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3 **Temperature effect on performance and methane**

4 **emissions of highly controlled replicate septic tanks**

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15 **Abstract**

16 Septic tanks are widely used for decentralized wastewater treatment but remain poorly
17 characterized with respect to greenhouse-gas emissions, particularly under variable temperature
18 regimes. Understanding how temperature influences treatment performance and methane
19 production is essential for improving both emission inventories and environmental sustainability
20 through tailored mitigation strategies. This study used a unique set of twelve full-scale, replicate
21 septic tanks fed with real domestic wastewater to isolate the effect of four controlled temperature
22 conditions (ambient, insulated, 20 °C, 30 °C). Continuous monitoring of headspace gas, dissolved
23 methane and water quality enabled a complete carbon balance across gaseous, liquid, and
24 sludge pathways. Higher temperatures enhanced organic degradation and established stronger
25 anaerobic conditions, increasing methane production from ~42% under insulated conditions to
26 ~54% at 30 °C. Critically, temperature governed methane partitioning: at 30 °C, desorption was
27 favored and ~70% of the methane accumulated in the headspace, whereas at lower temperatures
28 (≤ 20 °C), a greater fraction (often >80% of methane) remained dissolved and was discharged
29 with the effluent. This behavior reveals that dissolved methane can represent a substantial fraction
30 of total methane generated in septic tanks, yet it is typically not quantified separately in standard
31 emission assessments. By jointly accounting for gaseous and dissolved pathways, our results
32 show that total methane release from septic tanks may be higher than estimates based solely on
33 headspace measurements. These findings highlight the importance of explicitly considering
34 dissolved-phase dynamics when refining emission factors and developing mitigation strategies.

35

36 **Keywords**

37 Decentralized sanitation; septic tanks; temperature effect; methane partitioning.

38

39 **1. INTRODUCTION**

40 Septic tanks have long been a cornerstone of basic, decentralized wastewater treatment
41 systems, particularly in areas where centralized treatment systems are impractical or
42 unavailable. They are low-cost and are used to manage domestic wastewater for millions of
43 households worldwide. A standard septic tank is a very simple technology, consisting of a tank
44 made of one or more chambers. Wastewater separates into three layers: solids settle at the
45 bottom, clarified water remains in the middle, and floating materials, including fats, form a foam
46 layer at the top. Partial microbial degradation of pollutants in the wastewater and sludge occurs
47 through anaerobic digestion (AD). The AD generates more microbial cells (biomass) and gases,
48 such as methane and carbon dioxide. Septic tank effluent is usually discharged using a network
49 of pipelines into a drainage field, where it undergoes additional treatment through filtration, gas
50 desorption, and microbial degradation in the soil. Despite their prevalence, septic tanks are
51 poorly monitored and regulated and often fail to meet discharge standards (Ahmed et al., 2005;
52 Ravi and Johnson, 2021; Soewondo et al., 2025; Withers et al., 2012), which leads to pollutant
53 hotspots in the surrounding environment (Richards et al., 2016; Scott and Parsons, 2005).
54 Furthermore, it has been suggested that the emissions of methane from septic tanks could
55 constitute a significant portion of global emissions of greenhouse gas from wastewater
56 treatment (Cheng et al., 2022; Manga and Muoghalu, 2024), although direct measurements of
57 septic tank emissions are rare. The juxtaposition of needing affordable decentralised sanitation
58 with the potential for septic tanks to pollute local environments and the global atmosphere
59 makes experimentally quantifying the treatment outcomes and methane emissions an
60 imperative.

61 The former of these, treatment outcomes, has been addressed, in a large part, by a long but
62 sparse literature on research into septic tanks' performance and their environmental
63 implications, dating back to the 1970s (DeWalle and Schaff, 1980; Lawrence, 1973; Yates,

64 1985). This has led to new designs, with enhanced treatment, to meet increasingly strict
65 regulatory standards on discharges (Koottatep et al., 2025, 2020; Saeed et al., 2024; Singh et
66 al., 2019; Sorenson et al., 2023). Notwithstanding these innovations, it is widely accepted that
67 failure to regularly remove the sludge from tanks is a major cause of downstream septic tank
68 pollution (Tan et al., 2021; Wardhani et al., 2024). One way of mitigating this is to enhance the
69 biodegradation within the tank using AD processes to reduce the rate of sludge accumulation
70 and hence the frequency with which the tank needs to be emptied and thus reduce
71 homeowners' maintenance costs (Mahon et al., 2022; Pussayanavin et al., 2015). Typically,
72 microbial activity correlates positively with temperature (Viessman Jr. and Hammer, 1999) and
73 so methods of increasing the temperature of the tank have been deployed to increase sludge
74 degradation. Polprasert et al. (2018) demonstrated the efficacy of this approach, using solar
75 thermal energy to increase the tank temperature, with Connelly et al. (2019) correlating the
76 increased degradation with increased abundance in key organisms, such as hydrolysers.
77 However, inevitably, by increasing the AD of organic material the rate of methane production is
78 increased. And, given the potency of methane as a greenhouse gas, this improvement of
79 treatment at the expense of increased methane emissions presents a dichotomy.
80 In recent years, there have been some efforts to estimate the extent of methane emissions from
81 decentralized sanitation using models. So, for example, in USA, where approximately 25% of
82 the population rely on on-site systems, the methane emissions produced have been estimated
83 to 44% of the total emissions from domestic wastewater treatment systems (EPA, 2024). A
84 similar proportion of the population of continental Europe rely on septic tanks. In Scotland,
85 where more than 200,000 septic tanks, serve approximately 10% of the population, 90% of
86 whom are located in rural areas (Lawson et al., 2024), it has been shown that the carbon
87 footprint of conventional septic tanks can be 7 times higher per capita than for large urban
88 wastewater treatment plants (Gupta et al., 2024). However, the models used in these studies

89 are based on very sparse empirical data and, as a result, the predictions are subject to
90 significant uncertainties.

