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1 **Comparative analysis of the digestibility of sewage fine sieved fraction and**  
2 **hygiene paper produced from virgin fibers and recycled fibers**

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18

19 **Abstract**

20 Sewage fine sieved fraction (FSF) is a heterogeneous substrate consisting of mainly toilet  
21 paper fibers sequestered from municipal raw sewage by a fine screen. In earlier studies, a  
22 maximum biodegradation of 62% and 57% of the sewage FSF was found under thermophilic  
23 (55°C) and mesophilic (35°C) conditions, respectively. In order to research this limited  
24 biodegradability of sewage FSF, this study investigates the biodegradation of different types  
25 of cellulosic fibers-based hygiene papers including virgin fibers based toilet paper (VTP),  
26 recycled fiber based toilet paper (RTP), virgin pulp for paper production (VPPP) as a raw  
27 material, as well as microcrystalline cellulose (MCC) as a kind of fiberless reference material.  
28 The anaerobic biodegradation or digestibility tests were conducted under thermophilic and  
29 mesophilic conditions. Results of the experiments showed different biomethane potential  
30 (BMP) values for each tested cellulose fiber-based substrate, which might be associated with  
31 the physical characteristics of the fibers, type of pulping, presence of lignin encrusted fibers,  
32 and/or the presence of additive chemicals and refractory compounds. Higher hydrolysis rates  
33 ( $K_h$ ), higher specific methane production rates (SMPR) and shorter required incubation times  
34 to achieve 90% of the BMP ( $t_{90\%CH_4}$ ), were achieved under thermophilic conditions for all  
35 examined substrates compared to the mesophilic ones. Furthermore, the biodegradability of  
36 all employed cellulose fiber-based substrates was in the same range, 38%-45%, under both  
37 conditions and less than the observed FSF biodegradability, i.e. 57%-62%. MCC achieved the  
38 highest BMP and biodegradability, 86%-91%, among all cellulosic substrates.

39 **Key words:** Anaerobic digestion; thermophilic; mesophilic; biomethane potential, virgin pulp,  
40 toilet paper, fine sieved fraction

41

## 42 **1. Introduction**

43 At the sewage treatment plant (STP) Blaricum, the Netherlands, a 350  $\mu\text{m}$  mesh size fine  
44 sieve (Salsnes Filter, Norway) for raw sewage pretreatment is installed, immediately after the  
45 6 mm coarse screen. The fine sieve is implemented as a compact alternative to primary  
46 clarification to separate suspended solids from sewage prior to biological nutrient removal.  
47 The produced cake layer or fine sieved fraction (FSF) has a very heterogeneous composition  
48 but is presumed to contain mainly cellulosic fibers originating from toilet paper (Ruiken et al.,  
49 2013). Considering its nature and high energy content, FSF receives growing interest in  
50 countries like the Netherlands, either for cellulose fiber recovery or as feedstock for energy  
51 recovery (STOWA, 2010). Regarding the latter, increasing effort is put on onsite energy  
52 recovery for closing the energy balance, eventually realizing an energy neutral or energy  
53 producing STP.

54 Toilet paper or toilet tissue is one of the mostly used hygiene products, particularly in  
55 Northern Americas, and European countries, whereas it is less used in large parts of Asia and  
56 Africa (<http://www.worldwatch.org/node/5142>). The major component of all hygiene papers  
57 is fibrous cellulose, mostly from tree origin. Toilet papers are available in different qualities;  
58 they are generally smooth and can be embossed, unprinted or patterned, tinted, purely white  
59 or off-white (Holik, 2006).

60 Toilet paper is either made from virgin pulp, which is mainly extracted from wood and partly  
61 from non-wood cellulose (e.g., bamboo) and is called virgin fibers based toilet paper (VTP),  
62 or it is made from recycled paper fibers, which is known as recycled fibers based toilet paper  
63 (RTP). The type of pulp and paper chemicals used has an influence on the final quality of the  
64 tissue paper, e.g. softness, strength, absorbency and appearance. In the process of making

