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1 **Anaerobic digestion of soft drink beverage waste and sewage sludge**

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14 **Abstract**

15 Soft drink beverage waste (BW) was evaluated as a potential substrate for anaerobic co-
16 digestion with sewage sludge to increase biogas production. Results from this study show that
17 the increase in biogas production is proportional to the increase in organic loading rate (OLR)
18 rate due to BW addition. The OLR increase of 86 and 171% corresponding to 10 and 20%
19 BW by volume in the feed resulted in 89 and 191% increase in biogas production,
20 respectively. Under a stable condition, anaerobic co-digestion with BW did not lead to any
21 significant impact on digestate quality (in terms of COD removal and biosolids odour) and
22 biogas composition. **The results suggest that existing nutrients in sewage sludge can support**
23 **an increase in OLR by about 2 kg COD/m³/d from a carbon rich substrate such as soft drink**
24 **BW without inhibition or excessive impact on subsequent handling of the digestate.**

25 **Keywords:** Anaerobic co-digestion, beverage waste, sewage sludge, biogas, organic loading
26 rate.

27 **1. Introduction**

28 Anaerobic digestion is an integral component of municipal wastewater treatment, providing
29 the efficient stabilisation and volume minimisation of sewage sludge through biological
30 degradation (Wan et al., 2011, Sawatdeenarunat et al., 2016). In most wastewater treatment
31 plants (WWTPs), sewage sludge is currently digested on its own. In recent years, concern
32 about climate change and energy security has renewed the interest in anaerobic digestion as a
33 platform for renewable energy production from organic wastes and sewage sludge (Berkessa
34 et al., 2018, Tuyet et al., 2016, Li et al., 2017, Dennehy et al., 2018, Chu et al., 2015, Nguyen
35 et al., 2017). Indeed, research activities in anaerobic co-digestion have gained significant
36 momentum over the past decade (Luostarinen et al., 2009, Nghiem et al., 2017). Several
37 water utilities around the world have begun to explore the possibility of co-digesting organic
38 waste with sewage sludge using the spare digestion capacity at existing WWTPs (Tampio et
39 al., 2016, Nghiem et al., 2017).

40 Anaerobic co-digestion involves the pairing of two or more organic wastes with
41 complementary characteristics (Xie et al., 2016). In the context of a WWTP, sewage sludge is
42 rich in nutrients (i.e. nitrogen and phosphorus) and contains all necessary micronutrients for
43 the anaerobic process. On the other hand, organic wastes are a source of carbon for methane
44 production but are often deficient in nutrients including micronutrients. Co-digestion can also
45 benefit the anaerobic digestion process through the dilution of inhibitory substances that may
46 originate from either sewage sludge or organic waste co-substrates (Mata-Alvarez et al.,
47 2011). Furthermore, co-digestion presents an array of environmental and economic benefits.
48 These include the diversion of putrescible wastes from landfill or incineration, the increase in
49 the generation and feasibility of onsite renewable energy production and the added revenue
50 offered through charging gate fees (Nghiem et al., 2017).

51 The selection for suitable co-substrate pairing and the optimisation of mixing ratios and
52 organic loading rates (OLRs) are paramount to the widespread adoption of the practice. The
53 prevailing substrate selection parameter for AD co-digestion in literature concerns the total
54 organic carbon to total nitrogen (C/N) ratio of the feed solution, with the ideal ratio generally
55 accepted to fall in the range of 15:1 and 30:1 (Weiland, 2010). Because substrates generally
56 do not possess an ideal C/N ratio, co-digestion can mutually improve overall performance
57 and stability. In general, sewage sludge has a low C/N ratio. Despite a somewhat limited
58 biomethane potential, sewage sludge can provide a high buffering capacity and all the

59 necessary micronutrients for the anaerobic digestion process (Mata-Alvarez et al., 2014).
60 Consequently, sewage sludge is arguably the most prevalent co-substrate in the current
61 anaerobic digestion literature (Mata-Alvarez et al., 2011). In addition, due to the low carbon
62 content in sewage sludge, the digesters at most WWTPs are operated at a low OLR.

63 The C/N ratio is not the only parameter that is important in regulating anaerobic co-digestion
64 performance. Excessive inclusion of a carbon rich substrate (that can be rapidly hydrolysed
65 into volatile fatty acids (VFAs), which are an intermediate product) can destabilise the
66 anaerobic digestion process. Indeed, severe accumulation of these acids can cause the
67 acidification of the reactor and subsequent inhibition of further digestion, i.e.
68 methanogenesis. Furthermore, whilst it is attractive to utilise the spare digestion capacity in
69 WWTPs for anaerobic co-digestion to enhance biogas production, there are some concerns
70 regarding potential inhibition or negative implications for biosolids (solid fraction of digested
71 sludge after dewatering) properties due to co-substrate addition. Thus the identification and
72 demonstration of suitable co-substrates for sewage sludge co-digestion remain essential for
73 the widespread uptake of the practice. Inhibition can lead to the reduction and even collapse
74 of the biodegradation process. The primary cause of instability is the imbalance between
75 methanogenic and acidogenic functional microbial groups, resulting from their variable
76 requirements and growth kinetics (Chen et al., 2008). From an operational perspective,
77 inhibition in co-digestion is seen to derive from both substrate selection and the mixing ratio
78 adopted. These parameters can be represented through the OLR, which is subsequently the
79 primary comparative measure for the operational ranges of different substrates.

