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1 **On-site domestic wastewater treatment system using shredded waste plastic**
2 **bottles as biofilter media: pilot-scale study for effluent standard in Bhutan**

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10 **Abstract:**

11 In this study, a 1,000 L/d capacity one-off on-site wastewater treatment system was
12 operated for over a year as a pilot alternative to the conventional on-site treatment as
13 currently used in urban Bhutan. An up-flow anaerobic sludge blanket (UASB) was used for
14 blackwater treatment (to replace “septic tank followed by an anaerobic biofilter (ABF) (to
15 replace soak pits) for the treatment of a mixture of greywater and UASB effluent. Shredded
16 waste plastic bottles were used as the novel biofilter media in the ABF. During a yearlong
17 operation, the pilot system produced a final treated effluent from ABF with average BOD 5
18 28 mg/L, COD 38 mg/L, TSS 85 mg/L and 5 log units of *Escherichia coli* These effluents meet
19 three out of four of the national effluent discharge limits of Bhutan, but unsuccessful to
20 meet the *Escherichia coli* standard over a yearlong operation. Further process optimisation

21 may enable more significant *Escherichia coli* removal. An economic analysis indicates that
22 the total unit cost (capital and operating expenditures) of this alternative wastewater
23 treatment system for more than 50 users ranges between USD 0.27 - 0.37/person/month
24 comparable to USD 0.29 - 0.42/person/month for the current predominant on-site system,
25 i.e., “septic tanks”. This pilot study, therefore, indicates that this wastewater treatment
26 system using shredded waste plastic biofilter media has high potential to replace the
27 current conventional treatment, i.e., “septic tanks”, which are often overloaded with grey
28 water and discharging effluent which does not meet the national standards.

29 **Keywords:** ABF, domestic wastewater, on-site sanitation, plastic wastes, pollution, UASB

30 **1. Introduction**

31 Providing safe drinking water and improved sanitation facilities is listed under the
32 Goal 6 (clean water and sanitation) of the UN Sustainable Development Goals to safeguard
33 public health and well-being while at the same time protecting and restoring water-related
34 ecosystems (UN 2017). Conventional activated sludge processes are the most widely
35 adopted treatment technology in the world for human faeces (Tchobanoglous 2014).
36 However, most recently, aerobic granular sludge processes have been gaining significant
37 interest because of their compactness and greater cost-effectiveness (Nancharaiah &
38 Sarvajith 2019). However, these mainstream treatment technologies are still capital and
39 energy-intensive (Hahn & Figueroa 2015), making them less affordable for most developing
40 countries. Anaerobic treatment of municipal wastewater is considered to be the most
41 sustainable because this treatment approach requires no or very little energy to operate
42 and generates energy in the form of biogas (Verstraete, Van de Caveye & Diamantis 2009).
43 Anaerobic processes for municipal wastewater treatment with biogas generation are

44 commonly adopted in tropical and sub-tropical regions for waste with chemical oxygen
45 demand (COD) removal ranging from 40 - 75% (Heffernan, Van Lier & Van Der Lubbe 2011).
46 However, anaerobic treatment technology is not yet investigated in temperate climates
47 such as Bhutan. Simple anaerobic bioreactors such as ABF using local wastes or biofilters
48 provide a long solid retention time (SRT) decoupled from hydraulic retention time (HRT)
49 which facilitates low-temperature treatment of wastewater.

50 At the same time, plastic waste has become a global problem and has also been
51 growing in Bhutan over the years. Plastic makes up 13% of the municipal solid waste in
52 Bhutan (Phuntsho et al. 2010) accounting for 40.3 tonnes per day of total municipal waste
53 generated in the capital Thimphu alone (RGOB 2019), which is a significant waste for a small
54 country like Bhutan. The problem in Bhutan is also deteriorated by the absence of industries
55 to recycle waste plastic, and road transportation to India via mountainous terrain has
56 proven to be expensive. As a result, waste plastic bottles are dumped into landfills, posing
57 significant environmental risks as harmful leachates as well as taking up valuable landfill
58 capacity. Recently in 2019, there has been a trial in Bhutan to use plastic wastes as a road
59 surfacing material for paved roads to reduce the need for expensive fossil fuel-based
60 bitumen usually used as road surfacing binder. Other innovative solutions are required to
61 reuse plastic wastes to minimise the mounting non-biodegradable waste problem in Bhutan.

62 Bhutan has experienced rapid urbanisation, putting enormous pressure on the
63 government to meet demands for urban infrastructure and services including for improved
64 urban sanitation. With limited government resources, providing a public sewerage system to
65 all urban centres as is the current policy goal will be a significant challenge. Our recent study
66 observed that over 80% of the towns in Bhutan rely on conventional on-site sanitation
67 treatment systems, i.e., “septic tanks” to dispose of their domestic wastewater (Dorji et al.

2019). One of the major concerns is that about 40% of this on-site treatment was found to be inadequate due to omission of the sub-soil treatment phase due to lack of space in the urban areas. Instead, effluent is allowed to flow into surface drains, posing a significant risk to public health. Furthermore “septic tanks” are only designed to treat blackwater. At the same time, the greywater is discharged openly to surface drains or is directed into septic tanks compromising their anaerobic function as well as leading to overflow events.

Recognising the need for an alternative on-site treatment that addresses all the issues and challenges in unsewered areas of urban Bhutan, a novel on-site domestic wastewater treatment system was evaluated through a lab-scale study (Dorji et al. 2021). The on-site treatment system consisted of a UASB for primary treatment of blackwater (BW) as an alternative to the “septic tank”, followed by an ABF to treat combined primary effluent from UASB and household greywater (GW). The ABF was packed with shredded waste plastic bottle flakes as the biofilter media. This lab-scale study using synthetic wastewater revealed that a combined UASB and ABF process using shredded waste plastic bottles as biofilter media could be an effective on-site treatment system for domestic wastewater (Dorji et al. 2021). Shredded plastic bottles were selected as biofilter media because they are readily available in Bhutan, have been found to be effective biofilter media due to the large surface area to volume ratio, high mass transfer efficiency as media due to their lightweight, the possibility of greater depth of construction (Dacewicz & Chmielowski 2018) in addition to its long life. Also, plastics have a low density and more excellent specific surface when compared to gravel and other conventional biofilter media (do Couto et al. 2015).

UASB reactors have a simple design, low capital and operating costs while providing better retention of solids compared to other anaerobic reactors (Bal & Dhagat 2001). UASB

92 reactors are often described as “improved septic tanks”, having better treatment efficiency
93 due to the development of a sludge bed which behaves like a physical media, trapping the
94 active biomass under an up-flow hydraulic regime (Coelho et al. 2004). The passage of
95 wastewater through the height of the sludge blanket allows for natural separation of
96 microorganisms that perform the biochemical steps of hydrolysis, acidogenesis,
97 acetogenesis, and methanogenesis, converting the complex organics into biogas comprising
98 mostly CH₄ and CO₂ (Alptekin 2008; Hahn & Figueroa 2015; Mahmoud et al. 2004). UASB
99 reactors can handle large volumes of biosolids without clogging, with sound biogas
100 generation and collection from the gas-liquid-solid-separator (GLSS) (Haandel, Catunda &
101 Lettinga 1996). However, UASB provides only primary treatment of BW and requires further
102 treatment for the removal of organics remaining in the effluent. For the secondary
103 treatment of the UASB effluent, ABFs appear to be suitable due to their cost-effectiveness,
104 and recycling of filter materials used in two-step treatment systems for developing countries
105 (Chernicharo & Machado 1998).

106 The objective of this study is, therefore, to conduct a long-term field operation and
107 performance demonstration of a full-scale pilot on-site wastewater treatment plant for the
108 treatment of both BW and GW in Bhutan. The outcomes of this pilot study could provide
109 scientific evidence for policymakers in Bhutan to recognise and promote alternative
110 technology options to address environmental pollution from poorly maintained on-site
111 sanitation and treatment. This pilot on-site wastewater treatment system consisted of a
112 UASB and ABF filled with shredded waste plastic bottles connected to treat domestic
113 wastewater generated from a residential building. Much of the previous research on UASBs
114 or ABFs at low temperature has been done at a much smaller scale using smaller reactor
115 sizes of less than 25 L (Uemura & Harada 2000), short-term operation and usually treating

116 synthetic wastewater (Mahmoud et al. 2004). To the best of our knowledge, there has been
117 no report until now on the pilot-scale study using waste plastic flakes as biofilter media for a
118 real domestic wastewater treatment including greywater treatment although the treatment
119 processes such as UASB and biofilter itself are already well established. This study will also
120 be the first-ever pilot-scale on-site wastewater treatment technology demonstration in
121 Bhutan.