65 virgin pulp as a raw material for paper production (VPPP), one type of wood is generally  
66 usually used, i.e. either soft or hard wood. However, in the production of VTP a combination  
67 of soft (long fiber for strength) and hard wood (short fiber for softness) is employed.  
68 Depending on the required specifications, paper makers choose their fiber source (long fibers,  
69 short fibers and combinations). RTP, which completely or partially consists of recycled fibers,  
70 may originate from different sources, such as mixed office waste, or old newspapers. Paper  
71 production using recycled fibers in the paper mill follows various process steps such as  
72 pulping, screening and de-inking stages (Kamali and Khodaparast, 2014). The majority of  
73 paper tissue used in the Netherlands is recycled fibers based. The ratio virgin fibers relative to  
74 recycled fibers determines the level of softness of the end product. However, application of  
75 specific chemicals and process steps can improve the strength, softness, brightness, etc., of  
76 any tissue product, regardless the fibers used (WRAP, 2005). During pulp making, pulp  
77 processing and paper-making, certain types of chemicals are used as presented in Table 1.  
78 However, every papermaking factory deviates according to their applied raw materials,  
79 desired products and process optimization. Generally speaking, these additives can be divided  
80 in two categories: (1) additives used during the process (2) additives for product improvement  
81 (Table 1). Theoretically, both could end up within the product, which however, is more likely  
82 for the 'product additives' (Bos et al., 1995). Therefore, there is no standard composition of  
83 toilet paper and very likely, also the biodegradability will vary with its composition.

84 Cellulose is the main constituent of toilet paper and its biodegradability likely depends on its  
85 fibrous content and its crystallinity. Maximum biodegradability is expected when no fibers are  
86 present, i.e. when the cellulose consists of powdered cellulose (PC) or microcrystalline  
87 cellulose (MCC). The chemical composition and physical structure of MCC fully depend on

88 the characteristics of the virgin material from which the cellulose is obtained as well as on the  
89 manufacturing conditions (Landin et al., 1993). As a result, several grades of MCC are  
90 available on the market with different physicochemical and thermal properties, exhibiting  
91 different functional parameters and applications (Azubuiké and Okhamafe, 2012). MCCs are  
92 prepared by acid hydrolysis under mild conditions of native cellulose to a critical degree of  
93 polymerization (DP) (Shcherbakova et al., 2012).

94 Fibers originating from tissue paper can be screened from the waterline before biological  
95 sewage treatment, in order to reduce aeration energy requirements and to generate  
96 possibilities to (re-)use these fibers or its energy content. One of the processing routes of the  
97 FSF of sewage influent is digestion (Ghasimi et al., 2015). Although the exact composition of  
98 our FSF substrate was not measured, an approximate composition can be deduced from  
99 Appliedcleantech ([www.appliedcleantech.com](http://www.appliedcleantech.com), accessed on 22 December 2015): 60-80% of  
100 cellulose, 5-10% of hemi-cellulose, 5-10% of lignin, 5-10% of oil and the rest accounted for  
101 inorganic salts (5-10%)”.

102 The FSF biodegradability was investigated in our previous researches in batch reactors,  
103 applying mesophilic and thermophilic conditions. Results of our previous study revealed a  
104 maximum biodegradability of 57% and 62% for mesophilic and thermophilic FSF digestion,  
105 respectively (Ghasimi et al., 2016). These low biodegradabilities raised the question about the  
106 actual biodegradability of the source materials used in the different toilet papers and the  
107 contribution of other organic matter to FSF digestibility. Therefore, series of batch anaerobic  
108 digestion tests were conducted under both thermophilic and mesophilic conditions to  
109 investigate the ultimate methane potential yield (BMP), specific methane production rate  
110 (SMPR), apparent hydrolysis rate ( $K_h$ ), incubation time needed to achieve 90% of the BMP

111 (t<sub>90%</sub>CH<sub>4</sub>) as well as anaerobic biodegradability (AnBD) of designated cellulose fiber-based  
112 substrates including VPPP, VTP, RTP and MCC as a fiberless reference material. The results  
113 were compared with FSF digestion results from previous studies.

114

## 115 **2. Materials and Methods**

### 116 *2.1. Cellulose fibers-based substrates*

117 VPPP, VTP and RTP samples were supplied from Dutch paper factories and were considered  
118 the cellulose fiber-based substrates in our experiments, whereas MCC was purchased from  
119 Sigma Aldrich (98% purity, Germany). Prior to conducting the experiments, VPPP, VTP and  
120 RTP were cut into 1-2 mm pieces. These pieces were mixed with demineralized water and  
121 blended for about 15 minutes to form a soft bulky substrate (Fig.1). Table 3 presents the  
122 characteristics of these substances.