80 The potential impact of co-digestion of sewage sludge upon biosolids quality and volume can
81 restricts the implementation of the practice at full scale WWTPs. Biosolids management
82 accounts for as much as 50% of the operational cost at some WWTPs (Semblante et al.,
83 2014). Whilst in Europe biosolids are generally incinerated, in countries such as Australia
84 and the USA, biosolids volume and quality are of higher importance as they are most
85 commonly used for land application (Nghiem et al., 2017). In addition, some co-substrates
86 may contain contaminants such as heavy metals and persistent organic chemicals. The
87 occurrence of these contaminants in biosolids at a high concentration can render them
88 unsuitable for land applications (Demirel et al., 2013, Bonetta et al., 2014). At the same time,
89 there are a range of organic wastes in the urban environment that are both abundant and
90 benign, making them highly attractive as a co-substrate for anaerobic co-digestion.

91 Beverage waste is a major source of organic substrate in metropolitan areas and a potential
92 candidate for co-digestion with sewage sludge. It includes soft drink, alcoholic beverage, pre-
93 mixed drink, and juice. Beverage waste such as soft drink consists primarily of water along
94 with approximately 10-12% w/v dissolved carbon, mostly in the form of sugar (Isla et al.,
95 2013). The volume of beverage waste produced annually is enormous. About 4.5 million
96 m³/year of beverage waste is produced in Argentina. In the UK, it is estimated that 200,000
97 million m³ of beverage waste was produced in 2012 (Quested et al., 2013, Isla et al., 2013).
98 Disposal of beverage production waste typically involves dilution into municipal wastewater
99 streams, onsite treatment, or land spreading. **These all constitute a loss in the potential**
100 **recoverable energy and may result in environmental pollution.** Despite the significant volume
101 of beverage waste and its potential as a co-substrate for biogas production, the co-digestion of
102 beverage waste has yet to be demonstrated in the current literature.

103 This work focuses on the optimisation of the co-digestion ratio between beverage waste and
104 sewage sludge and the overall OLR in terms of biomethane production and system stability.
105 The study further seeks to determine the likely type of inhibition associated with excessive
106 concentrations of the co-substrate. Particular emphasis is directed toward elucidating the
107 impact of co-digestion on the digestate quality in terms of biosolids odour potential.

108 **2. Materials and Methods**

109 **2.1 Substrates**

110 Anaerobically digested sludge was obtained from the Wollongong wastewater treatment plant
111 (WWTP) in New South Wales (NSW) Australia and used as the inoculum. Primary sludge
112 was obtained from the same plant every fortnight. A mixture of carbonated soft drinks was
113 obtained from a commercial waste collector in NSW Australia. These soft drinks did not
114 meet market requirements (e.g. out of date, damaged packaging, and contamination) and thus
115 had to be destroyed and disposed. Diet and sugar free soft drinks were excluded from this
116 study. The primary sludge and beverage waste were stored at 4 °C in the dark. Any unused
117 portion of these substrates was discarded after two weeks of storage.

118 **2.2 Experimental systems**

119 Three identical anaerobic digesters were operated in parallel in this study. Each digester
120 consisted of a 28 L stainless steel conical shape reactor, a peristaltic hose pump (DULCO®
121 Flex from Prominent Fluid Controls, Australia), a biogas counter (Ritter Company™,

122 MilliGascounter), a thermal probe and a gas trap for biogas sampling. A temperature control
 123 unit (Neslab RTE 7, Thermo Fisher Scientific, Newington, USA) was used to maintain the
 124 reactor temperature at 35 ± 1 °C. This was achieved by circulating hot water from the
 125 temperature control unit through a rubber tube that was firmly wrapped around the reactor.
 126 The reactor and pipeline were encased in polystyrene foam for insulation. The peristaltic hose
 127 pump was continuously operated to circulate the digestate at 60 L/h for mixing. Further
 128 details of these anaerobic digesters are available elsewhere (Yang et al., 2017).

129 2.3 Experimental Protocol

130 The working volume of each reactor was set at 20 L. At the beginning of this study, all three
 131 reactors were seeded with digestate from the Wollongong WWTP and were flushed with N₂
 132 gas for 5 min. Unless otherwise stated, the hydraulic retention time (HRT) was set at 20 days.
 133 Each day, 1 L of digestate was removed from the digester and then 1 L of co-substrate (either
 134 primary sludge or a combination of primary sludge and beverage waste) was fed into the
 135 digester via the peristaltic pump.

136 **Table 1:** Operating conditions of the three anaerobic digesters over the 3 experiment stages.

