 OMPs in novel WWTPs.

455 3.7. Viruses removal

456 Recovery efficiencies were generally high, demonstrating minimal viral genome loss
457 during the extraction–RT-qPCR process. With the exception of HAV which was not
458 detected, all the other enteric viruses analysed were detected in untreated and treated
459 wastewater (Figure 5). GI and GII NoV were found at mean concentrations of 6.70 log₁₀
460 copies/L and 6.05 log₁₀ copies/L, respectively, whereas SaV was detected at mean
461 concentration of 4.52 log₁₀ copies/L. These values are in the range of those found in
462 wastewaters in different geographic areas, including USA, Japan or Tunisia (Ito et al.,
463 2017; Schmitz et al., 2016; Varela et al., 2018).



464

465 **Figure 5.** Removal of NoV GI (■), NoV GII (■) and SaV (■) in CEPT and HRAS,

466 estimated by the decrease of concentration of viral genome copies/L.

467

468 3.7.1. Chemically enhanced primary treatment

469 The results show that NoV GI experienced the greatest reduction values (1.3 log units;

470 94.5%), followed by NoV GII (0.5 log units; 66.5%) and SaV (0.4 log units; 62.2%)
471 (Figure 5). Such removal efficiencies were similar to those reported by other authors in
472 a WWTPs based on CAS reactors or trickling filters for a variety of viruses including
473 NoV, SaV or Aichi virus (Schmitz et al., 2016; Varela et al., 2018).

474 3.7.2. High-rate activated sludge

475 Viral reductions in the HRAS treatment were, in general, higher than those yielded in
476 the CEPT unit (Figure 5). Mean reductions for NoV GI, NOV GII and SaV were of 1.3
477 log units, 1.4 log units and 1.2 log units, respectively. These values are more in line with
478 those reported elsewhere in WWTPs based on advanced processes, such as five-stage
479 Bardenpho treatment (Schmitz et al., 2016). This system was originally designed to
480 enhance nutrient removal, and viral reduction could be related with a greater virus
481 adsorption to suspended particles than in other treatments.

482 Some differences in the removal efficiencies of viruses in the HRAS reactor were
483 observed between the sampling campaigns, indicating that slight variations in the
484 physico-chemical parameters (pH, TSS, COD, divalent cations, SRT, etc) may
485 differentially affect the removal of the diverse viral types. Likewise, for OMPs,
486 reduction of virus concentration was unnoticeable after treating the effluents of the
487 HRAS reactors with 30 mg/L of FeCl₃ (data not shown). The influence of WWTP
488 configurations and conditions on the efficiency of viral removal has been previously
489 indicated by different authors (Li et al., 2011; Schmitz et al., 2016), who also suggested
490 virus aggregation/disaggregation as one of the main reasons explaining the great viral
491 survival and resistance to diverse chemical compounds (Gerba and Betancourt, 2017).
492 Viral aggregation can also be responsible of inaccurate estimations of removal during
493 different treatment processes, such as ultrafiltration and reverse osmosis, usually
494 considered as more efficient than CAS.

495 It is important to point out that the procedures employed in this study cannot
496 differentiate whether the virus detected are infective or if the reductions observed are
497 due to physical removal or damage to the nucleic acid, making it no longer detectable
498 by qPCR. Further studies are needed in order to clarify these aspects.

499 **4. Conclusions**

500 Similar COD removal efficiencies are achieved in HRAS and CEPT, although the
501 former reaches lower recovery due to the partial oxidation of the influent COD.
502 Whereas in CEPT a high phosphate removal efficiency (>99%) is obtained, in HRAS it
503 is quite limited (13%), although it can be enhanced to 99% after a post-treatment with
504 30 mg/L of FeCl₃. Under these conditions, the HRAS-based configuration leads to
505 higher energy demand than that based on CEPT, however, the former results in lower
506 operational costs. In general, higher OMPs removal efficiencies were achieved in the
507 HRAS system, although for those recalcitrant they were in both systems quite
508 comparable and very low. HRAS presents higher potential for viral removal than CEPT,
509 but significant variations of performance against the different viruses were observed. All
510 in all, HRAS appears as a better technology than CEPT in novel WWTPs.

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