123

### 124 *2.2. Fine sieved fraction (FSF)*

125 FSF was collected from the 350  $\mu\text{m}$  mesh fine sieve (Salsnes, Norway) at the sewage  
126 treatment plant (STP) Blaricum, the Netherlands, and was stored at 4°C prior to conduct the  
127 BMP tests. Total solids (TS) and volatile solids (VS) were measured on weight base (g/L)  
128 according to the standard methods for the examination of water and wastewater (APHA,  
129 2005). Chemical oxygen demand (COD) was measured using Merck photometric cell tests  
130 (500-10,000mg/L, Merck, Germany). All analyses were done in triplicate.

### 131 *2.3. Inoculum*

132 As inoculum for the batch tests, well-adapted and highly active sludge was used. Fresh  
133 inoculums were sampled from thermophilic and mesophilic mixed FSF fed-batch digesters  
134 (working volume of 8L), which were operated for over 500 days. The characterization of both  
135 inoculates was done according to the methods described in the previous paragraph. Initial pH  
136 of the thermophilic and mesophilic inoculum sludge were  $7.4 \pm 0.2$  and  $7.0 \pm 0.1$ , respectively.  
137 Characteristics of the used substrates are given in Table 2.



138 *2.4. Biomethane potential (BMP) assays*

139 The anaerobic biodegradation of the FSF was performed using the anaerobic methane  
140 potential test (AMPTS-II), (Lund, Sweden), applying adopted protocols as suggested by  
141 Angelidaki et al. (2006, 2009). The 250 and 650mL batch flasks containing thermophilic and  
142 mesophilic inoculum, respectively, and designated substrates were incubated in a temperature  
143 controlled rotational shaker (New Brunswick™ Biological Shakers Innova® 44/44R, USA) at  
144 150 rpm, instead of using the AMPTS-II individual mixers. The gases CO<sub>2</sub> and H<sub>2</sub>S were  
145 stripped from the biogas by leading the biogas through 100 mL bottles containing a 3M  
146 NaOH solution. Hereafter the remaining gas, containing methane, flows into a gas flow cell  
147 with a calibrated volume. When the gas volume equals the calibrated volume of the flow cell,  
148 the gas was released and recorded as one normalized volume at time t. The test is finished at  
149 the moment gas production stops. Biodegradation experiments were performed in triplicate  
150 for all inoculum to substrate ratios ( $R_{I/S}$ ) and every batch flask contained the same amount of  
151 inoculum. After adding the required amounts of inoculum and substrate, each bottle was filled  
152 with a medium including macro-nutrients, micro-nutrients and buffer solution following the  
153 protocols of Angelidaki et al. (2006, 2009), and liquid volumes were adjusted accordingly.

154 The BMP is the net methane production per gram substrate VS added during the entire  
155 incubation period (subtracting the blank methane production) at standard temperature and  
156 pressure, which has the unit of mL CH<sub>4</sub>/gVS<sub>added</sub>.

157 The BMP tests were conducted at an inoculum to substrate ratio ( $R_{I/S}$ ) of 3 under both  
158 conditions. Table 2 shows the dosed inoculum and substrate concentrations for the BMP tests  
159 at thermophilic and mesophilic conditions, as well as its VS content per sample. Working  
160 volumes of the digestion bottles were 0.2L and 0.4L for the thermophilic and mesophilic

161 digestion series, respectively. The final inoculum concentration in the batch digestion bottles  
162 was 21.9 and 7.7 g VS/L and the substrate concentration (VS basis) was 7.3 and 2.6 g VS/L,  
163 both for the thermophilic and mesophilic conditions, respectively. It is noted that the TS and  
164 VS values of examined substrates were different under both conditions since the experiments  
165 were not performed simultaneously and new substrates were made for each condition. Owing  
166 to the used different volumes of the serum bottles, the amounts of TS and VS were higher  
167 under thermophilic conditions for all substrates except MCC (Table 3), however, the COD/VS  
168 ratio was constant under both conditions. The results of the BMP assays using different  
169 cellulosic fiber-substances and MCC were compared to the BMP of FSF under both  
170 conditions as presented elsewhere (Ghasimi et al., 2016).

#### 171 *2.5. Specific methane potential rate (SMPR)*

172 Specific methane production rate (SMPR) (expressed in mL CH<sub>4</sub>/g VS<sub>inoc</sub>.d) was obtained by  
173 dividing the daily methane volume per gram added VS of inoculum.