122 **2. Materials and Methods**

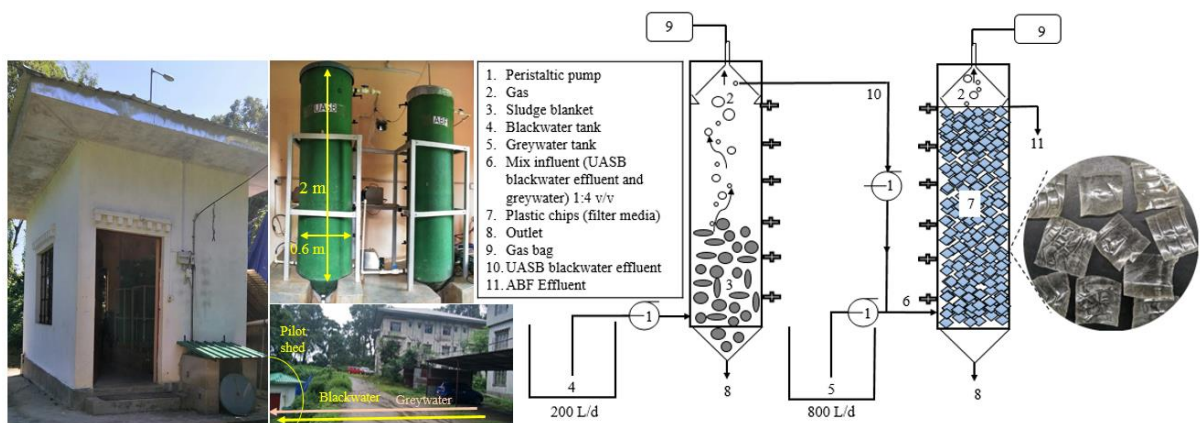
123 **2.1 Location and description of the pilot site**

124 The pilot site is located at 26° 50'59.33" N, 89° 23' 48.96" E within the campus of the
125 College of Science and Technology (CST), Rinchending, Bhutan, at an elevation of 1600 m
126 above the sea level and 5 km from Phuentsholing, the second-largest city in Bhutan,
127 bordering the Indian State of West Bengal. A sultry, humid subtropical climate characterises
128 this area with summer (June - August) mean minimum temperature of 18.0 - 25.3°C and
129 mean maximum temperature of 29.1 - 34.7°C in the last ten years (2008 - 2017). The winter
130 (December - February) is colder with a mean minimum temperature of 9.4 - 21.7°C and
131 mean maximum temperature of 22.9 - 30.8°C (NCHM 2018). The annual rainfall recorded
132 between 2008 and 2017 ranged between 1,681 - 4,979 mm (NCHM 2018). **Figure 1** shows
133 the location of the pilot shed within the CST campus.

134 **2.2 Set-up of the pilot on-site wastewater treatment system**

135 The pilot on-site wastewater treatment system consisted of two anaerobic
136 bioreactor tanks: a UASB followed by an ABF connected in series and housed inside a closed
137 shed located below two CST staff buildings of 12 household units. Both the bioreactors were
138 made from fibre reinforced glass each with a hydraulic volume of 595 L (2 m high and 0.6 m

139 diameter) with a wall thickness of 4 mm. The base of the reactors was cone-shaped resting
 140 on the plain cement concrete for easy desludging. Chlorinated polyvinyl chloride (CPVC)
 141 pipes and fittings were used for the inflow and outflow of wastewater. A stainless steel
 142 deflector and gas-liquid-solid separator (GLSS) were placed on the top portion of the UASB
 143 reactor (refer to **Figure 1**). A mild steel weir was used in the ABF treated with anti-corrosive
 144 paint. Inside the bottom of the ABF, rectangular CPVC pipes formed a pipe grid with holes
 145 drilled on the underside (to prevent clogging of pores with sludge) was constructed for
 146 “sparged” distribution of the influent. The reactor design of UASB and ABF was based on a
 147 previous lab-scale study which had identified optimum HRTs: 2-d HRT for UASB treating
 148 blackwater and 8.8 hours for ABF treating mixed UASB blackwater effluents and greywater
 149 (Dorji et al. 2021).



150
 151 **Figure 1:** Schematic diagram of UASB-ABF pilot system with roughened plastic filter media.

152 The pilot on-site wastewater treatment system was designed for a total capacity of
 153 1000 L/d, assuming 200 L/day of blackwater and 800 L/day of greywater for 10 persons or
 154 about two household units, considering 100 L/p/d wastewater generation, consisting of 20
 155 L/p/d of BW and 80 L/p/d GW.

156 A small portion of the BW from the residential buildings that currently goes to an
157 existing “septic tank” was diverted to the BW balance tank (200 L plastic tank) from where it
158 was then pumped to the UASB reactor using a peristaltic pump (Lead Fluid, China) set at a
159 fixed flow rate of 139 mL/min (200 L/d). Although in a real-life application, the wastewater
160 can be gravity fed to the bioreactors or placed underground from a reservoir tank using
161 non-return valves, pumps were used in this study to control the flow rates going to each
162 bioreactor. The balance tank also served as a pre-treatment in removing heavier grit, large
163 objects (installed screen mesh of 5 mm x 5 mm) and was fitted with a stirrer (DLH Overhead
164 Stirrer VELS Scientifica, Italy) to achieve proper mixing of the BW and to break up faecal
165 solids to prevent choking of pipes and peristaltic pumps. An overflow pipe in the BW
166 balance tank ensured that the detention time did not exceed 0.25 d to avoid odour issues.
167 An outlet from the sloping bottom of the tank was provided to flush out any settled grit and
168 excess solids under gravity. The overflow from the BW balance tank was sent to the make-
169 shift “septic tank” from where the final effluent was then disposed using a soak-pit filled
170 with broken bricks, providing further soil treatment.

171 Currently, the GW (from kitchens, bathrooms and laundries) from the buildings is
172 simply discharged into surface water drains. A new 100 mm diameter CPVC pipe was
173 installed to collect GW from the building and sent to the pilot treatment plant. The GW was
174 first collected in a GW balance tank (500 L plastic tank) from where it was then pumped to
175 the ABF reactor with the help of a peristaltic pump (Lead Fluid, China). The overflow system
176 of the GW balance tank was adjusted to have as short hydraulic detention time as possible
177 (0.125 d in this case) and the overflowing GW was sent to the make-shift “septic tanks” for
178 final disposal. The sloped bottom of the balance tank contained an outlet to flush out any

179 settled heavy grit and solids. **Figure 1** shows the process diagram of the pilot on-site
180 wastewater treatment system adopted in this work.

181 The ABF reactor was filled with 46 kg of waste plastic bottles flakes as biofilter media
182 as presented in **Figure 1** with a total reactor hydraulic volume of 595 L. Waste plastic bottles
183 were collected from Phuentsholing City municipality, cleaned and manually cut into flake
184 sizes of about 40 mm x 40 mm since no shredding equipment is available. Although, in our
185 earlier lab-scale study, the largest plastic flake size used was 30 mm x 30 mm (Dorji et al.
186 2021), a slightly larger flake size was used for the pilot to provide a greater void volume and
187 prevent choking from biomass accumulation during long-term operation using real domestic
188 wastewater. However, increasing the flake sizes to 40 mm x 40 mm, the specific surface
189 area of the biofilter media decreased to 347 m²/m³ from 1,425 m²/m³ (30 mm x 30 mm),
190 1,623 m²/m³ (20 mm x 20 mm) and 1,903 m²/m³ (10 mm x 10 mm) (Dorji et al. 2021).

191 The plastic bottle flakes were mixed with sharp stone gravel and subjected to
192 abrasive action in a concrete mixer for about 30 minutes (HEICO, India). This resulted in
193 rougher flake surfaces to help accelerate attached growth of biomass to the media. The
194 properties of the ABF media are presented in **Table 1**.

195 **Table 1:** Properties of the ABF media used in this study.

Media properties	Values
Media flake size	40 mm x 40 mm
Thickness of plastic flakes	~ 0.20 - 0.40 mm
Specific surface area of packed media	347 m ² /m ³
Packed media porosity	92%
Packed media density	78.5 kg/m ³

Types of plastic bottles	PET and PP
--------------------------	------------

196 **2.3 Acclimatisation and operation of the pilot anaerobic bioreactors**

197 The seeding and inoculation of the bioreactors were conducted using anaerobic
198 sludge obtained from anaerobic ponds of the Phuentsholing City waste stabilisation pond
199 (WSP), and the start-up procedure was followed as described below (de Lemos Chernicharo
200 2007). The seed sludge in the bioreactors was left unfed for 24 hours to develop anaerobic
201 conditions and adapt to the environment inside the bioreactors. The UASB reactor was then
202 filled with blackwater and the ABF with greywater diverted from the households to half of
203 the ABF reactor volume. After 48 hours of seeding and inoculation period, the reactors were
204 filled with wastewater and operated as a batch system for another 48 hours (Behling et al.
205 1997) followed by a continuous flow-regime (Behling et al. 1997). After reaching constant
206 hydraulic loading of the bioreactors, the pilot system was then operated under steady
207 design HRT; 2 d for the UASB (~ 200 L/day) for 262 days and 0.37 d (~ 8.8 h) for the ABF (800
208 L/d) throughout a year.

209 **2.4 Wastewater analysis and performance monitoring**

210 The performance of the pilot system was monitored rigorously, recording various
211 parameters including pH, oxidation-potential (ORP), dissolved oxygen (DO) and temperature
212 (all measured using Vernier Lab Quest, USA) and turbidity (HACH, USA). The total suspended
213 solids (TSS) and the volatile suspended solids (VSS) were determined as per US standards
214 (APHA 1998). Organics including total COD (COD_t) and soluble COD (COD_s) were measured
215 using analytical test kits (HACH, USA) and DR3900 Spectrophotometer (HACH, USA). BOD₅
216 was measured using OxiTop® OC100 (Germany). *Escherichia coli* was measured as per the
217 standard operating procedure at the Royal Centre for Disease Control – the National Water
218 Reference Laboratory in Bhutan – to US standards (APHA 1998). Biogas samples were

219 collected from the GLSS in the UASB and ABF using 10 L and 5 L Tedlar gas bags, respectively
220 (Sigma Aldrich, Australia). The biogas and its composition were analysed using a Biogas
221 Analyser 500 (GeoTech, UK). Influent and effluent grab samples were collected from the
222 UASB and ABF fortnightly in the morning (9 AM) where the generation of wastewater was at
223 its peak, to obtain uniform and representative samples. Due to sheer volume of the samples
224 involved, samples were analysed in duplicates for BOD, COD, TSS and VSS only during the
225 initial stages of the pilot operations while later only single measurements were carried out
226 except when analytical errors are envisaged. For other parameters such as pH, DO and ORP,
227 samples were measured in triplicates.