		Reactor 1	Reactor 2	Reactor 3
Stage 1	OLR (kg COD/m ³ /d)	1.16	1.16	1.16
	Beverage waste (%)	0	0	0
	HRT (d)	20	20	20
	Duration (d)	52	52	52
Stage 2	OLR (kg COD/m ³ /d)	3.03	2.08	1.16
	Beverage waste (%)	20	10	0
	HRT (d)	20	20	20
	Duration (d)	31	31	31
Stage 3	OLR (kg COD/m ³ /d)	3.80	3.88	1.16
	Beverage waste (%)	30	20	0
	HRT (d)	20	15	20
	Duration (d)	25	25	25

137 The experiment was conducted over three stages (Table 1). In Stage 1, all three reactors were
 138 fed with primary sludge for 7 weeks to establish the baseline conditions. In the subsequent
 139 stages, Reactor 3 was used as the control system (same operating condition as in Stage 1)
 140 while Reactors 1 and 2 were used to evaluate the co-digestion of sewage sludge and beverage
 141 waste. In Stage 2, in addition to primary sludge, beverage waste was also fed into Reactors 1
 142 and 2 at 20 and 10% (vol/vol) of the total feed, respectively. In Stage 3, the portion of
 143 beverage waste fed into Reactors 1 and 2 was increased further to 30 and 20% (vol/vol)

144 respectively. It is noted that the HRT of Reactor 2 was shortened to 15 d to achieve a similar
145 OLR in both reactors.

146 **2.4 Analytical Methods**

147 TS, VS, alkalinity, total COD and soluble COD, pH and total organic acids (TOA) of the
148 digestate were measured weekly. The primary sludge feed and beverage waste were also
149 characterised on a weekly basis. COD measurements were conducted using a Hatch DRB200
150 COD Reactor and Hatch DR3900 spectrophotometer (program number 435 COD HR)
151 following the US-EPA Standard Method 5220. Biogas composition analysis was conducted
152 on a weekly basis by a portable gas analyser (GA5000 Gas Analyser, Geotechnical
153 Instruments (UK) Ltd., England) using the gas trap to store the required 1 L gas sample prior
154 to measurement. The details of these analytical techniques are available elsewhere (Yang et
155 al., 2016, Nghiem et al., 2014).

156 Odour measurement was conducted based on an incubation technique previously reported by
157 Glindemann et al. (2006) This method allows for the monitoring of hydrogen sulphite and
158 six other sulphur bearing odour compounds. In brief, digestate was dewatered by laboratory
159 centrifuge using the method previously developed by To et al. (2016). Then, 25 g of biosolids
160 cake was collected into a 500 mL PET bottle. The bottle was sealed using a rubber cap and
161 incubated at $28\pm 1^\circ\text{C}$. The head space was extracted using a syringe at a specific time interval
162 for Gas Chromatography – Mass Spectrometry analysis. The results are reported as
163 volumetric concentration in the incubation bottle headspace.

164 **3. Results and Discussion**

165 **3.1 Substrate Characteristics**

166 Key properties of the inoculum, primary sludge and beverage waste are summarised in Table
167 2. Beverage waste contained a significantly higher organic fraction mostly in the form of
168 dissolved sugars than the primary sludge. On the other hand, VS of the beverage waste is
169 only marginally higher than that of primary sludge (Table 2). Given the high carbon content
170 and easily degradable organics (owing to the high sugar content) of beverage waste, its co-
171 digestion with nutrient rich, high buffering capacity primary sludge can provide
172 complementary benefits to both co-substrates. Co-digestion is necessary to achieve a balance
173 between organic carbon and nutrients to prevent pH-derived inhibition based on the rapid
174 formation of intermediate products, specifically volatile fatty acids.

175 **Table 2:** Characteristics of the inoculum, primary sludge feed, and beverage waste (mean \pm
 176 standard deviation of at least 5 samples).

	Inoculum	Primary sludge	Beverage waste
TS (%) (fresh weight)	0.85	1.7 \pm 0.2	4.73 \pm 0.5
VS (%) (fresh weight)	0.56	1.3 \pm 0.1	4.59 \pm 0.5
VS/TS (%)	65	78 \pm 1	92 \pm 1
pH	6.6	6.1 \pm 0.5	3.3 \pm 0.1
COD (mg/L fresh weight)	8300	22300 \pm 1750	204000 \pm 27000