#### 174 *2.6. Apparent hydrolysis rate (K<sub>h</sub>)*

175 Calculation of apparent K<sub>h</sub> was performed according to the protocol published by Angelidaki  
176 et al. ( 2009). The apparent K<sub>h</sub> describes the hydrolysis rate and typically follows first-order  
177 kinetics assuming normal growth (no inhibition, no lack of macro-nutrients or micro-  
178 nutrients) (Koch and Drewes, 2014; Pfeffer, 1974; Tong et al., 1990). When no intermediates  
179 accumulate, substrate hydrolysis can be regarded the rate-limiting step. The K<sub>h</sub> can then be  
180 derived from the accumulating methane production curve using a first-order kinetic model as  
181 expressed in Eq.(1):

$$182 \quad P=P_{\max}[1-\exp(-K_h.t)] \quad (1)$$

183 Where,  $P$ =cumulative methane production from the BMP assay at time  $t$  (mL),  $P_{\max}$ = ultimate  
184 methane yield from BMP assay at the end of the incubation time (mL),  $K_h$ = first-order  
185 hydrolysis rate (1/d). The apparent  $K_h$  can be derived from the slope of the linear regression  
186 line plotted for the net accumulated methane production against time for each substrate at  $R_{1/S}$   
187 of 3.

### 188 2.7. Anaerobic biodegradability (AnBD)

189 The relationship between anaerobic biodegradability (AnBD) and BMP is given in Eq.(2)  
190 (Buffiere et al., 2006):

$$191 \text{AnBD} = \frac{\text{BMP}(\text{mLCH}_4 / \text{gVS})}{350 \times \text{COD}_{\text{substrate}}(\text{gCOD} / \text{gVS})} \quad (2)$$

192 Giving the conversion  $1 \text{ CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$ , 1 g COD equals 350 mL of  $\text{CH}_4$  at  
193 standard temperature (273 K) and pressure (100 kPa). It is noted that this theoretical approach  
194 does not take into account the needs for bacterial cell growth and their maintenance, which  
195 has been reported typically 5-10% of organic material degraded (Angelidaki and Sanders,  
196 2004), meaning that not all biodegraded COD is transformed into methane. Moreover, during  
197 bioconversion non-methanised biodegradable or non-biodegradable intermediates may occur,  
198 lowering the actual methane yield of the substrate. In the latter case  $K_h$  must be calculated  
199 taking the accumulating intermediates into account.

200

### 201 3. Results and Discussion

202 Dry weight and ash content of the inoculum and substrates that were used in the experiments  
203 are presented in Table 3. Lowest and highest COD/VS ratios were found for MCC and VPPP,  
204 with values of 1.17 and 1.84, respectively. The high COD/VS ratio of VTTP, was rather  
205 surprising and possibly can be explained by the use of reduced chemicals during the paper  
206 production process. The Danish EPA conducted a survey on the possible chemical substances  
207 used in the paper making process, with handkerchiefs and toilet paper as end products  
208 (Abildgaard et al., 2003). They reported that, in general, up to 800 different chemical  
209 substances are used in the paper manufacturing. However, in the toilet paper and paper  
210 handkerchiefs production the variety of the chemicals used is somewhat narrower. The exact  
211 composition differs per factory and is unknown.

212 TS and VS concentrations of the cellulose-based substrates, except cellulose, differ between  
213 the mesophilic and thermophilic experiment since the thermophilic and mesophilic  
214 experiments were not performed at the same time and thus fresh substrates were made for  
215 each experiment.

#### 216 3.1. Biomethane potential (BMP)

217 The BMP, or ultimate methane yield tests, giving the maximum amount of mL CH<sub>4</sub>/g VS<sub>added</sub>,  
218 were conducted under mesophilic and thermophilic conditions for all substrates. Thermophilic  
219 and mesophilic digestion presented different substrate degradation characteristics.  
220 With respect to the assessed BMP, the values for RTP, MCC and FSF were higher under  
221 thermophilic conditions compared to the mesophilic digesters, whereas VPPP and VTP  
222 obtained higher BMP values under mesophilic conditions. As expected, the highest BMP was

223 found for MCC (369±5 mL CH<sub>4</sub>/g VS) and the lowest for VTP (200±10 mL CH<sub>4</sub>/g VS), both  
224 under thermophilic conditions. The second highest BMP was found for FSF with values  
225 reaching 338±8 and 309±5 mL CH<sub>4</sub>/g VS under thermophilic and mesophilic conditions,  
226 respectively (Ghasimi et al., 2016) . FSF is more heterogeneous than the tested papers and  
227 virgin materials, since other particulate matter originating from the raw sewage, e.g. lipids and  
228 proteins will stay behind on the fine sieve. These compounds might have contributed to the  
229 overall higher BMP values for FSF (Table 4).