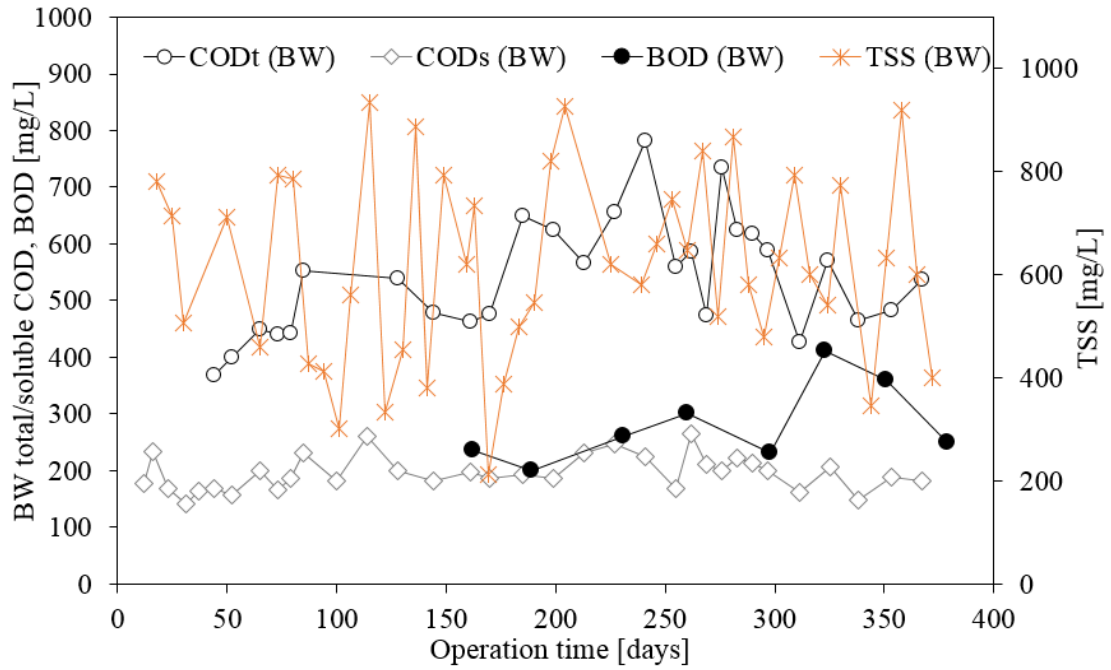
228 **3. Results and Discussions**

229 **3.1 Wastewater characteristics**

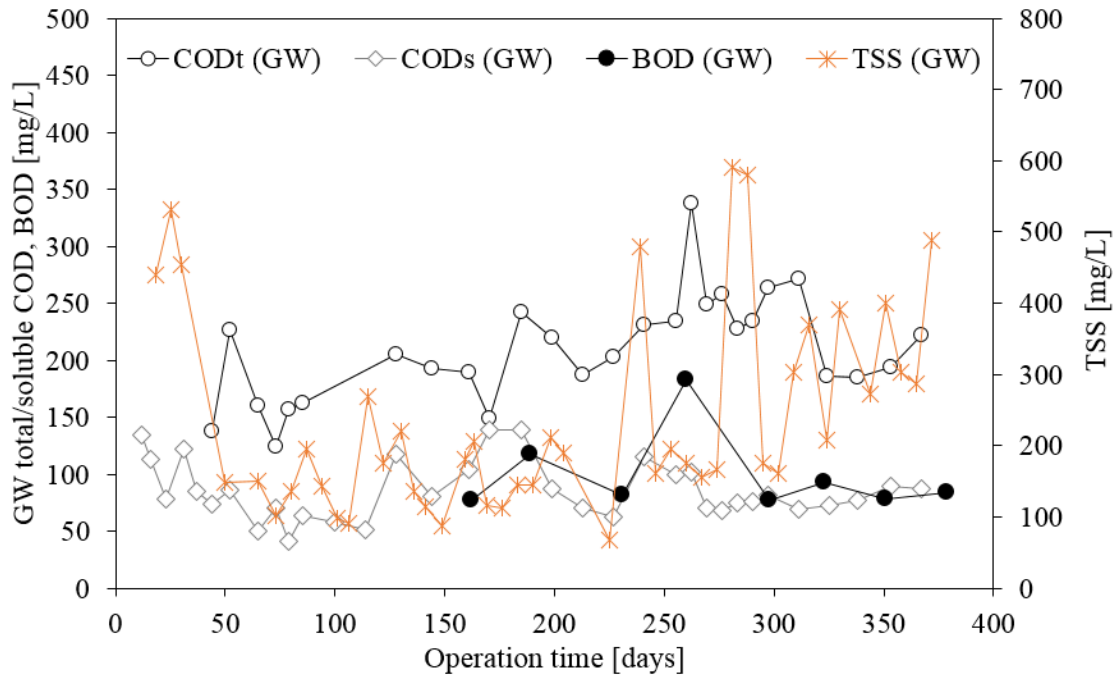
230 The characteristics of the wastewater (grab samples) from the residential building
231 being treated by the pilot treatment plant were continuously monitored, and their features
232 are presented in **Figure 2 (a)** for BW and **Figure 2 (b)** for GW. The COD_t of the blackwater
233 ranged between 367 - 782 mg/L (average of 539 mg/L) while the soluble COD (COD_s) ranged
234 between 140 - 265 mg/L (average of 183 mg/L), which can be classified as medium-strength
235 domestic wastewater (Tchobanoglous 2014). The differences between the COD_t and COD_s
236 indicate the presence of a significant concentration of suspended or other undissolved
237 organics present in the BW. These average COD values are similar to the values reported in
238 countries, including Turkey and the Netherlands, as presented in **Table S1** (Alptekin 2008) of
239 the supplementary material. The BOD of the BW varied between 200 - 410 mg/L; however,
240 due to limitations of the lab at CST, the BOD sampling and analysis was only possible in the
241 later stage of the pilot. The average VSS/TSS ratio of 0.79 found is high compared with the
242 sewage characteristics in other countries, probably due to the starch-rich diet (rice-based)

243 of most Bhutaneese. However, the low VSS/TSS compared to Egypt suggests more diluted
244 wastewater in Bhutan than in arid Egypt.

245



(a)



(b)

Figure 2: Characteristics of the wastewater generated from the CST staff residential building and used as influent for the pilot treatment system. (a) Blackwater and (b) Greywater

246

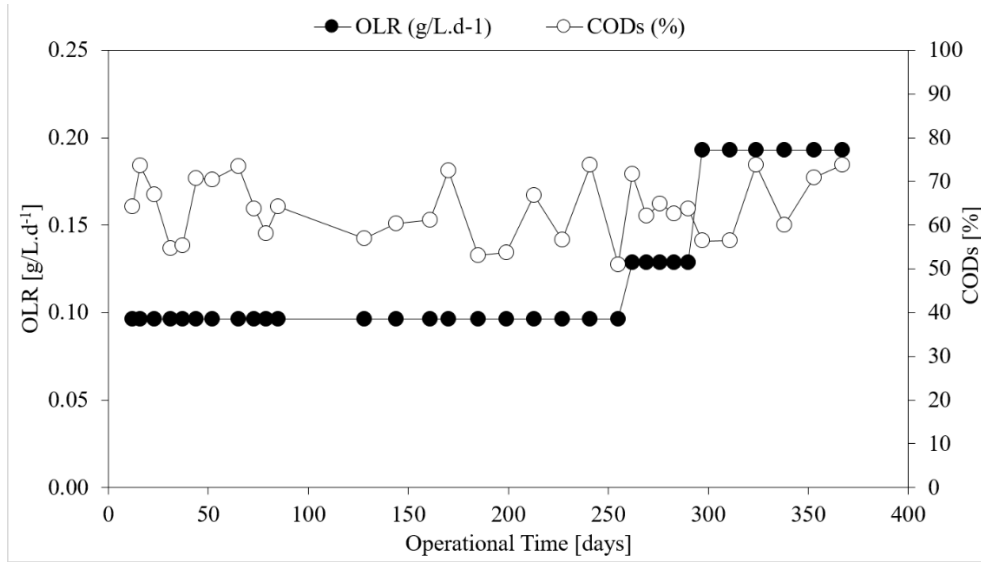
247 **3.2 Start-up operations of the anaerobic bioreactors**

248 Following start-up, the UASB reactor was operated continuously at 2-d HRT, and
249 sludge acclimatisation was achieved after 262 days, indicated by consistent COD removal as
250 shown in **Figure 3 (a)**. The ABF was operated continuously for more than 300 days at 8.8 h
251 HRT to achieve full acclimatisation as presented in **Figure 3 (b)**. Anaerobic processes such as
252 UASB and ABF usually take a long to achieve acclimatisation, which is one of the main
253 challenges of anaerobic treatment.

254 Anaerobic processes are sensitive to pH, and rising acidity as acidogenesis takes
255 place. The influent alkalinity likely provided an adequate buffer for maintaining consistent
256 pH in both the reactors (UASB influent pH 7.6 ± 0.4 , effluent pH 7.2 ± 0.2 ; ABF influent pH
257 7.1 ± 0.2 and effluent pH: 6.8 ± 0.2 ; n=122) without the risk of souring even after a year of
258 operation. This can be attributed to concurrent metabolic activities taking place during the
259 anaerobic digestion process, where CO₂ generated as a by-product is partially dissolved in
260 the wastewater to form bicarbonates that enhance the pH buffering capacity of the
261 wastewater (Chiappero et al. 2019; Lettinga & Van Haandel 1994).