91 It is imperative, therefore, that we measure emissions (Poudel et al., 2023). Historically,
92 emissions from on-site technologies such as septic tanks have been neglected, and there is a
93 shortage of direct field measurements (Burchart-Korol and Zawartka, 2019). Diaz-Valbuena et
94 al. (2011) conducted a study in real septic tanks to assess methane emission rates, considering
95 both direct emissions (measured using a gas flux chamber and directly from the vent pipes) and
96 dissolved methane. Whilst their measurements from dissolved methane accounted for only a
97 small fraction (up to 11%) of the total in water temperatures from 12 to 27 °C, it has been shown
98 that methane can be supersaturated at low temperatures, increasing the environmental impact
99 of technologies performing anaerobic digestion (Gómez-Borraz et al., 2022). In STs where
100 biogas is not recovered, the emission becomes the total amount of methane produced, which
101 includes both the gas measured in the gas phase and the fraction that remains in the liquid, as
102 dissolved methane. Because methane solubility in water increases at lower temperatures,
103 dissolved methane poses a problem when septic tanks operate under low temperatures
104 (<15°C), which is common in much of the global north (Mahon et al., 2022). One of the
105 difficulties in drawing conclusions on the factors affecting emissions in real septic tanks is that
106 each one is different and subject to the vagaries of, amongst other things, local weather and
107 influent composition (Connelly et al., 2019). Field studies of methane emissions have been
108 complemented by laboratory studies, which do provide valuable insights, but fail to capture the
109 complexity of real systems (Dubois et al., 2022; Shaw and Dorea, 2021). The ideal, therefore, is
110 a system of closely monitored, field-based, replicate septic tanks that retain the complexity of
111 real influent but where elements of the operating conditions can be controlled.

112 The aim of this study was to understand the trade-offs between enhanced treatment and
113 methane emissions using a unique system of twelve identical real-scale septic tanks fed from

114 the same waste stream. The tanks were operated as groups of three replicates under four
115 different temperature regimes, with the intention of varying the biological degradation rates, and
116 were instrumented to accurately and continuously measure emissions along with a range of
117 physical and chemical characteristics. The use of triplicate tanks and a common waste stream
118 give confidence that the conclusions we draw on treatment outcomes and emissions are a result
119 of controlling temperature and not an artifact of tank-specific complex biochemistry.

120

121 2. MATERIALS AND METHODS

122 2.1 System set-up and operation

123 The study was conducted in Gauldry, a small rural community in Fife, Scotland, with
124 approximately 600 people. It has a temperate marine climate and is located at sea level. The
125 average yearly temperature is 10 °C, with average-daily temperatures ranging from 14 to 19 °C
126 during summer (June to August) and typically 5 to 10 °C during winter (November to April).

127 There, a unique system consisting of 12, 1-m³ septic tanks (STs) was constructed and operated
128 for eight months, from April to November 2023 (Figure 1). The effective volume of each ST was
129 900 L (0.75 m height x 1 m wide x 1.2 m long), and it consisted of a one-chamber reactor with a
130 water seal for the inlet and outlet to prevent the gases from escaping the STs' headspace. Each
131 ST included four sample ports on the side for water and sludge sampling at different height
132 levels (0.5 m, 0.25 m, 0.5 m, and 0.70 m from the bottom of the ST). The STs were inoculated
133 with 80-90 L of primary sludge from the local wastewater treatment plant.

134 The STs were fed with real domestic wastewater collected after a coarse screen and a grit
135 channel. It consisted primarily of domestic raw sewage augmented by some rainwater runoff, as
136 there is no industrial activity in the village. A macerating pump fed the raw wastewater to a
137 manifold, which distributed the influent into 12 independent 100-L conical tanks. Using

138 electrically controlled valves, 100 L of wastewater were gradually released into each ST, twice a
139 day, simulating a 4.5-day hydraulic retention time (HRT). Each 'feeding period' lasted
140 approximately 50 minutes by doing 24 cycles as follows: 10 seconds on (valve open)/120
141 seconds off (valve closed), with an approximate inlet water flow of 0.42 L/s.

142 Three conditions were tested using real triplicates, including a set of insulated STs (INS) and
143 two heated sets using thermal jackets (Total Thermal Services, UK) to hold the water
144 temperature close to 20°C (20C) and 30°C (30C). A triplicate for the control (CON) was
145 operated under ambient conditions. The heated tanks were regulated by a 0.5-m thermocouple
146 attached to the top of each ST, measuring the water temperature at a height of 0.5 m.

147



148 **Figure 1.** Septic tank system location in Scotland (Gauldry) and experimental set-up.

149

150 2.2 System monitoring, sampling and analytical methods

151 Every ST unit featured real-time monitoring of pH, water temperature, and headspace methane
152 concentration. The monitoring system was 5G-enabled, allowing valves and sensors to be
153 controlled remotely from a computer, and ensuring that all data were continuously uploaded to

154 the cloud. A control box was integrated per ST triplicate to record the data remotely every 5
155 minutes for pH and temperature, and every 4 hours for the headspace methane concentration.
156 Each control box consisted of a plastic container with a microcontroller, the methane monitoring
157 unit (gas pump, return valve, methane sensor, and filters), pH probe inputs, and thermocouple
158 inputs. For the pH measurement, a pH probe was attached to the top of each ST and
159 submerged approximately 0.3 m below the water level; whereas for the temperature, two
160 thermocouples measuring 0.5 and 1 m in length were attached to the top of each ST unit. An
161 additional thermocouple was set outside one of the STs to record the ambient temperature. A
162 Gascard NG infrared methane gas sensor (Edinburgh Instruments Ltd.) was installed to
163 measure the headspace methane concentration on each ST. A water trap and silica beads filter
164 were installed before the sensor inlet to avoid moisture (>95% relative humidity) from entering
165 the methane sensor. A headspace gas sample from each ST was taken using a gas pump with
166 a flow rate of 0.6 L/h for 5 min and returned to the same ST through a one-way valve. After that,
167 a clean air stream (with the same flow rate) was passed through the filter and sensor to clean
168 the line. The procedure was repeated until all the STs' headspaces were measured.
169 Additionally, a Geotech Biogas 5000 portable analyzer (Cadmus, UK) was used monthly to
170 measure the concentration of methane, carbon dioxide, oxygen, and hydrogen sulfide from the
171 STs' headspace using the same ports for the on-line methane sensor monitoring.
172 Once a week, one liter of raw sewage (influent) and effluent samples from each ST were taken
173 in triplicate and stored at 4°C until their analysis (no later than 24 h) to monitor water quality
174 parameters according to the Standard Methods (APHA, 2017). The analyses performed
175 included weekly tests for total and soluble chemical oxygen demand (tCOD, sCOD), total
176 suspended solids (TSS), total organic carbon (TON), and total nitrogen (TN); bi-weekly tests for
177 nitrate, nitrite, ammonia, sulfate and total phosphorous (total P); and monthly samples for
178 biochemical oxygen demand (BOD_5) and alkalinity. Additionally, pathogen removal was

179 evaluated through the presence or absence of fecal coliform after incubation using the
180 membrane method, where raw wastewater was compared to treated effluent for each condition.
181 The removal efficiency corresponds to the percentage of reduction of each parameter after the
182 treatment in the ST.