177 3.2 Process Performance

178 3.2.1 Biomethane Production

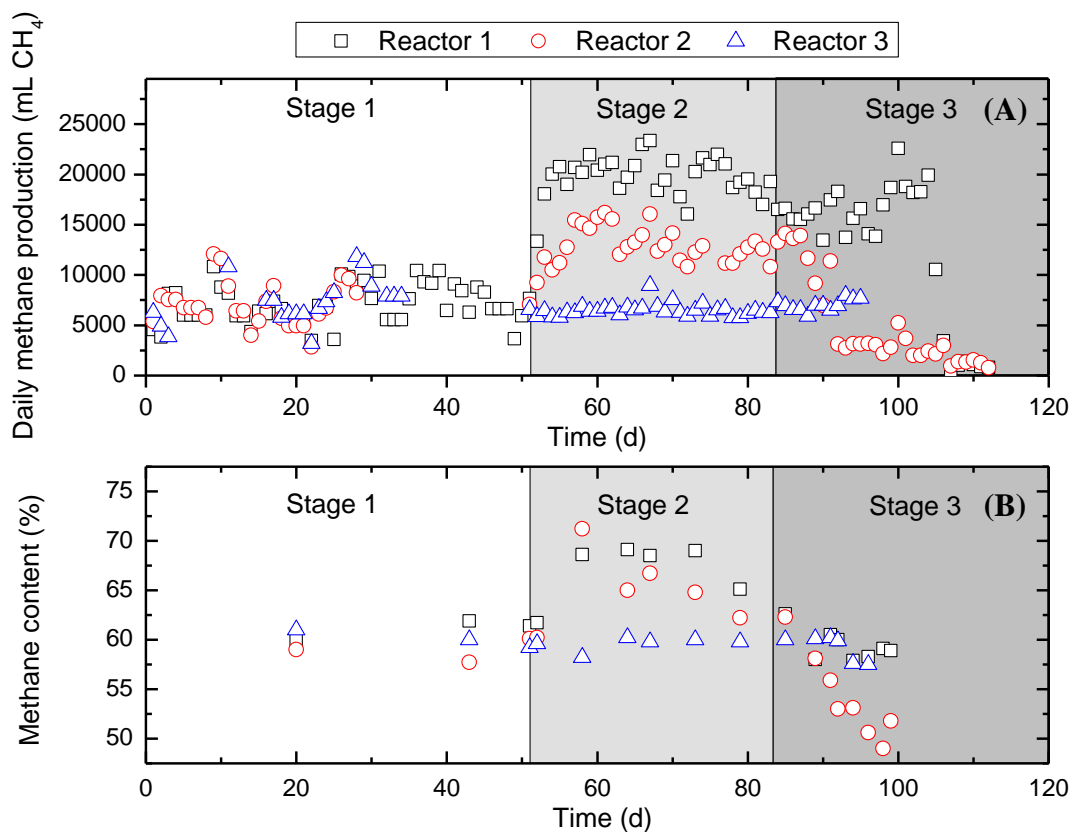
179 In Stage 1, the three reactors were preconditioned under the same operating conditions. As
 180 expected, biomethane production from all three reactors was almost identical during this
 181 stage (Figure 1 A). In Stage 2, stable biomethane production was observed during the co-
 182 digestion of sewage sludge with beverage waste co-digestion ratios of 10 and 20%,
 183 corresponding to an increase in OLR of 86 and 171% and in overall biogas production of 89
 184 and 191% (compared to the control reactor).

185 During the early phase of Stage 2, an elevated methane content of approximately 70% was
 186 observed in the biogas produced from Reactors 1 and 2 compared to the baseline value of
 187 60% from Reactor 3 (Figure 1 B). However, at the end of Stage 2, the methane content in
 188 biogas from Reactors 1 and 2 returned to the baseline. The brevity of this increase in methane
 189 content suggests the change may result from the transitory condition of co-digestion with
 190 carbohydrate rich co-substrates. A permanent increase in methane content was observed by
 191 Jang et al. (2016) during the co-digestion of food waste and waste activated sludge,
 192 corresponding to higher food waste mixing ratios. However, it is noted that the initial
 193 methane content recorded in previous feeding conditions in Jang et al. (2016) was usually
 194 low (i.e. only 50%) and their observations concern the co-digestion of different co-substrates.
 195 The disparity between our findings and that from Jang et al. (2016) highlights the need for
 196 further research to ascertain the potential effect of co-digestion on methane content.

197 In addition to the overall biomethane production, co-digestion may also affect the specific
 198 methane yield. Over the duration of the experiment the methane yield of the sewage sludge
 199 was 284 mL CH₄/g COD added, suggesting a highly degradable sludge substrate compared to
 200 typical values in literature, often in the range of 188 to 214 mL CH₄/g COD (Astals et al.,
 201 2013). It is noted that in Stage 2, the specific methane yield of the control (Reactor 3)

202 decreased slightly from 300 to 275 mL/g COD added. On the other hand, the specific methane
 203 yield of Reactors 1 and 2 were stable despite the addition of beverage waste (Figure 2). This
 204 is consistent with the findings by Razaviarani et al. (2013), who observed a slight increase in
 205 the specific methane yield when co-digesting sewage sludge with glycerine. Further results
 206 suggest the increase was likely due to the higher digestible fraction of beverage waste relative
 207 to sewage sludge, rather than due to any synergistic effect.