262 During the pilot operation period, the influent wastewater temperature ranged
263 between 17 - 29°C, a sub-mesophilic temperature suitable for UASB operations (Ayaz et al.
264 2012). The ORP values of the wastewater inside both bioreactors were < -150 mV, indicating
265 the presence of an anaerobic environment as presented in **Figure 3 (c)** and further
266 supported by the methane production as shown in **Figure 4**. Wastewater with ORP below -

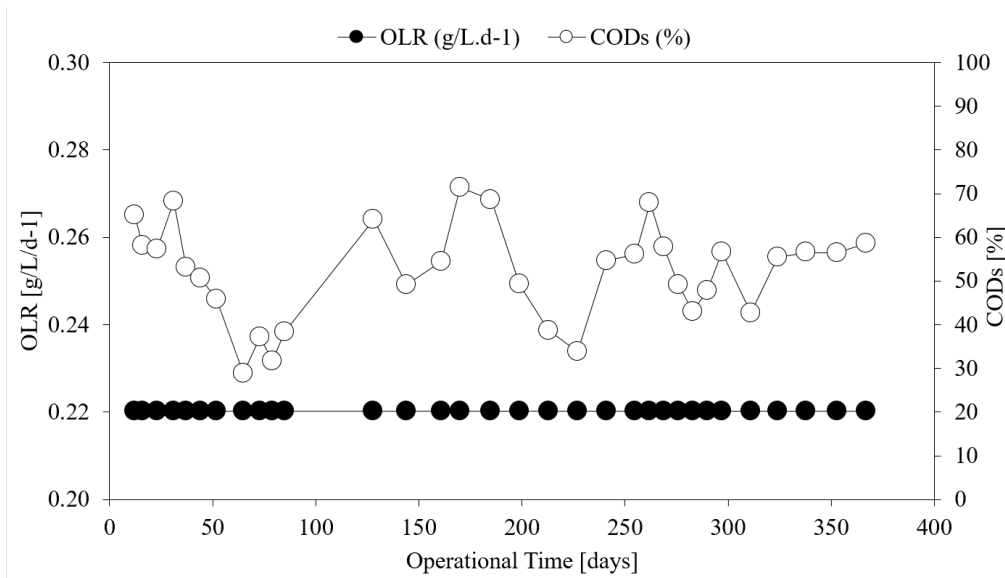
267 200 mV is an indication of high anaerobic conditions, but with limited potential for
268 production of CH₄ (Srivastava 2020), since methanogenesis generally occurs within the ORP
269 range of -230 mV to -210 mV (Santiago-Díaz & Salazar-Peláez 2017).



270

271

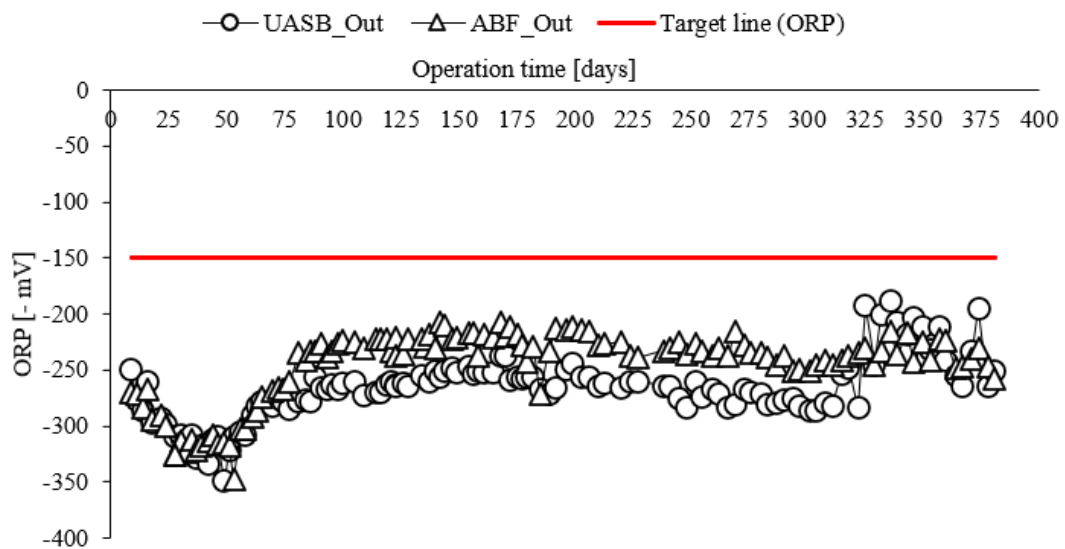
(a)



272

273

(b)



274

275

(c)

276 **Figure 3:** The OLR and the CODt removal of the (a) UASB reactor (b) ABF reactor throughout
 277 the pilot operation since the start-up stage and, (c) Oxidation-Reduction Potential (ORP)
 278 from the effluents of UASB and ABF.

279

280 3.3 Performance of UASB bioreactor for primary treatment of blackwater

281 3.3.1 Organic (BOD/COD) removal and biogas production

282 The BOD₅/COD removal and the biogas production from the UASB reactor are
 283 presented in **Figure 4**. The removal efficiency of BOD₅ at 2 d HRT ranged between 73 - 75%,
 284 with a resulting effluent BOD of 54 - 75 mg/L, as shown in **Figure 4 (a)**. The effluent BOD₅
 285 improved (46 - 55 mg/L) when the UASB was operated at HRT of 1 day, with a removal
 286 efficiency of 76 - 89%. This is likely due to the increased up-flow velocity causing improved
 287 mixing and distribution, optimising contact with biomass in the sludge granules (Mahmoud
 288 et al. 2003). This significant BOD₅ removal indicates the satisfactory methanogenic activity
 289 occurred within the sludge bed even under psychrophilic conditions suggesting that UASB
 290 treatment is suitable for conditions in Bhutan. For conventional primary clarification, the

291 BOD₅ removal typically ranges between 25 - 35% (O'Connor et al. 2015). BOD₅ removal of 73
 292 - 89% achieved in this study with the UASB is better than the primary clarifier option. Based
 293 on the BOD₅ removal, 1-d HRT is adequate for the primary treatment of BW using a UASB,
 294 with the remaining BOD removed in the ABF to reach a target final effluent BOD₅ of 30
 295 mg/L, the effluent discharge standard in Bhutan as shown in **Table 2**. These standards are
 296 based on the American Public Health Association (APHA) and the World Health Organization (WHO)
 297 (NEC 2020).

298 **Table 2:** Sewage treatment plant (STP) discharge standards (NEC 2020) .

Parameter	Unit	Standards
COD	mg/L	125
BOD		30
TSS		100
pH	pH scale	6.5 - 9
Faecal coliform	CFU/100 mL	1000

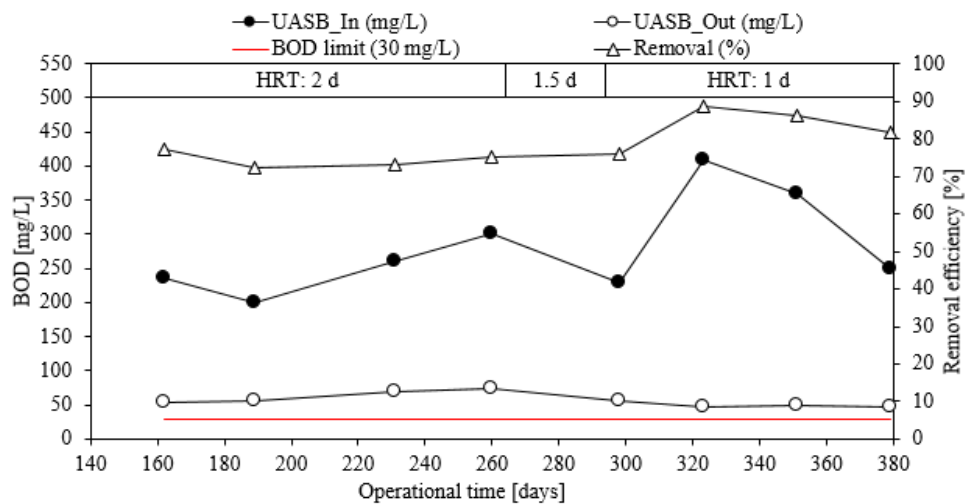
299 At an HRT of 2 days, the UASB effluent COD varied between 49 - 107 mg/L (average
 300 COD of 71 mg/L) which translates to a COD removal of 53 and 74% (average of 63%) as
 301 presented in **Figure 4 (a)**. The COD removal did not vary significantly when the HRT was
 302 reduced to 1.5-d HRT. Similar to BOD₅ removal described earlier, decrease in HRT to 1 day
 303 improved the effluent COD to 56 mg/L, equivalent to COD removal of 70%, which almost
 304 meets the Bhutan effluent discharge limit for COD of 50 mg/L. Assuming a BOD/COD ratio of
 305 0.6, and a minimum BOD discharge limit for sewage effluent in Bhutan of 30 mg/L. Similar to
 306 BOD removal, this improved COD removal at 1-d HRT is probably due to enhanced mixing
 307 and diffusion of organics within the granular sludge biomass (Mahmoud et al. 2003). This
 308 study, therefore, suggests that the optimum 1-d HRT produces effluent quality that almost

309 meets the Bhutan effluent discharge standard in terms of BOD/COD. These results further
310 demonstrate that UASB has the potential for the primary treatment of BW.