183 Monthly 0.5-L samples were taken from the sample ports (0.1, 0.25, 0.5, and 0.70 m) to monitor
184 the stratification of dissolved oxygen (DO) and oxidation-reduction potential (ORP) using a Go-
185 direct optical dissolved oxygen (Vernier, USA) and ORP probes (Hanna Instruments, USA),
186 respectively. Finally, the sludge depth was monitored at the beginning and end of the
187 experiment using a 5-ft sludge judge (Cole-Parmer, USA).

188

189 2.3 Carbon mass balance using COD and dissolved methane measurements

190 Lobato et al. (2012) proposed a model for a carbon mass balance considering COD for
191 wastewater treatment in anaerobic reactors that, for the first time, integrated both the methane
192 lost with the effluent (dissolved methane) and the portion used in sulfate reduction. Following
193 their model, we incorporated into our results the estimation of the COD fraction used in sulfate
194 reduction, considering the influent sulfate concentration, biomass production (sludge retained in
195 the reactor), and the dissolved methane. So then, the complete COD mass balance included the
196 fractions (%) of soluble COD lost in the effluent ($COD_{not_converted}$), for biomass production
197 (COD_{sludge}), for methane production, both in the biogas ($COD_{CH_4_biogas}$) and dissolved
198 ($COD_{CH_4_dissolved}$), and used for sulfate reduction (COD_{SO_4}), Thus,

$$199 COD_{influent} = COD_{not_converted} + COD_{sludge} + COD_{CH_4_biogas} + COD_{CH_4_dissolved} + COD_{SO_4}, \\ 200 (1)$$

201 where $COD_{influent}$ represented the total amount of COD fed to each ST. The $COD_{not_converted}$
202 fraction refers to the portion of influent COD that was not removed or transformed within the

203 system and therefore remained in the effluent samples. Additional grab liquid samples were
204 taken by each set of STs in triplicate, to corroborate the above dissolved methane estimation
205 following the methodology proposed by Souza et al. (2011). The gas samples were analyzed in
206 an FID gas chromatograph (Agilent Technologies 7890B) using a HayeSep Q column.

207

208 **3. RESULTS AND DISCUSSION**

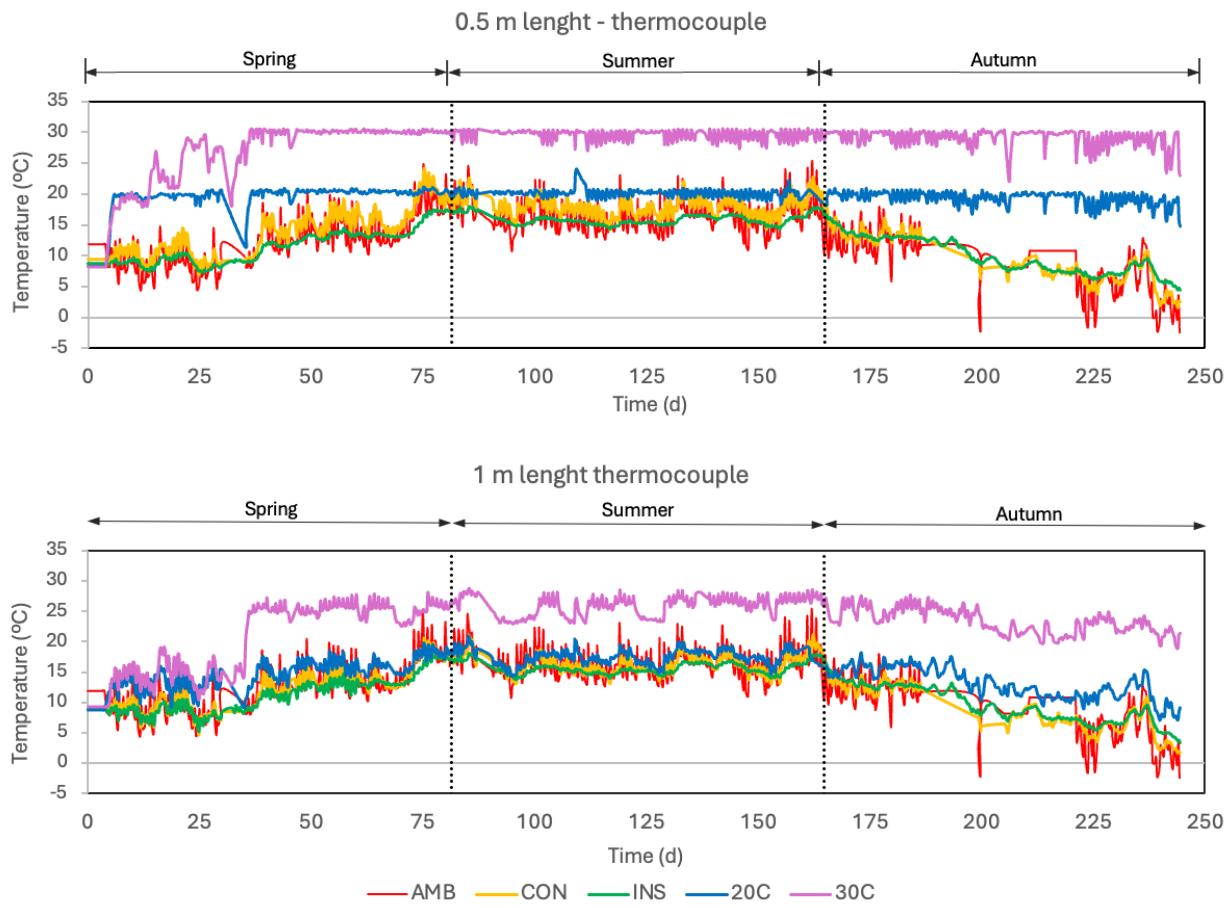
209 **3.1 Temperature and pH monitoring**

210 The STs were operated for 244 days. During this time, the ambient temperature was recorded
211 and compared to the bulk-water temperature of the control (CON), insulated (INS), and heated
212 at 20 (20C) and 30 °C (30C) STs. As mentioned above, two thermocouples were installed to
213 measure the water temperature, where the 0.5 m long one was used to control the heated STs,
214 and the 1m long one measured the temperature close to the bottom of the ST. Figure 2 shows
215 the average temperature profiles for each triplicate at the two locations of the thermocouples
216 (0.5 and 1 m depth). For the 0.5 m thermocouples, the CON STs presented a slightly higher
217 temperature compared to the INS STs during spring and summer. In contrast, the insulation in
218 the INS STs provided a thermal buffering effect, reducing the impact of low ambient temperature
219 in the colder months (after day 200).