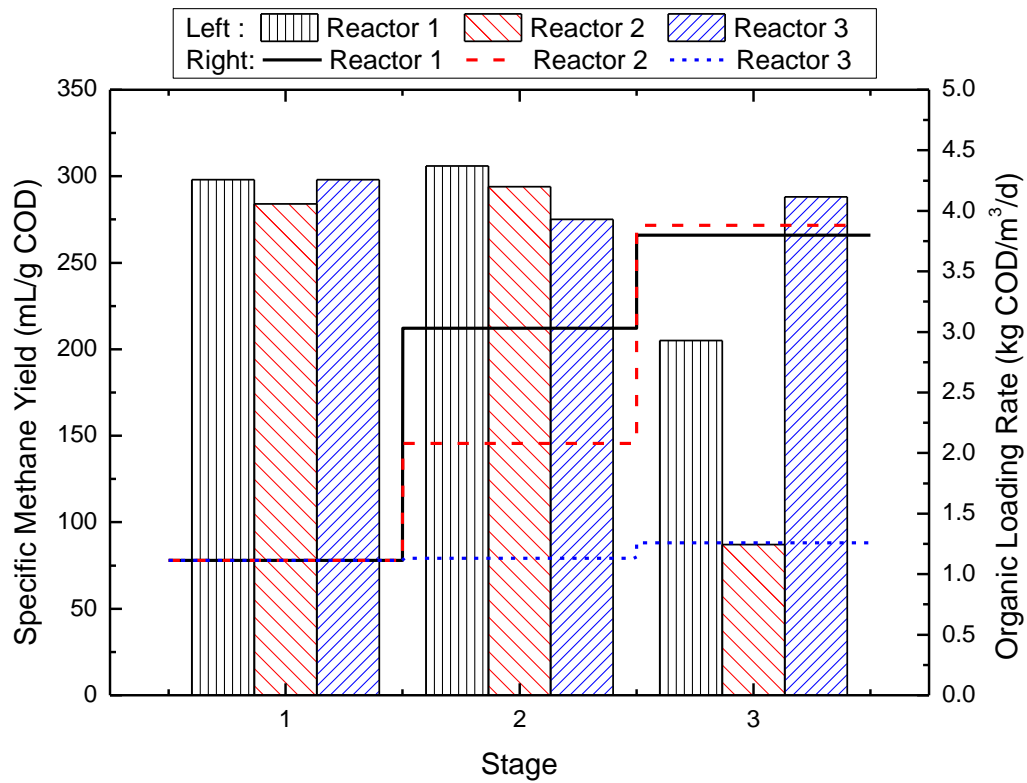
208 Inhibition of both co-digestion reactors was observed during Stage 3 at an OLR of ~3.8-3.9
 209 kg COD/m³/d. During Stage 3, both the volume and methane content of the biogas declined
 210 sharply in Reactors 1 and 2. When applying mono-digestion of a similar soft-drink
 211 wastewater, Redzwan and Banks (2007) observed complete inhibition of methanogenic
 212 processes at a loading rate of 1.33 kg COD/m³ in batch experiments, which was attributed to
 213 alkalinity loss (from 2300 mg/L to 1000 mg/L) and accumulation of volatile fatty acids,
 214 which reached a concentration of 1500 mg/L. Whilst this inhibitory OLR value was obtained
 215 in batch experiments and therefore is not directly comparable, it demonstrates the risk of
 216 overloading when co-digesting with organic rich co-substrates. Furthermore, the stable
 217 operation demonstrated at a much higher OLR in this study suggests that co-digestion of the
 218 substrate with sewage sludge can improve digestion stability.



219

220 **Figure 1:** Biomethane Production: (A) Daily biomethane production at different
 221 experimental stages and (B) Methane content in biogas during different experimental stages
 222 (temperature = 35 ± 1 °C; other experimental conditions are as described in Table 1).

223



224

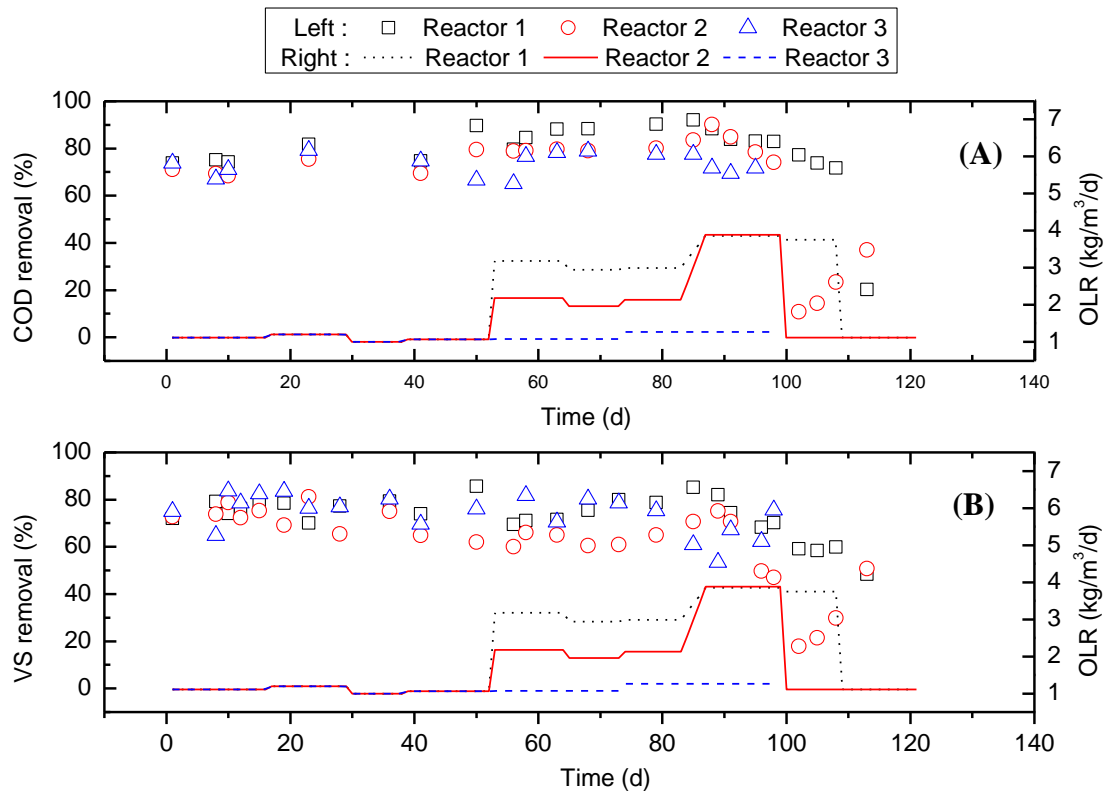
225 **Figure 2:** Specific methane yields at each OLR value (temperature = 35 ± 1 °C; other
 226 experimental conditions are as described in Table 1).