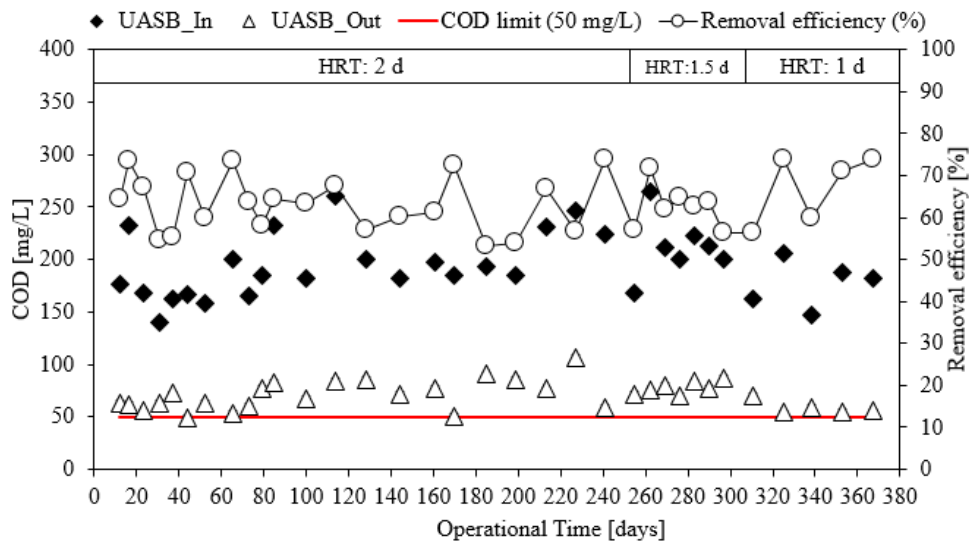
311 At the start of the UASB operation, methane generation varied widely ranging
312 between 43 - 230 L CH₄/kg COD (or of COD removed) with an overall average of 128 L
313 CH₄/kg COD during the entire pilot operation of as shown in **Figure 4 (b)**. This irregular
314 methane generation could be due to random release of biogas that was already present
315 either in dissolved form or in the form of micro-bubbles within the active anaerobic seed
316 sludge collected from the anaerobic pond when subjected to up-flow turbulence (Feng et al.
317 2020). These methane generation values are similar to those reported in the literature (Ayaz
318 et al. 2012). The methane generation and its proportion of the biogas increased from
319 seeding day 65 - 85, probably as the seed sludge becomes more stable having adapted to
320 the UASB environment. However, the methane generation then decreased between day 80
321 and 170, presumably responding to decreasing temperature with the start of winter, giving
322 rise to psychrophilic conditions. Methanogens are sensitive to a lower temperature, and
323 their biological activities can be significantly affected. From day 170 - 227, methane
324 generation gradually increased with the start of the summer.

325 When the HRT of the UASB was reduced to 1.5 d, the methane generation
326 decreased, and this is most likely due to increased OLR inducing shock loading of the UASB
327 reactor as observed during the UASB treatment of cattle slaughterhouse wastewater (Musa
328 et al. 2020). However, after day 276, methane generation and proportion recovered from
329 the shock and operation became more stable. Although the biogas production was slightly
330 lower at 1.5-d HRT compared to 2-d HRT, COD removal was consistent with a steady
331 methane proportion, indicating the stability of the UASB granular sludge bed. Systems with

332 higher rates of COD do not always also show increased biogas release (Manariotis &
 333 Grigoropoulos 2006). The proportion of methane in the biogas followed a similar trend as
 334 the methane generation, as presented in **Figure 4 (c)**. The methane proportion ranged
 335 between 45% (in cold winter months) to as high as 78% during the warmer months.
 336



(a)



(b)

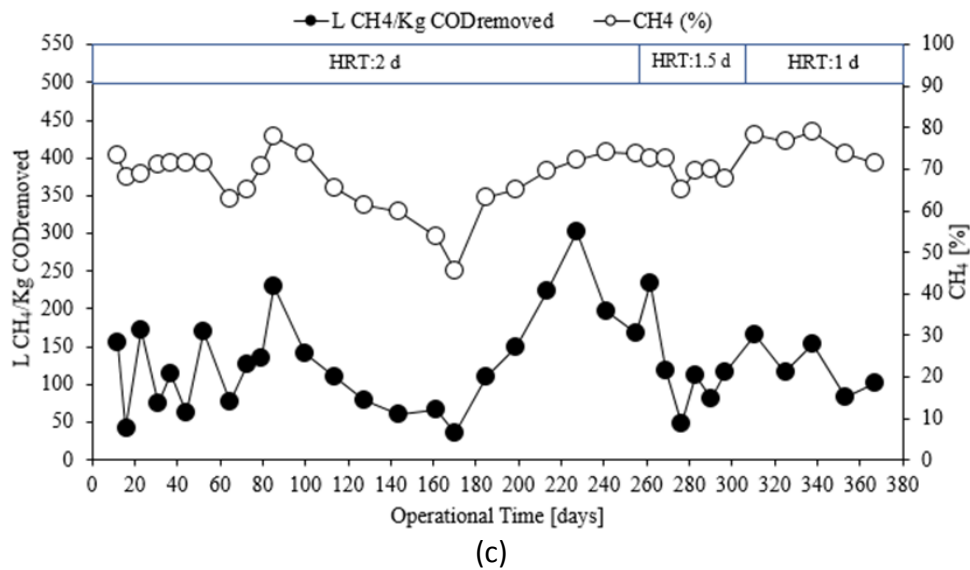


Figure 4: Performance of the pilot UASB reactor for the primary treatment of BW.

(Organic removal in (a) BOD, (b) COD and (c) biogas production. Average reactor temperature ($23.4 \pm 3.5^\circ\text{C}$) and ambient room temperature ($23.7 \pm 3.7^\circ\text{C}$) with an average influent COD: 195 mg/L (as measured).

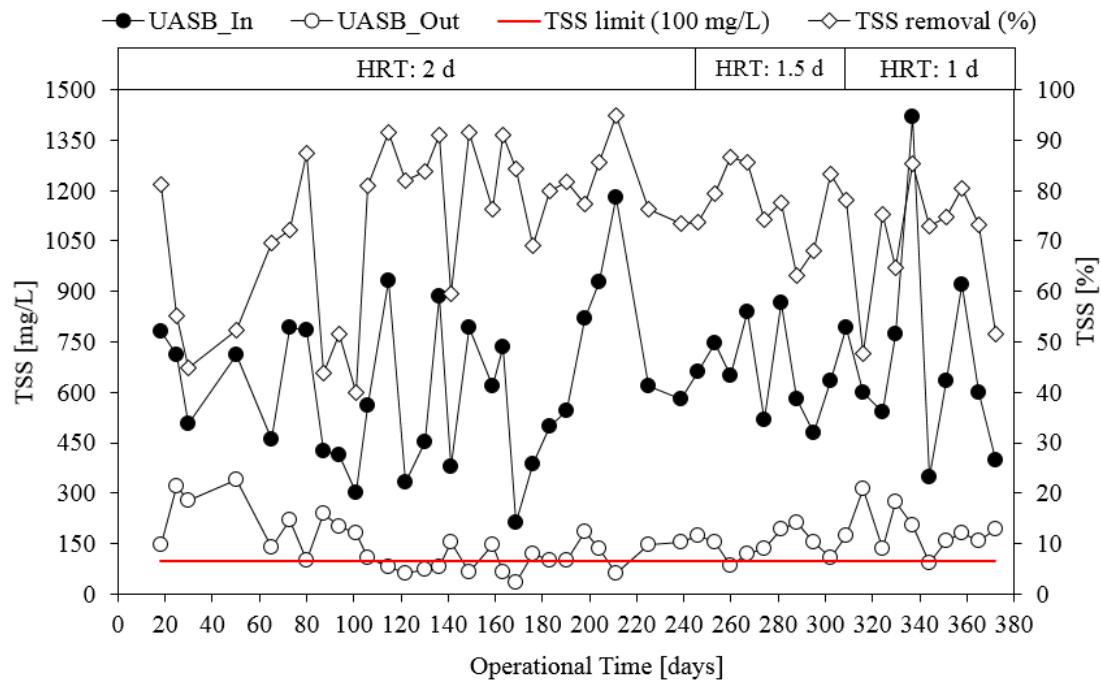
337 3.3.2 TSS removal

338 The influent and effluent TSS in the UASB averaged 612 ± 236 mg/L and 125 ± 55
 339 mg/L respectively with a removal efficiency of about 80% during the stable period of pilot
 340 operations at 2-d HRT (see **Figure 5**). During the initial stages of UASB operation
 341 (acclimatisation period) at 2-d HRT, the TSS removal efficiency decreased, which is
 342 presumed to be due to washout of seed sludge from the UASB reactor. However, after 50
 343 days of operation, the effluent TSS concentration started to decrease. It stabilised within a
 344 range of 33 - 240 mg/L (averaging 125 ± 55 mg/L and 79.6% removal), which is close to
 345 Bhutan's effluent discharge standard of 100 mg/L. When the UASB was operated at a
 346 reduced HRT of 1.5 d, the effluent TSS increased slightly to an average 148 ± 41 mg/L (78%).
 347 However, when the HRT from 1.5 d was decreased to 1 day, the TSS slightly spiked to an
 348 average of 232 ± 79 mg/L (72% removal) before achieving a stable effluent TSS of 157 ± 38
 349 mg/L (73% removal). This slight spiking of the effluent TSS concentration is attributed to the

350 increased up-flow velocity of the wastewater giving rise to turbulence might have disturbed
351 the sludge bed resulting in temporary sludge washout. However, this stability under the HRT
352 of 1 day resulted in a stable effluent TSS of 157 ± 38 mg/L (73% removal) which was higher
353 than the average effluent TSS of 125 ± 55 mg/L (80% removal) at 2-d HRT. Similar TSS
354 removal efficiency ranging between 45 - 84% have been reported for UASB in the other
355 studies (Heffernan, Van Lier & Van Der Lubbe 2011).