220 The 20C was the most stable condition in terms of temperature, with variations within 5 degrees
221 from the desired temperature due to the incoming lower-temperature raw wastewater during the
222 feeding times. Meanwhile, for the 30C STs, it was necessary to add a supplementary heating
223 element beneath them at the beginning of the experimental work to reach the desired
224 temperature on day 32. From day 37 onwards, the temperature remained stable in the 30C set.

225 Since the mixing effect is extremely low in septic tanks (Mahon et al., 2022), the temperature
226 recorded at the bottom of the STs followed very closely the ambient temperature, except for the
227 30C that had the extra heating element integrated. Similar to the INS STs, the insulation jacket
228 provided a thermal buffering effect to the heated STs.

229 According to Shaw and Dorea (2021), a large number of studies on septic tanks indicate that
230 the operating temperature of these systems is within 10-40 °C, with a median of 24 °C.



231 **Figure 2.** Average temperatures recorded at the 0.5 and 1 m length thermocouples in the
 232 control (CON), insulated (INS), and 20 (20C) and 30 °C (30C) heated septic tanks, compared to
 233 ambient temperature (AMB).

234 The pH in the STs was very stable during the experimentation. The average values recorded
 235 were 7.1 ± 0.5 , 7.0 ± 0.4 , 6.7 ± 0.2 and 6.6 ± 0.3 for the CON, INS, 20C and 30C STs,
 236 respectively. Some algal growth was observed in the control STs since the tank (white colour)
 237 was not covered by any jacket and received direct sunlight. That could have influenced the
 238 slightly higher pH values, while the lower pH in the heated STs is an indication of the enhanced
 239 microbial activity, which means a higher concentration of volatile fatty acids and carbonic acid
 240 (from the produced CO_2) in the bulk liquid (Wang et al., 2019).

241

242 **3.2 Water quality analysis**

243 The influent can be considered a low-medium strength domestic wastewater. The seasonal
244 variation of the raw wastewater used is presented in Table 1. High sCOD and tCOD
245 concentrations were found during summer and declined in autumn. This trend may be attributed
246 to increased biological activity in warmer months and dilution effects from rainfall in autumn. For
247 example, BOD, tCOD and SST values ranged from 25 to 262 mg/L, 71 to 855 mg_{tCOD}/L and 26
248 to 442 mg_{SST}/L, with the lowest values occurring in autumn after rain events and the highest in
249 summer. It is noteworthy that, because the raw sewage was drawn from an open channel
250 leading into an open primary sedimentation tank, ambient conditions influenced the wastewater
251 strength, including dilution by rainwater during autumn. In general, all the parameters are in
252 accordance with previous reports for this type of wastewater (domestic with rainfall influence) in
253 the UK (Martin Garcia et al., 2013; Trego et al., 2021).

254 Table 1. Seasonal variation of domestic wastewater quality parameters (Mean values ±
255 standard deviation).

Parameter	Unit	Season		
		Spring	Summer	Autumn
BOD	mg/L	199 ± 80	191 ± 101	117 ± 11
tCOD	mg _{tCOD} /L	474 ± 195	549 ± 241	203 ± 140
sCOD	mg _{sCOD} /L	143 ± 51	205 ± 93	89 ± 56
SST	mg/L	198 ± 91	217 ± 117	88 ± 65
Total N	mg/L	37 ± 21	55 ± 25	18 ± 14
NO ₂ ⁻	mg/L	0.5 ± 0.4	0.0 ± 0.0	0.3 ± 0.2

NO_3^-	mg/L	0.8 ± 0.5	0.4 ± 0.0	1.4 ± 1.0
NH_4^+	mg/L	31 ± 19	42 ± 19	19 ± 9
Total P	mg/L	4.7 ± 1.9	6.1 ± 2.5	2.5 ± 2.1
SO_4^{2-}	mg/L	45 ± 10	49 ± 4	28 ± 17
Conductivity	mg/L	0.7 ± 0.0	0.8 ± 0.2	0.4 ± 0.3
Alkalinity	mg/L	286 ± 0	335 ± 124	136 ± 117

256

257 The effectiveness of septic tanks in waste removal varies significantly based on several factors,
 258 including the type of wastewater (blackwater alone or combined), the number of chambers, and
 259 the frequency of sludge removal, among others. Therefore, this experiment can be considered a
 260 typical example of a one-chamber septic tank treating real domestic wastewater under good
 261 maintenance practices, operating under different temperature regimes.

262 According to Dasgupta and Agarwal (2021) BOD removal in a conventional ST is expected to
 263 be between 30 and 50%. Our findings showed BOD removals above 55% for all the treatments,
 264 with averages of 55 ± 12 , 55 ± 13 , 62 ± 12 and 73 ± 13 % for the CON, INS, 20C, and 30C
 265 conditions, respectively. These values corresponded to average BOD concentrations in the
 266 effluent ranging from 53 to 90 mg/L, with absolute concentrations varying between 28 and 157
 267 mg/L (from ST 20C and INS). Figure 3 shows the seasonal variation for tCOD, sCOD and TSS
 268 removal efficiency for the control (CON), insulated (INS), heated at 20 (20C) and 30°C (30C)
 269 triplicates. A positive correlation was observed between operational temperature and RE across
 270 all parameters. The 30C condition consistently demonstrated the highest RE. However, all the
 271 conditions exhibited their highest RE for the three parameters during the spring season. This
 272 could be related to the start-up period, during which a higher amount of solids and organic
 273 matter retention may have occurred in the system. Both tCOD and TSS removal reached a

274 semi-steady state from the beginning of the operation, despite variations in the influent strength,
275 demonstrating the robustness of the technology. The average tCOD removal in the heated STs
276 was above 65%, with effluent tCOD concentrations ranging from 78 to 461 mg/L. In comparison,
277 the CON and INS STs achieved a tCOD removal efficiency of 56-60%, with the lowest values
278 being 107 and 102 mg_{tCOD}/L, respectively. The TSS removal was above 80% and up to 92 ± 3%
279 in the 30C STs, whereas the CON and INS STs showed peaks in the effluent concentration
280 following the trend from the inlet wastewater (data not shown).