227

228 3.2.2 Digestate Quality

229 The removal efficiency of both VS and COD was constant throughout Stages 1 and 2 of the
 230 experiment, indicating that co-digestion does not significantly impact on the performance of
 231 anaerobic digestion (Figure 3). Nevertheless, a small increase in VS and COD in the digestate
 232 from Reactors 1 and 2 during Stage 2 could be observed. At steady-state condition in Stage 2,
 233 the average VS content in the digestate from Reactor 1 and 2 by 21% and 27% respectively,
 234 compared to Stage 1. Similarly, the average total COD in the digestate from Reactor 1 and 2
 235 also increased by 18% and 23% compared to Stage 1. This is a small increase compared to
 236 the increase in OLR of 171 and 86% in Reactor 1 and 2, respectively. Indeed, this small

237 increase in VS and COD in the digestate is expected and can be attributed to a high OLR
 238 value. During Stage 2 the ratio of VS/TS in the digestate was relatively constant in Reactors
 239 1, 2 and 3, with ratios of 63, 65 and 62% respectively.



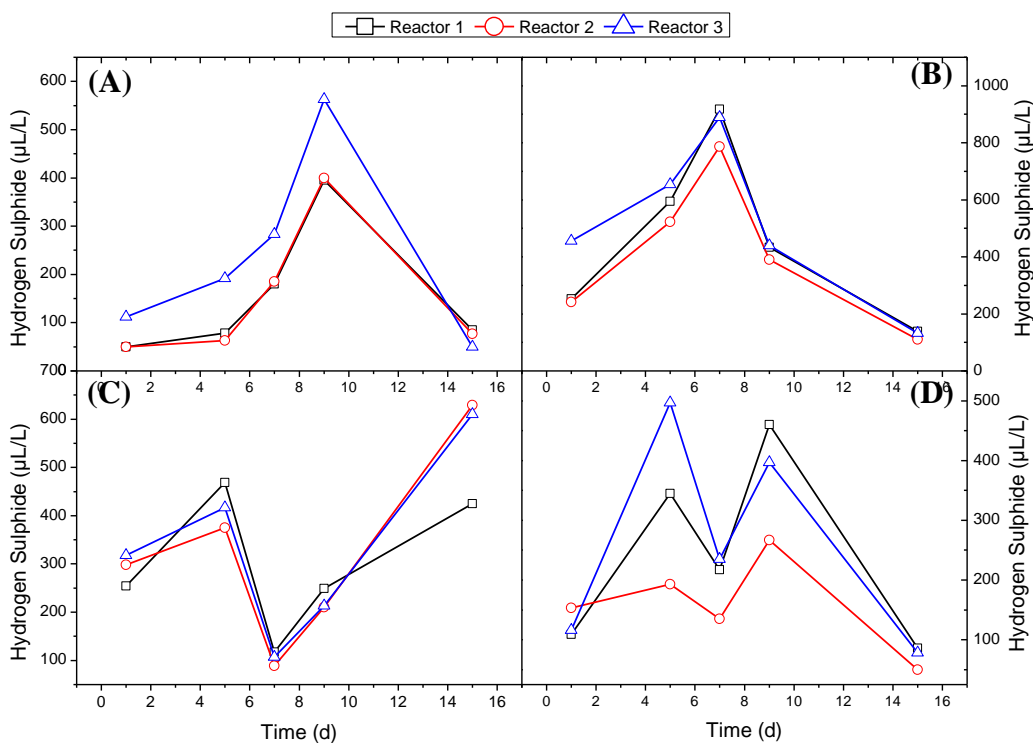
240

241 **Figure 3:** Biosolids stabilisation: (A) Volatile removal efficiency and (B) total COD removal
 242 efficiency with corresponding OLR value (temperature = 35 ± 1 °C; other experimental
 243 conditions are as described in Table 1).

244 Soft drinks only contain sugar and flavours, thus as expected, their co-digestion with sewage
 245 sludge does not result in any notable impact on biosolids odour. Similar to the VS and COD
 246 removal data, under a stable condition (Stage 2), the odour potential of biosolids samples
 247 from all three Reactors was almost identical (Figure 4). It is also noteworthy that among the
 248 seven sulphur bearing odour compounds (namely H_2S , CH_4S , C_2H_6S , $C_2H_6S_2$, CS_2 , $(CH_3)_2S$,
 249 COS) from biosolids monitored in this study, only hydrogen sulfide (H_2S) was prevalent in
 250 all samples. In most instances, the concentration of H_2S in the head space of biosolids
 251 samples from Reactor 3 (mono digestion) was slightly higher than that from Reactor 1 and 2
 252 (co-digestion).