356 This anaerobic pilot operation was carried under ambient temperatures, and no
357 correlation was observed between TSS removal and environmental temperatures. Similar
358 observations were made by Hahn & Figueroa (2015)) during their 2-year long pilot
359 treatment of wastewater using an anaerobic baffled reactor (ABR). The TSS removal (40 -
360 95%) is comparable to the highest reduction observed with the chemically added enhanced
361 primary treatment ranging from 60 - 90% (Mau & Jeyanayagam 2008). However, the current
362 treatment study does not require the addition of chemicals and neither wasting of solids
363 from the reactor. The TSS removal exceeded the typical 50 - 65% removal for conventional
364 primary clarification (Mau & Jeyanayagam 2008) indicating the advantage of using UASB as
365 a primary treatment for domestic wastewater.

366



367

368

Figure 5: TSS removal in UASB under ambient conditions.

369

370 **3.3.3 *Escherichia coli* removal**

371

With an *Escherichia coli* discharge standard of 1000 cfu/100 mL, *Escherichia coli* is a

372

critical water quality indicator because of the health hazard it poses. The log reduction of

373

Escherichia coli from the UASB treatment of blackwater was 0.81 - 1.12 log values,

374

equivalent to 84.6 - 92.4% removal efficiency as presented in **Figure 6**. The log removal did

375

not vary significantly on the reduction of UASB HRT, with a relatively consistent average of

376

0.92 log units under 1-d HRT. This log reduction of *Escherichia coli* is lower than the log

377

reduction of 1.90 units reported for a UASB reactor in Sweden for municipal wastewater

378

treatment under HRT of less than 24 h (Samhan, Al-Sa'ed & Mahmoud 2007).

379

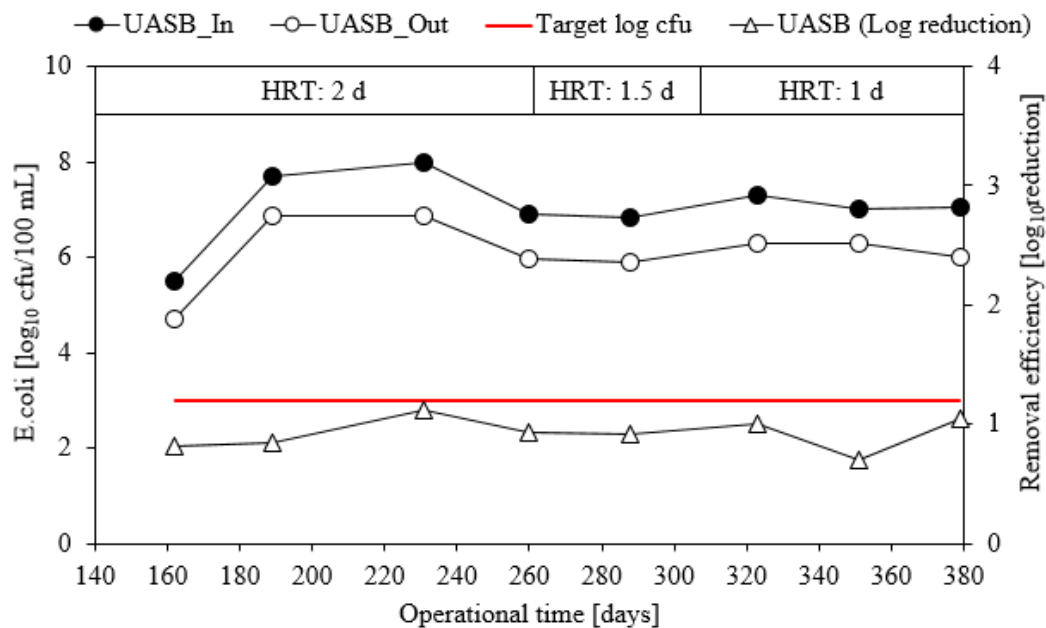


Figure 6: Performance of the pilot UASB reactor in the removal of *Escherichia coli*. The *Escherichia coli* data before day 161 are not available due to issue with the analytical equipment.

380

381 3.4 Performance of ABF for secondary treatment of mixed UASB effluents and greywater

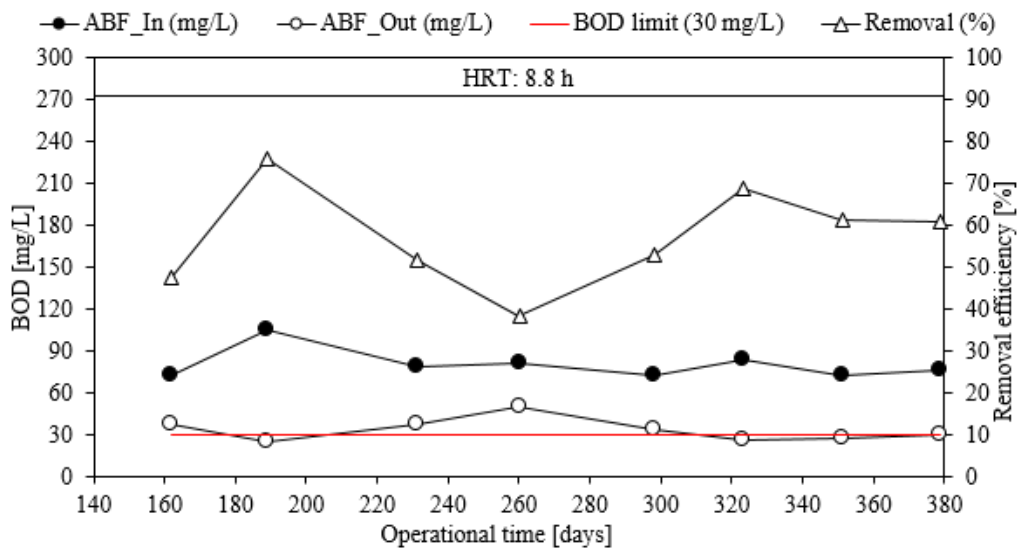
382 3.4.1 Organics removal and biogas production

383 The long-term performance of the ABF for BOD₅ and COD removal and methane
 384 generation at a constant HRT of 8.8 h are presented in **Figure 7**. BOD₅ sample testing could
 385 only commence after 162 days of ABF operation. Although the effluent BOD₅ from the ABF
 386 was initially mostly above 30 mg/L; after day 300 after the initial effluent BOD₅ test from
 387 day 162, the effluent BOD₅ was consistently below 30 mg/L (average BOD₅ of 28 mg/L and
 388 60 - 70% removal) as shown in **Fig 7(a)**, thereby meeting the effluent discharge standard for
 389 Bhutan. The BOD₅ removal rate of the ABF is slightly lower than the UASB reactor which is
 390 expected because the ABF influent consists of a mixture of UASB effluent (BOD₅: 46 - 75
 391 mg/L) and greywater (BOD₅: 77 - 93 mg/L), having a resulting BOD₅ of 72 - 84 mg/L during
 392 the operation period. The BOD₅ removal of 60 - 70% was slightly lower than the 80% BOD₅

393 removal reported for an up-flow anaerobic filter comprising tezontle biofilter media treating
394 municipal wastewater (Merino-Solís et al. 2015). This pilot study, therefore, suggests that
395 shredded waste plastic bottles have the potential to be a suitable biofilter media for
396 domestic wastewater treatment. The ABF was operated at a fixed HRT of 8.8 h based on our
397 earlier lab-scale study; however, further process optimisation may enhance organic removal
398 to achieve lower final effluent BOD₅.

399 **Figure 7 (b)** also shows that at a constant HRT of 8.8 h, the ABF produced an effluent
400 COD ranging between 29 - 47 mg/L (29% and 72% removal efficiencies) and consistently
401 100% of the samples were below the target limit of 50 mg/L for Bhutan. These effluent COD
402 (average of 38 ± 5 mg/L) and COD removal efficiencies (29 - 72%) were not significantly
403 different from those observed during the lab-scale study (Dorji et al. 2021). The large
404 variations in the removal efficiencies of 29 - 72% are attributed to the large fluctuations in
405 the incoming domestic greywater COD (48 - 128 mg/L) (see **Figure 2 (b)**). These COD data
406 and the BOD data presented earlier indicate the likely potential of the shredded waste
407 plastic bottles as a promising low cost, locally available biofilter media for the treatment of
408 both the mixture of primary effluent and the greywater. The COD removal performances of
409 the biofilter can be attributed to several factors including the large surface area provided by
410 thin plastic bottles for biological attached growth, the curved nature of the bottle shreds
411 and the roughened texture of the plastic media that supported the development of the
412 microbes. Compared to the surface area of biofilters such as coconut shells ($100 \text{ m}^2/\text{m}^3$)
413 (Tonon et al. 2015), shredded waste plastic bottles provided a large surface area of 347
414 m^2/m^3 and high porosity (92%) for the growth of biomass that enhanced biological
415 degradation of organics.