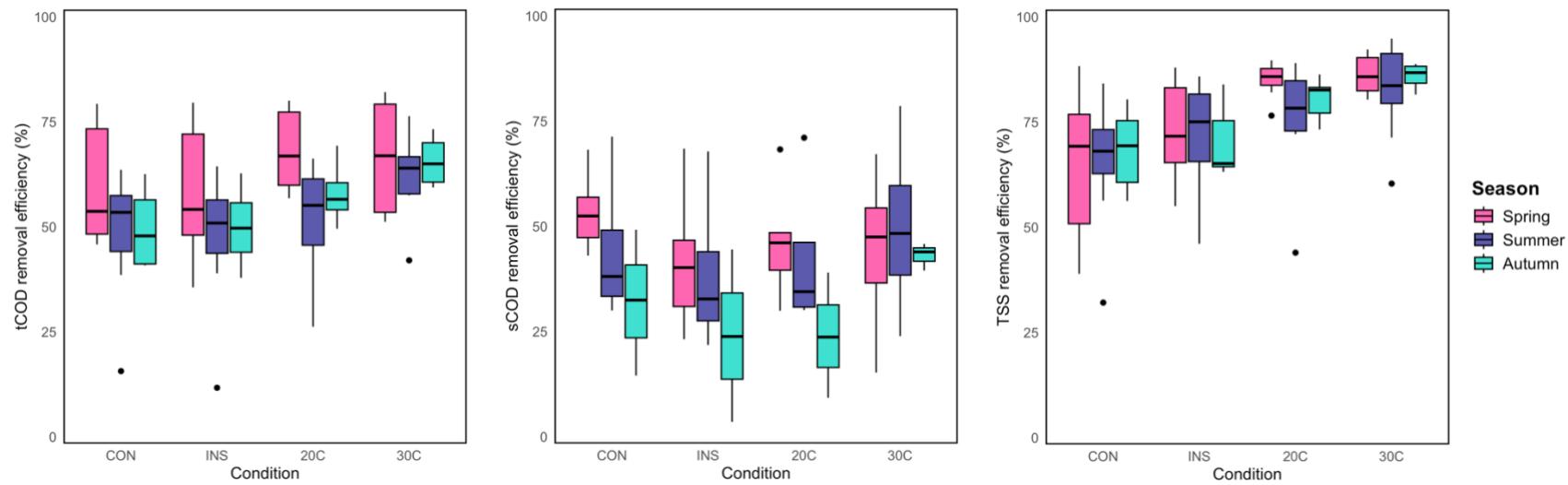
281 In the case of sCOD, the highest removal efficiency was observed in the 30C STs, followed by
282 the CON condition. This may be due to the algal biofilm growth seen on the walls of the STs,
283 which was promoted by the absence of covering (jacket) in this condition, potentially facilitating
284 aerobic processes within those STs. Similar results were found by Nasr and Mikhaeil (2013)
285 using domestic wastewater in conventional (one chamber) septic tanks under a similar HRT (72
286 h), at an average temperature of 27.8 ± 4 °C. They reported average removal efficiencies of
287 65% both for tCOD and TSS, with average influent concentrations of 960 and 295 mg/L,
288 respectively. In this study, the highest tCOD and SST influent concentrations were observed
289 during the summer, at 549 and 217 mg/L, respectively. The removal efficiencies were 64% for
290 tCOD and 85% for SST. Overall, the heated septic tanks (20C and 30C) removed higher
291 concentrations of organic matter and suspended solids.

292 The seasonal effect was evident in the non-heated STs (CON and INS), especially when colder
293 ambient temperatures were recorded. Once the ambient temperatures dropped below 10 °C, a
294 decrease in both COD and solids removal was observed. Lettinga et al. (2001) described the
295 effects of psychrophilic temperatures in wastewater anaerobic processes: firstly, solids
296 separation due to sedimentation becomes slower under low temperatures when the liquid
297 viscosity increases; then, since the majority of known methanogenic microorganisms are
298 mesophilic and thermophilic, with optimal temperatures of 37 and 55 °C, respectively, it is

299 expected to have reduced microbial activity in psychrophilic conditions. Previous studies have
300 even reported that anaerobic activity decreases at least one-tenfold when the water-bulk
301 temperature drops in the range of 5 to 35 °C (Viessman Jr. and Hammer, 1999). Still, Halalsheh
302 et al. (2005) reported no significant effect of temperature, when it was between 18 and 25 °C for
303 tCOD and sCOD for UASB reactors fed with high-strength wastewater (tCOD ~ 1500 mg/L).

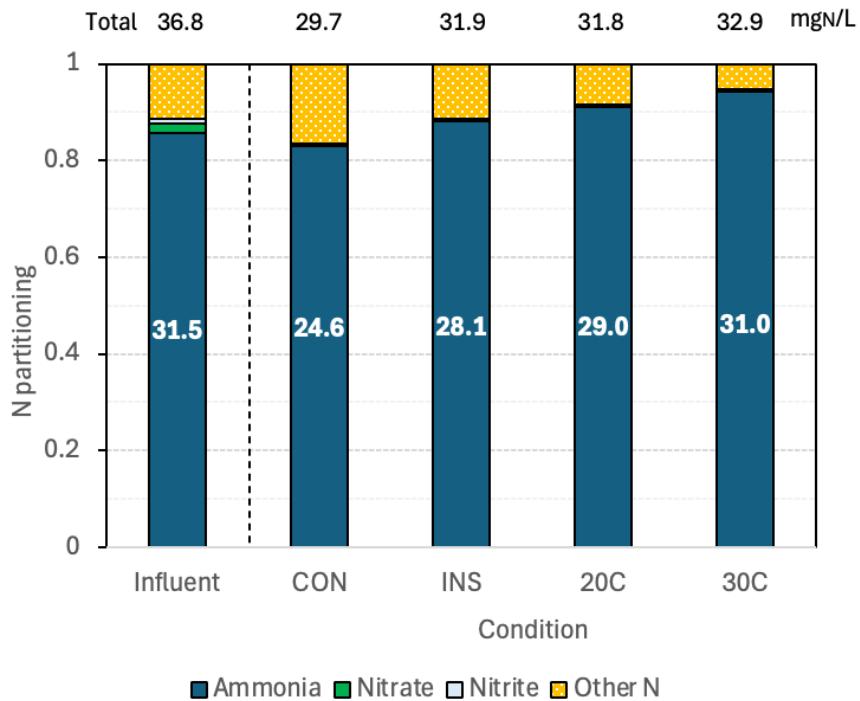
304 Nitrogen removal is a crucial step in wastewater treatment, and biologically, it is achieved
305 through a combination of aerobic and anaerobic processes. Therefore, it is natural to have very
306 low nitrogen removal rates after wastewater receives treatment in a septic tank (anaerobic
307 processes only). The main nitrogen compound in domestic wastewater is ammonia (NH₃), which
308 can also be present as ammonium ion (NH₄⁺) depending on the pH. In this study, total nitrogen,
309 ammonia, nitrates, and nitrites were measured both in the treated and untreated wastewater.
310 Results shown in Figure 4 indicate that ammonia represents the major nitrogen form in all the
311 samples. It ranged from 25 to 32 mg/L, representing around 86% in the influent and 83 to 94%
312 in the effluent samples, and in accordance with typical values for domestic wastewater (Körner
313 et al., 2001). Total nitrogen removal rates were below 15%, except for the CON STs where the
314 overall nitrogen removal was around 20%, similar to the 22% ammonia elimination. This result
315 can be explained by the fact that the algae biofilm growth in the CON STs' wall could act as
316 microzones where aerobic activity occurred. Overall, our results confirm that nitrification
317 processes and ammonia removal are improbable in STs, as are anammox reactions, due to the
318 slow growth of these microorganisms, which hampers their development in conventional STs
319 (Shaw and Dorea, 2021).