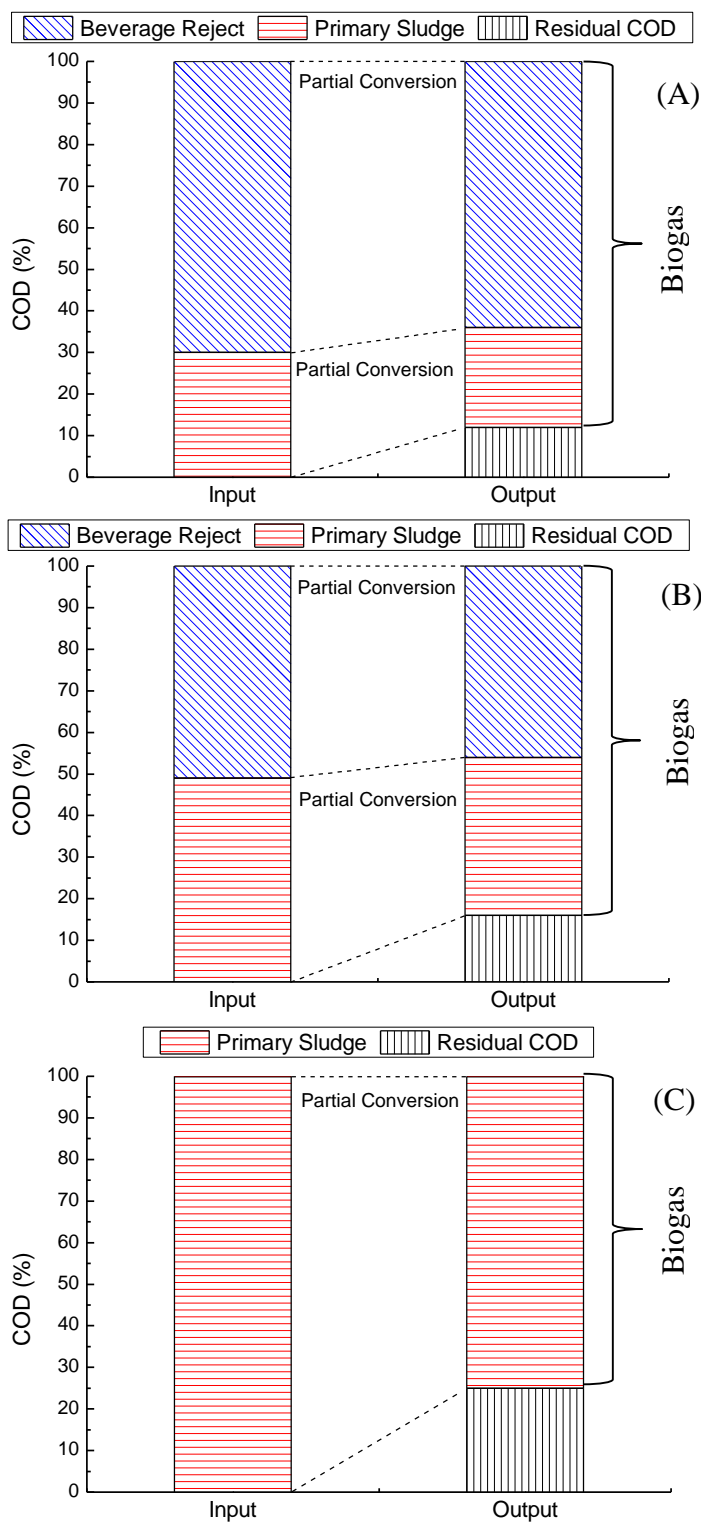
253 3.2.3 Impact of co-digestion on specific methane yield

254 The co-digestion of beverage waste with primary sludge was evaluated using a COD balance
 255 approach, previously adopted in Aichinger et al. (2015). A COD balance was used to
 256 represent the digestion performance of each of the reactors during Stage 2 (Figure 5). The
 257 specific biomethane yields and subsequent COD consumption were determined for the mono-
 258 digestion of each substrate. Reactor 3 demonstrated a biomethane yield of 275 mL/g COD
 259 added, which was used to represent sewage sludge COD consumption. Meanwhile a yield of
 260 321 mL/g COD was adopted for beverage waste, derived from previous biomethane potential
 261 evaluation of the substrate (Wickham et al., 2016). Based on this data, the conversion of
 262 COD into biomethane in each substrate precisely matched their performance during mono-
 263 digestion. Results in Figure 6 show that beverage waste was fully digested. In other words,
 264 beverage waste addition did not result in any discernible increase in the COD content of the
 265 final digestate. On the other hand, data in Figure 5 cannot be used to confirm the synergistic
 266 effect of sewage sludge and beverage waste co-digestion. The lack of observable synergism is
 267 not unexpected as substrates rich in rapidly degradable organic matter have been observed to
 268 produce little to no synergetic effects during co-digestion (Jensen et al., 2014).



269

270 **Figure 4:** Odour generation over 15 days from biosolids produced during Stage 2, sampled
 271 on (A) day 64, (B) day 71 (C), day 78 and (D) day 85 (Reactor 1: co-digestion with 20%
 272 (v/v) beverage waste, Reactor 2: co-digestion with 10% (v/v) beverage waste and Reactor 3:
 273 mono-digestion of primary sludge) temperature = 35 ± 1 °C; other experimental conditions
 274 are as described in Table 1.