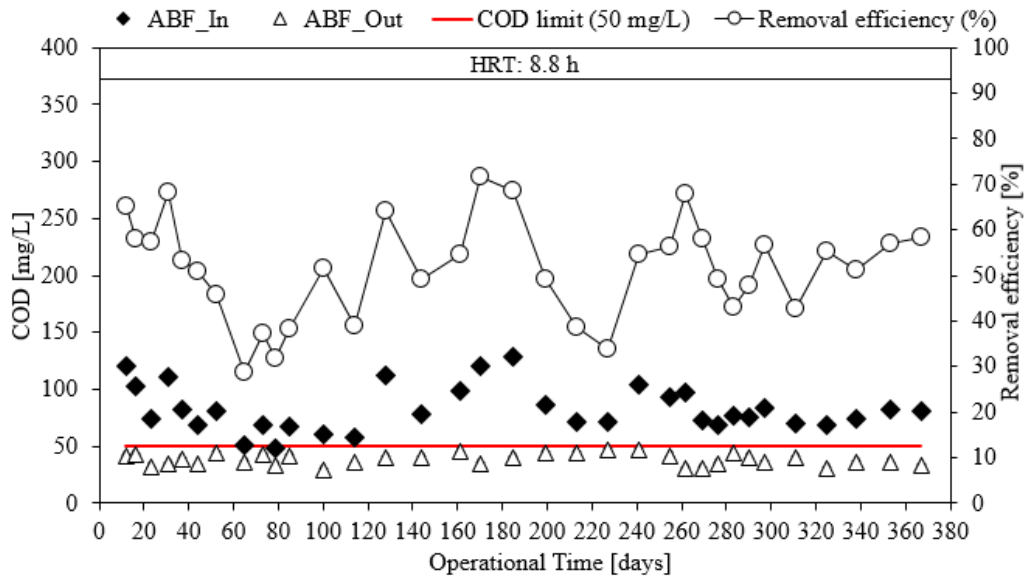
416 Due to some technical issues, biogas from the ABF reactor was only tested from day
 417 144, and the results in **Figure 7 (c)** shows that methane production increased gradually as
 418 the ABF system slowly stabilised. Between day 140 - 170, even during the winter (17 - 24°C)
 419 steady methane production indicates methanogenic activity occurred even under
 420 psychrophilic conditions which is attributed to fully developed biomass within the waste
 421 plastic biofilter matrix aiding methanogenesis. Similar observations were also made in
 422 (Lettinga, Rebac & Zeeman 2001). After day 170, as spring began, the fluctuations in
 423 methane production (17 - 110 L CH₄/kg COD) are attributed to the large fluctuations in the
 424 influent COD composition (48 - 129 mg/L) mainly due to the daily variations in the greywater
 425 composition. Consistent OLR is necessary for consistent methane generation from the
 426 bioreactors. The observed methane generation values are similar to those reported in the
 427 literature (Manariotis & Grigoropoulos 2006a).



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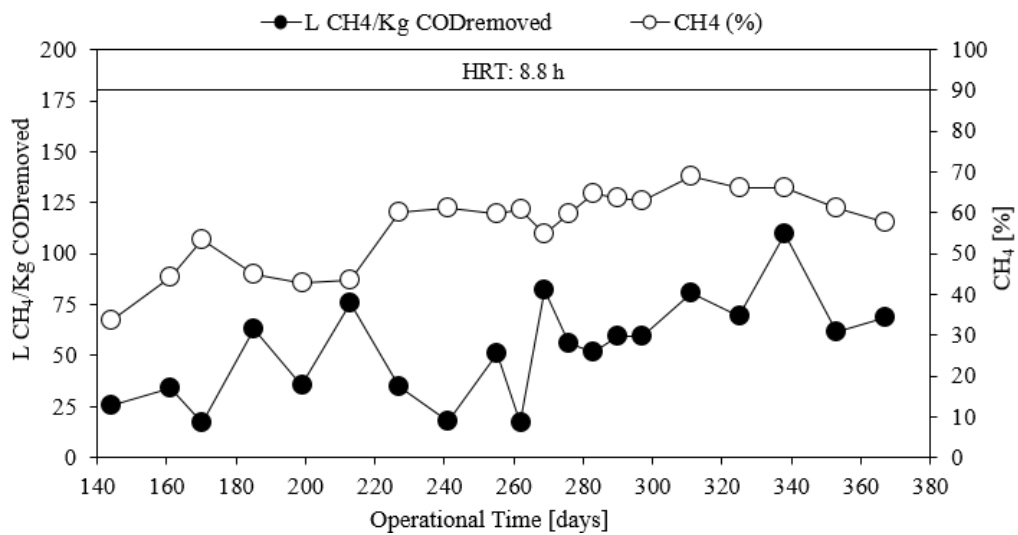
(a)



430

431

(b)



432

433

(c)

434 **Figure 7:** Performance of the pilot ABF reactor for treatment of a mixture of UASB effluent

435 and greywater (1:4 v/v mix ratio). Organic removal (a) in BOD, (b) COD and (c) Biogas

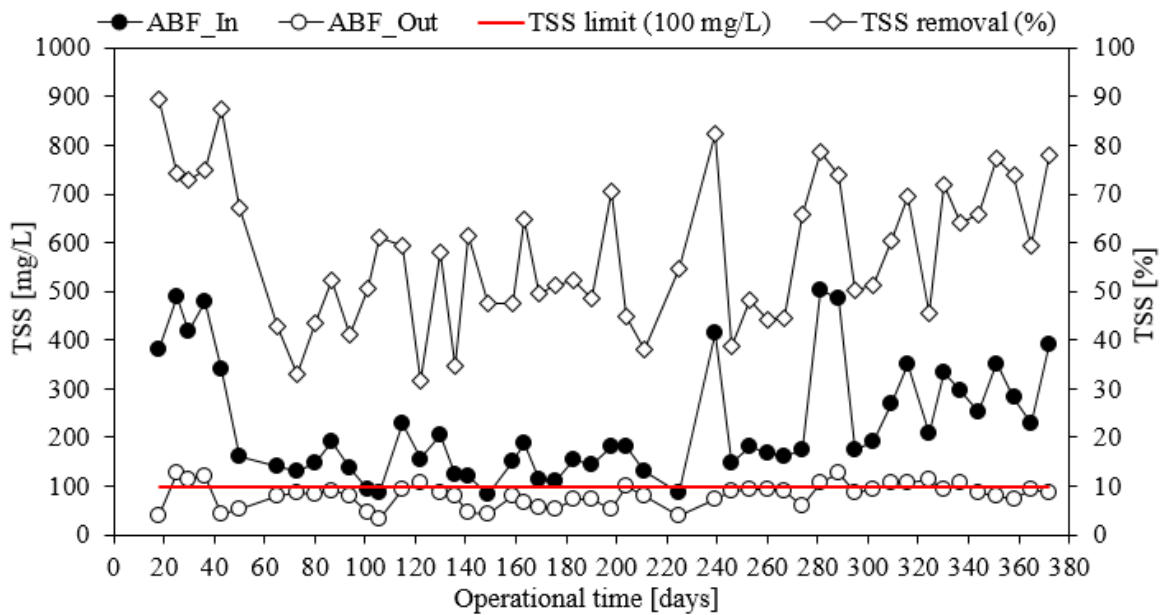
436 production. ABF reactor temperature ($23.37 \pm 3.48^\circ\text{C}$) at ambient temperature ($23.68 \pm$

437 3.65°C).

438 **3.4.2 TSS removal**

439 The TSS data in **Figure 8** shows that the influent TSS (mixed UASB effluent and
440 greywater) ranged between 83 - 502 mg/L which is explained by the highly variable nature
441 of the grey water coming from the residential buildings as the outflow of kitchen and
442 bathroom wastewater are unpredictable. During the early stages of ABF treatment,
443 following start-up operations, a significant spike in the influent TSS was observed and is
444 attributed to washout of the seed sludge from the UASB reactor (as discussed earlier under
445 **Section 3.3.2**) as shown in **Figure 5**. It is also likely that this seed sludge washout also
446 contributed to the higher effluent TSS from the ABF during initial stages of operation. After
447 day 40 of operations, the effluent TSS stabilised, but still varied significantly (33 - 127 mg/L)
448 with an average effluent TSS of 80 ± 22 mg/L (average removal efficiency of 62%). Over 80%
449 of the ABF effluent samples had TSS below 100 mg/L, which is the effluent discharge
450 standard of Bhutan. This demonstrates the suitability of shredded waste plastic bottles as a
451 possible biofilter media for removing TSS from domestic greywater. To achieve consistent
452 TSS below 100 mg/L, further process optimisation can be carried out; adjusting HRT
453 (probably increasing the ABF HRT), increasing the density of the biofilter media or providing
454 a second ABF reactor.

455



456

457 **Figure 8:** Performance of the ABF for the removal of TSS from the mixed UASB effluent and
 458 greywater.