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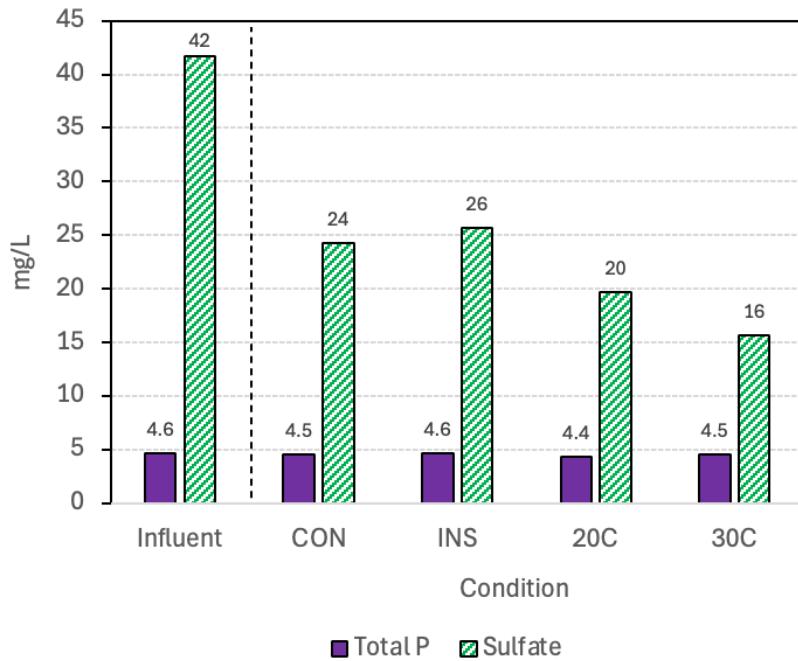
321

322 Figure 3. Total and soluble COD (tCOD, sCOD) and total suspended solids (TSS)
 323 removal efficiency (%) in the control (CON),
 insulated (INS), and 20 (20C) and 30 °C (30C) heated septic tanks.



324 Figure 4. Average nitrogen partitioning in the influent wastewater, and the treated effluent of the
 325 control (CON), insulated (INS), and 20 (20C) and 30 °C (30C) heated septic tanks.
 326 Additionally, total phosphorus and sulfate concentrations were measured as part of the nutrients
 327 found in raw domestic wastewater. Both nutrients were within the typical range reported by
 328 other studies for typical domestic wastewater, at $4.5 \pm 0.1 \text{ mg}_{\text{TotalP}}/\text{L}$ overall, and 41.7 and $21.3 \pm$
 329 $4.5 \text{ mg}_{\text{SO}_4^2-}/\text{L}$ for the raw and treated wastewater, respectively (Sahinkaya et al., 2018). It is
 330 known that anaerobic conditions in a septic tank promote the growth of sulfate-reducing
 331 bacteria. This set of bacteria
 332 Zuo et al. (2019) demonstrated a strong correlation between H_2S emission peaks and increased
 333 water flow, specifically water turbulence, which releases sulfide present in the liquid fraction.
 334 This may explain why, in our study, we could not detect H_2S production exceeding 5 ppmv
 335 under any of the septic tank conditions tested, despite a decrease in sulfate concentration.
 336 Monitoring sulfide and H_2S is crucial yet often overlooked in these systems. For instance,

337 concentrations as low as 2 mgS/L can compromise the septic tank's integrity by causing
338 corrosion, while H₂S emissions can present significant health risks.



339 Figure 5. Average total phosphorus (Total P) and sulfate concentration in the influent
340 wastewater, and the treated effluent of the control (CON), insulated (INS), and 20 (20C) and 30
341 °C (30C) heated septic tanks.

342 Pathogen content is one of the most important parameters often regulated in direct discharges
343 of treated effluents. In this study, the fecal coliform content was used as an indicator for
344 pathogens. Two sample campaigns were performed at the beginning of the experimental work
345 (May 15th), and once the septic tanks demonstrated a semi-steady-state operation (September
346 11th). The results are shown in Table 1. Fecal coliform levels in the raw wastewater ranged from
347 6 to 12 X 10⁷ CFU/100 mL. A one-tenth decrease was observed in the case of CON, INS and
348 20C STs. In terms of sanitation, heating the water temperature in a septic tank to 30 °C resulted
349 in favorable conditions for a higher fecal coliform removal. However, it is known that other
350 pathogens, such as *Salmonella* spp and helminth eggs, can survive and reproduce at higher
351 temperatures (Scaglia et al., 2014). Therefore, a broader monitoring of pathogens is

352 recommended depending on the final use of the treated wastewater. Still, fecal coliforms were
353 two orders of magnitude lower compared to Nasr and Mikhæil (2013), who reported a removal
354 efficiency of 86% at 27.5 ± 4.3 °C.

355 Table 1. Average values for fecal coliforms (CFU/100 mL) from the raw and treated wastewater
356 at the start and steady state operation.

	May	September
INLET	$6.3 \times 10^7 \pm 5.6 \times 10^6$	$9.0 \times 10^7 \pm 3.4 \times 10^7$
CON	$1.1 \times 10^6 \pm 9.1 \times 10^5$	$2.1 \times 10^6 \pm 1.8 \times 10^6$
INS	$2.9 \times 10^6 \pm 2.2 \times 10^6$	$1.5 \times 10^6 \pm 1.0 \times 10^6$
20C	$1.3 \times 10^6 \pm 7.7 \times 10^5$	$3.1 \times 10^6 \pm 7.8 \times 10^5$
30C	$5.0 \times 10^5 \pm 3.1 \times 10^5$	$2.9 \times 10^5 \pm 1.3 \times 10^5$

357

358 3.3 Anaerobic conditions and methane emissions monitoring

359 Dissolved oxygen (DO) and oxidation-reduction potential (ORP) were monitored inside the
360 septic tanks to assess the degree of anaerobicity achieved under each operational condition
361 (Table 2).

362 Table 2. Dissolved oxygen (DO) and Oxidation-Reduction potential (ORP) monitoring inside the
363 control (CON), insulated (INS), and 20°C (20) and 30°C (30C) heated septic tanks.