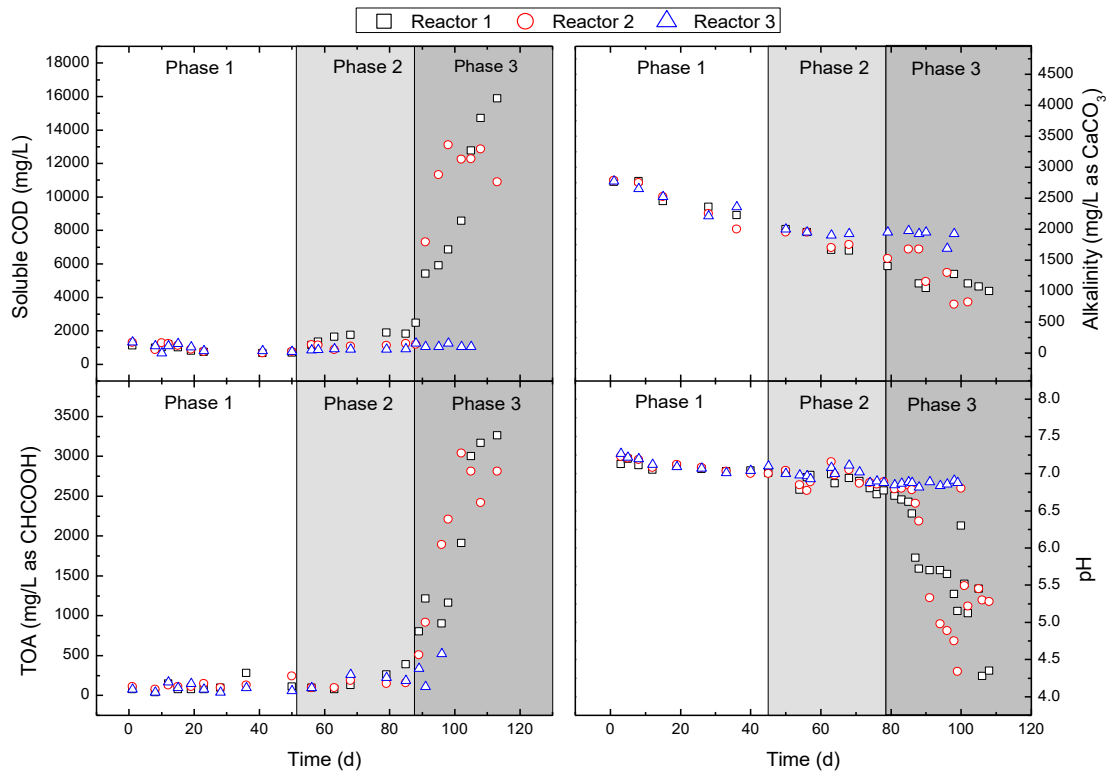


275 **Figure 5:** Average substrate mixture and corresponding gas production represented as COD
 276 balance during Stage 2 for (A) Reactor 1 (co-digestion with 20% (v/v) beverage waste), (B)
 277

278 Reactor 2 (co-digestion with 10% (v/v) beverage waste) and (C) Reactor 2 (mono-digestion
279 of primary sludge) Temperature = 35 ± 1 °C; other experimental conditions are as described
280 in Table 1.

281 **3.3 Process Stability**

282 Process stability was evaluated through the measurement of soluble COD, alkalinity, TOA,
283 and pH. Stable co-digestion was observed in each of the parameters throughout Stages 1 and
284 2 (Figure 6). A slight decline in alkalinity in Reactors 1 and 2 relative to the control was
285 observed. However; this was not accompanied by any accumulation of TOAs or significant
286 drop in pH, indicating the degradation of BW generates less alkalinity than that of sewage
287 sludge. A sharp decline in the stability of Reactors 1 and 2 occurred with the commencement
288 of Stage 3. The higher OLR values adopted in these reactors instigated the rapid
289 accumulation of COD in the form of organic acids, leading to the consumption of alkalinity
290 and sharp decline in pH. Indeed, in Stage 3, the profiles of soluble COD and TOA in Reactors
291 1 and 2 closely resemble each other (Figure 6). The progression of inhibition aligns well with
292 previous findings, as carbohydrate rich co-substrates are known to pose risks in the
293 accumulation of intermediaries such as volatile fatty acids (Astals et al., 2014). It is
294 noteworthy that a similar rate of inhibition was observed in both in Reactors 1 and 2. In Stage
295 3, these reactors have similar OLR value but different HRT (20 vs 15 days). These results
296 suggest that inhibition was intrinsically due to a high OLR value rather than the sudden
297 variation in organic loading.



298
 299 **Figure 6:** Basic stability parameters: Soluble COD, Alkalinity, TOA and pH (Temperature =
 300 35 ± 1 °C; other experimental conditions are as described in Table 1).

301

302 4. Conclusions

303 Soft drink beverage waste (BW) was evaluated for anaerobic co-digestion with sewage
 304 sludge for the first time. Biogas production increase was proportional to the increase in
 305 organic loading rate (OLR) from BW addition. The OLR increase of 171% corresponding to
 306 20%(v/v) BW in the feed was the optimum co-digestion ratio, and resulted in an biogas
 307 production increase of 191%. Under this optimum condition, co-digestion with BW did not
 308 result in any significant impact on digestate quality and biogas composition. The results
 309 suggest that sewage sludge can support about 2 kg COD/m³/d OLR increase from a carbon
 310 rich co-substrate.

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