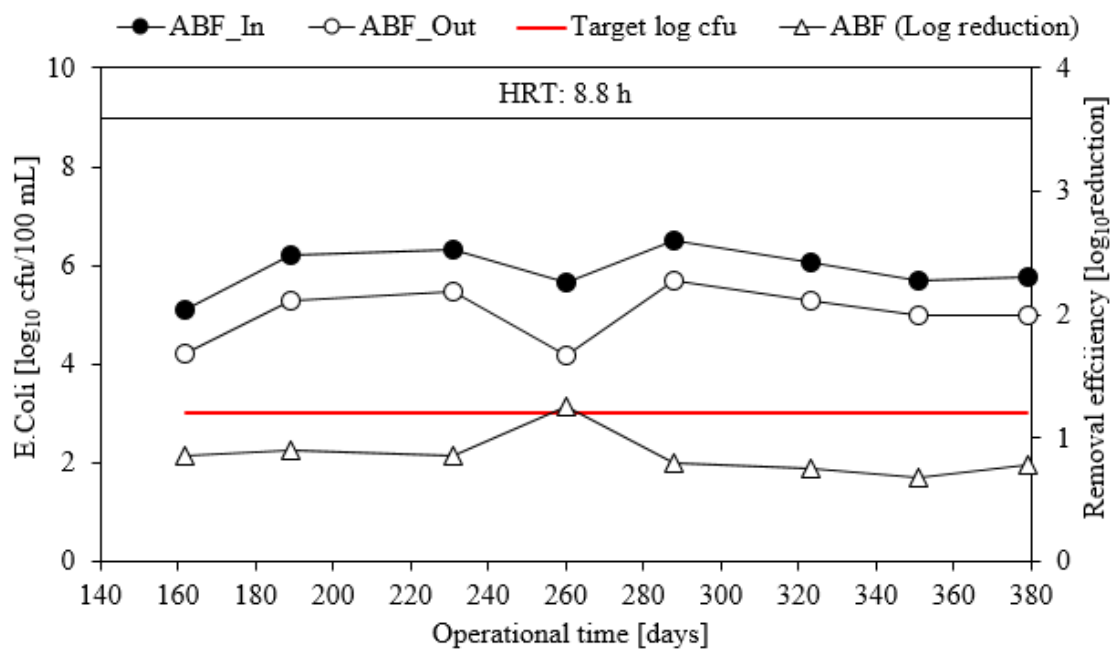
459 **3.4.3 *Escherichia coli***

460 The measured influent ABF and effluent *Escherichia coli* are presented in terms of
 461 their log units in **Figure 9**. The final average *Escherichia coli* reduction from ABF was 0.9 log
 462 units. This reduction in *Escherichia coli* are found to be in range between 0.78 - 1.02 log
 463 reduction units for other filter media such as biochar, woodchips, and gravel filters reported
 464 to range (Kaetzel et al. 2018). Biochar has been found to have better *Escherichia coli* removal
 465 because of higher pathogen adsorption rates on biochar particles due to its polarity. The
 466 final ABF effluent showed on average 5 log units of *Escherichia coli* ($\sim 10^5$ cfu/100 ml) which
 467 does not meet the allowable 3 log units (~ 1000 cfu/100 ml) effluent discharge limit of
 468 Bhutan.

469 This pilot of sequential UASB and ABF treatment using shredded waste plastic bottle
 470 media resulted in 0.4 - 1.5 log removal of *Escherichia coli*. A similar combined UASB and ABF
 471 however reported between 1 - 2 log removals using reticulated polyurethane foam as ABF

472 media in terms of *Escherichia coli* removal (Alrajoula, Halalsheh & Fayyad 2009). It appears
 473 that the performance of the ABF reactor has a significant influence on the potential
 474 reduction of *Escherichia coli*. Therefore, through better process design and optimisation of
 475 the UASB and ABF combination, *Escherichia coli* removal would need to be further improved
 476 to meet Bhutan’s standards.

477



478

479 **Figure 9:** Performance of the ABF in the removal of *Escherichia coli*

480 **3.5 Economic assessment**

481 A brief economic assessment was carried out to compare the capital expenditure
 482 (CAPEX) and operating expenditure (OPEX) of this alternative on-site wastewater treatment
 483 system to the conventional on-site “septic tanks” currently predominant in Bhutan. The
 484 capital costs of a reinforced cement concrete (RCC) based “septic tank”, and soak-pit were
 485 obtained from the Bhutan Schedule of Rates-2020 (MoWHS 2020). The unit costs of RCC
 486 work was then applied to estimate the capital cost of the UASB and ABF treatment system

487 with 30% added price to account for additional construction complexities of the UASB and
488 ABF reactor tanks. Further work, including plumbing, gas-liquid-solid-separator (GLSS) and
489 the effluent weirs were estimated based on the BSR 2020 (MoWHS 2020). The plumbing
490 from the residential buildings up to the “septic tank” or the UASB reactor is not included in
491 the estimate as this is required for both systems. Additional information, including
492 economic analysis worksheet, can be found in the supporting information (Refer to **Table S2**
493 & **Table S3**).

494 The results in **Figure 10 (a)** show unless treating population equivalents of at least 50
495 people, the total monthly cost (CAPEX + OPEX) is significantly higher than for the current
496 “septic tank” systems. For a 15 person system, the total unit cost of the new system is
497 estimated at USD 0.84/PE/month, which is about 60% higher compared to USD
498 0.52/PE/month for a conventional system. The total cost difference reduces to USD
499 0.58/PE/month for a 25 user system which is only 38% higher compared to USD
500 0.42/PE/month for the conventional system. However, for larger system such as 50, 75 and
501 100 PE, the total unit cost for the new system is cheaper by 7 - 10% compared to
502 conventional treatment. For a 100 user system, for example, the total cost of the new
503 system is USD 0.27/PE/month compared to USD 0.29/PE/month for a conventional system.
504 In terms of total cost per household, for 50 users system and above, the new system costs
505 USD 1.57/HH/month compared to USD 1.74/HH/month for a conventional system and USD
506 2.04/HH/month for a DEWATS (refer **Table S4**). Since most modern buildings in Bhutan
507 within the commercial and semi-commercial areas are expected to range within 50-100 PE,
508 the cost implications on the new owner is insignificant. The government could provide
509 limited subsidies to encourage adoption of new on-site treatment system for buildings with
510 PE lower than 50.

511 **Figure 10 (b)** shows the contributions of CAPEX and OPEX to the total cost of the
 512 system for different numbers of users. OPEX is the major cost component of the new on-site
 513 wastewater treatment system (53 - 66%) compared to “septic tanks” where the CAPEX
 514 forms the major cost component (68 - 78%). The per capita OPEX cost component of the
 515 new system decreases at a larger scale.

516 If sufficient methane is produced from the two bioreactors to be used as energy, it could
 517 mitigate the monthly operating costs of the treatment system. Based on the measured
 518 average of 128 L CH₄/kg COD for UASB and 54 L CH₄/kg COD for ABF, it would require about
 519 750 PE to produce enough methane to supply to one household (5 people) for their daily
 520 cooking usage in Bhutan, assuming a 14.2 kg LPG gas cylinder lasts about a month for each
 521 household (MoEA 2019). This indicates that methane capture and collection from this new
 522 wastewater treatment system cannot be claimed as a benefit for a single building. However,
 523 if the biogas from the on-site treatment system of all the buildings within the
 524 neighbourhood is collected, it may be able to generate enough biogas viable for supplies to
 525 several households and thereby helping prevent the release of the highly potent
 526 greenhouse gases from the treatment system.

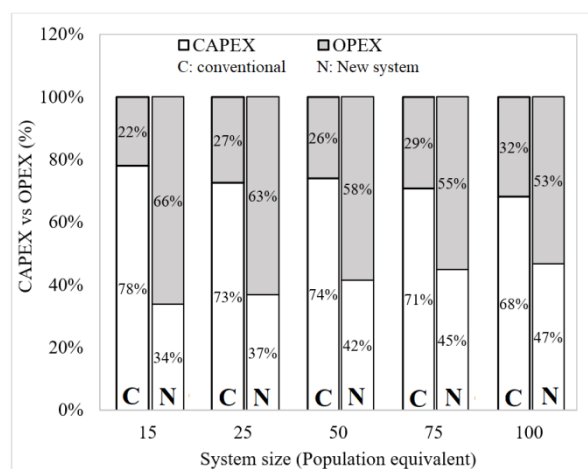
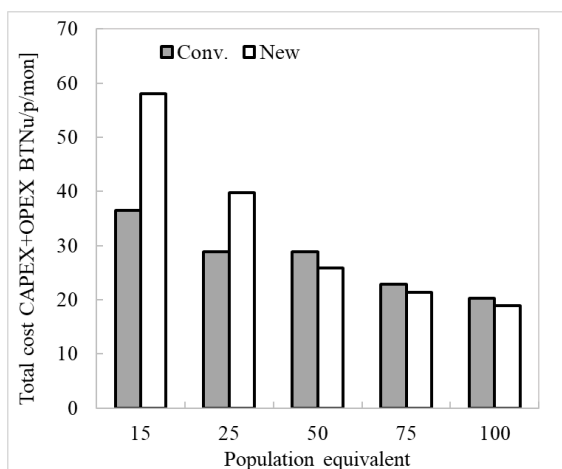


Figure 10: Comparative economic analysis of the new (N) improved on-site wastewater treatment system (UASB + ABF) and the conventional (C) on-site sanitation system (“septic tank” and soak pit). (a) Unit total cost (CAPEX + OPEX) and (b) Cost components of CAPEX and OPEX. Economic parameters: economic life 30 years & interest rate of 10%.

527 **4. Conclusions**

528 An on-site wastewater treatment system consisting of a combined UASB and ABF in
529 sequence with a total 1,000 L/d capacity was piloted for treating domestic wastewater for
530 about a year in Bhutan. The following conclusions are drawn from this study:

- 531 1. The pilot system produced a final treated effluent quality with an average BOD₅ of 28
532 mg/L, COD 38 mg/L, TSS 85 mg/L and 5 log units of *Escherichia coli*
- 533 2. The pilot effluent meets three out of four of the effluent discharge limits of Bhutan, and
534 could not meet the *Escherichia coli* standard. However, further process optimisation
535 may enable greater *Escherichia coli* removal and hence is an interest of future research.
- 536 3. An economic analysis indicates that, for a building with over 50 population equivalent,
537 the cost (CAPEX and OPEX) of the piloted wastewater treatment system is 7 - 10% lower
538 than conventional septic tanks.

539 This study, therefore, validates the earlier lab-scale study that a combined UASB and ABF
540 (using shredded waste plastic bottles) can be effective in the treatment of domestic
541 wastewater as an alternative to “septic tanks” that risk discharge of a poorly treated
542 effluent to the environment. This new wastewater treatment system could contribute to
543 protecting the environment from wastewater pollution, but also help reduce the financial
544 burden on the local government from the investment in expensive sewerage infrastructure.

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