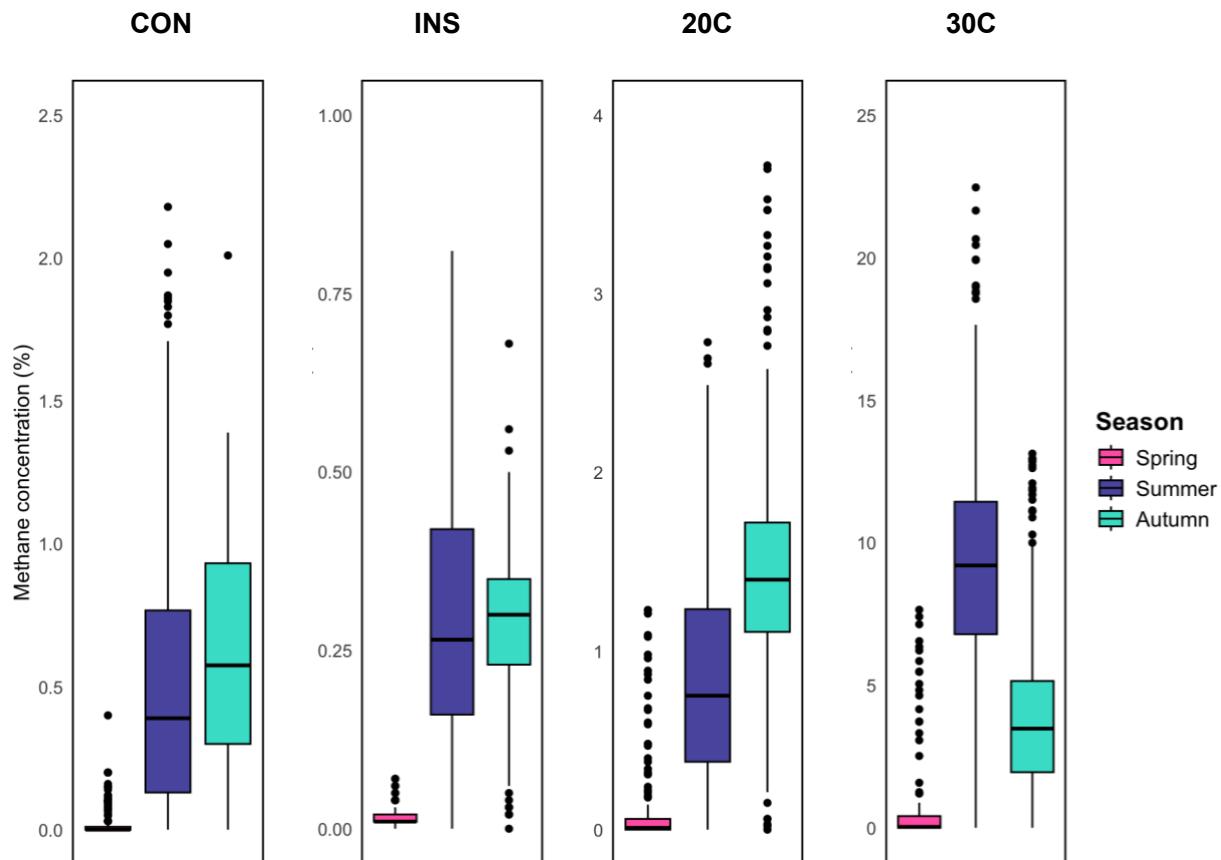
	DO range (mg/L)	ORP range (mV)
CON	1.12 – 3.01	-135 to -211
INS	1.20 – 3.04	-130 to -216
20C	0.88 – 2.10	-183 to -225
30C	0.43 – 2.08	-206 to -261

364

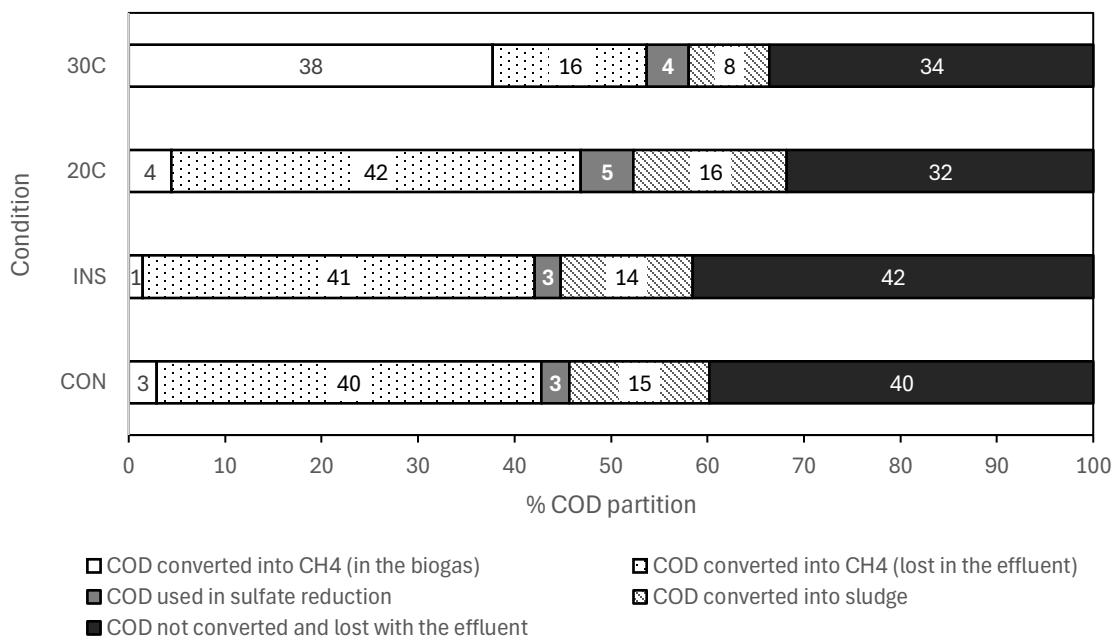
365 Results indicate that 30C STs exhibited the most strongly reducing environment, characterized
366 by the lowest DO (0.43 to 2.08 mg/L) and the most negative ORP values (-206 to -261 mV).
367 According to Huynh et al. (2021), lower ORP correlates strongly with higher methane emissions
368 ($R = -0.67$; $p = 0.034$). Particularly, STs with long empty intervals were found under complete
369 anaerobic conditions, demonstrated by ORP values ranging from -230 to -489, with 3.9 to 19
370 years of storage (Huynh et al., 2021). Another study found ORP values ranged from -150 to -
371 210 mV in conventional STs in the USA (Diaz-Valbuena et al., 2011). Our results overlap with
372 these ranges, with the 30C condition being the most effective at fostering anaerobic conditions.
373 The 20C condition also enhanced anaerobic conditions compared to the CON and INS STs,
374 suggesting a moderate increase in methanogenic potential.

375 The progressive decrease in ORP with increasing temperature supports the notion that
376 temperature primarily affects methane production indirectly by accelerating organic degradation
377 and depleting residual oxygen. Once anaerobic conditions are firmly established ($ORP < -150$
378 mV), however, several authors have reported that further increases in temperature or reductions
379 in DO have limited impact on methane yield (Huynh et al., 2021; Moonkawin et al., 2023). For
380 example, comparative field observations between winter and summer (with ~ 9 °C temperature
381 difference) in STs showed negligible differences in methane emission rates, suggesting that
382 temperature alone (within sub-optimal ranges) may not strongly influence methane emissions if
383 anaerobic conditions are already established. However, studies with more extreme temperature
384 differences, such as mesophilic anaerobic digesters operated at 25 °C and up to 34 °C
385 demonstrate that while biogas yield increases with temperature, yields at 31 °C are still close to
386 those at 34 °C (~90%), whereas those at 25 °C are significantly lower (~70%) (Babaei and
387 Shayegan, 2019). Similar behavior was observed here: after the start-up phase, methane
388 generation in CON and INS tanks stabilized across summer and autumn, while greater
389 variability was found in the temperature-regulated reactors (20C and 30C), likely due to

390 transient cooling from the daily cold-water inputs. Nonetheless, the 30C STs consistently
391 produced biogas with methane concentrations reaching up to 23% v/v in the headspace,
392 confirming active methanogenesis under enhanced thermal conditions.



402 loading and limited mixing. In the last decades, the supersaturation term has been used to refer
 403 to a higher amount of dissolved methane than the one theoretically calculated using the
 404 equilibrium constant (Henry Law), temperature, and gas phase partial pressure data (Cookney
 405 et al., 2016; Noyola et al., 1988; Souza et al., 2011; Zhang et al., 2022). This dual pathway
 406 agrees with previous findings in anaerobic domestic wastewater systems, where dissolved
 407 methane can represent between 20% and 60% of total emissions depending on temperature,
 408 hydraulic retention and turbulence (Crone et al., 2016; Lobato et al., 2012; Souza et al., 2011).



409

410 Figure 7. Average COD partitioning in septic tanks.

411 In our study, the persistence of high dissolved methane concentrations, even under conditions
 412 where the headspace contained elevated methane levels, indicates a rapid but incomplete gas-
 413 liquid equilibration periodically disrupted by feeding events (turbulence) and therefore transient
 414 pressure variations. As expected, since methane solubility is temperature-dependent, this also
 415 highly influenced methane partitioning in STs. It is clear that higher temperature (30 °C)
 416 enhanced desorption from the liquid phase, leading to greater accumulation of methane in the
 417 headspace. Nevertheless, a considerable fraction of the total methane produced, corresponding

418 to approximately 30% of the total COD transformed into methane, remained in the liquid phase
419 and was subsequently discharged with the effluent. Under cooler conditions (CON and INS),
420 this fraction increased markedly (>90%), highlighting that temperature not only governs
421 methanogenic activity but also the balance between gaseous release and dissolved retention.
422 These results confirm that even in well-developed anaerobic environments, the dissolved phase
423 represents a substantial portion of methane-derived COD. This pathway is rarely measured
424 despite having clear implications for both energy recovery and greenhouse-gas accounting
425 (Crone et al., 2016; Gómez-Borraz et al., 2025, 2017; Huete et al., 2018). This observation
426 challenges conventional assumptions used in global inventories and current emission models.
427 While the IPCC methodology provides a simplified framework for estimating total methane
428 generation from septic tanks, it does not explicitly distinguish between methane released as
429 biogas and methane remaining dissolved in the effluent. Our findings show that, under certain
430 operating conditions, particularly at lower temperatures, a considerable amount of the methane
431 produced can remain in the liquid phase, underscoring the importance of accounting for this
432 route when assessing total methane releases.
433 The clear coupling observed between redox potential, temperature and methane partitioning
434 underscores that STs are dynamic emitters whose gaseous and dissolved fluxes respond to
435 subtle operational and environmental changes. These insights emphasize the need for
436 monitoring approaches that encompass both phases and for inventory frameworks that can
437 better reflect the multiple routes through which methane is released from on-site systems.
438 Recognizing and quantifying both pathways is essential for improving greenhouse-gas
439 assessments of decentralized sanitation systems and for guiding the development of future
440 emission factors and mitigation strategies.
441

442 **CONCLUSIONS**

443 This study demonstrates that temperature is a primary driver of both treatment performance and
444 methane dynamics in full-scale septic tanks treating real domestic wastewater. Higher water
445 temperature enhanced organic degradation and strengthened anaerobic conditions, increasing
446 methane generation: the fraction of influent COD converted to methane increased from
447 approximately 45% under insulated conditions to up to 54% at 30 °C. Crucially, temperature
448 also strongly influenced methane partitioning between dissolved and gaseous phases. Under
449 cooler conditions (≤ 20 °C), methane was predominantly retained in the liquid phase, with up to
450 98% of produced methane remaining dissolved and discharged with the effluent. In contrast, at
451 30 °C, enhanced desorption favored methane transfer to the gas phase, reducing the dissolved
452 fraction to around 30% and resulting in headspace accumulation. This highlights an emission
453 pathway that is rarely quantified in routine monitoring.

454 The coexistence of high dissolved and gaseous methane fluxes indicates that septic tanks
455 behave as dynamic dual emitters whose emissions cannot be reliably assessed from
456 headspace measurements alone. These findings underscore the importance of considering
457 dissolved-phase methane in greenhouse gas assessments of decentralized sanitation systems
458 and in future refinements of emission factors.

459 From a management perspective, strategies aimed at improving treatment performance by
460 increasing temperature must be evaluated alongside their potential to elevate methane
461 emissions. Integrating dissolved-methane monitoring, developing mitigation approaches (e.g.,
462 degassing or oxidation stages), and improving inventory methodologies will be essential for
463 accurately capturing the climate impact of septic tanks and informing sustainable sanitation
464 practices.

465

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475

476 **CRediT author contribution**

477 **Tania L. Gómez-Borraz**: Data curation, formal analysis, investigation, methodology,
478 supervision, writing-original draft, review and editing. **Calum Cuthill**: Data curation,
479 investigation, methodology. **Tymon Herzyk**: Investigation, methodology. **Stephanie Connolly**:
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482

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