230 The reasons for the observed differences in BMP between the 2 temperature conditions are  
231 not (yet) clear and might be related to the added process chemicals (Table 1). During  
232 digestion, paper additives might be released, possibly impacting the methanogenic consortia  
233 differently. Various researchers showed a higher sensitivity of thermophilic methanogenic  
234 consortia compared to mesophilic ones (dos Santos et al., 2005; Kalyuzhnyi et al., 2000).  
235 Strikingly, the BMP values for VPPP and VTP were lower under the applied thermophilic  
236 condition, which is generally regarded more effective for anaerobic digestion of  
237 lignocellulosic biomass (De Baere, 2000). However, possibly more additives are released  
238 under thermophilic conditions, limiting bioconversion. In addition, it should be noted that the  
239 substrate doses on COD basis for VPPP, VTP, RTP, MCC and FSF were 2.5, 2.9, 2.3, 2.8 and  
240 1.1 times higher for the thermophilic digesters compared to the mesophilic digesters,  
241 respectively (Table 2). Thus, the total quantity of possibly released additives and/or  
242 intermediate compounds might have been higher under thermophilic conditions, affecting the  
243 results.

244 Initial lag phases of almost 0.5 day and 1.2-2.0 days were found for all cellulose fiber-based  
245 substrates under thermophilic and mesophilic conditions, respectively, followed by a rapid

246 methane production, which was higher in thermophilic assays compared to the mesophilic  
247 ones. However, no lag phase was observed during digestion of FSF, likely because of: (1) the  
248 long adaptation period of the inoculum to FSF substrate (over 500 days) and (2) the presence  
249 of readily degradable matter in the FSF, like fat and proteins, that may have resulted in a  
250 steady methane generation from the start, masking any possible lag phase related to refractory  
251 fiber degradation. Previous studies achieved varying BMP values under mesophilic conditions  
252 for different types of paper: Paper and cardboard ranged between 109-128 mL CH<sub>4</sub>/g VS  
253 (Pommier et al., 2010), whereas paper bags were reported to have a BMP of 250 mL CH<sub>4</sub>/g  
254 VS (Hansen et al., 2004), office printer paper and newsprint paper gave a BMP of 340 and 58  
255 mLCH<sub>4</sub>/gVS, respectively (Jokela et al., 2005), newspaper (shredded) 92 mLCH<sub>4</sub>/gVS (Tong  
256 et al., 1990) and magazine paper 203 mLCH<sub>4</sub>/gVS (Owens and Chynoweth, 1993). For the  
257 commercial paper or cardboard, the range of lignin content is very wide: between 2% (office  
258 paper) and 24% (newspaper) according to Barlaz et al. (1990).

259 Since lignin is known to be persistent to anaerobic conversion, the variations in lignin content  
260 might partly explain the variations in reported BMP. Possibly, the low methane yield of  
261 lignin-rich substrates are rather related to lignin encrustation than to inhibitors like resin acids  
262 and sulphur-containing substances. A negative effect of possible inhibitors is found less  
263 plausible, since the substrates are highly diluted during the BMP test applying R<sub>1/S</sub> ratios of 3  
264 (VS basis). Given the fact that well-adapted inoculates were used, it is assumed that  
265 hydrolytic enzymes are sufficiently available, agreeing with literature observations  
266 (Hagelqvist, 2013). In general, the BMP values found for the tested virgin hygiene papers in  
267 this study are in the high range, which might be attributed to the relatively low lignin content  
268 and limited accumulation of inhibitory additives.

### 269 3.2 .Specific methane potential rate (SMPR)

270 The methane production rate varied over time, following the batch degradation of the  
271 substrate. The variation in SMPR, expressed in (mL CH<sub>4</sub>/g VS<sub>inoc.</sub>.d), during the digestion of  
272 the cellulose fiber-based substrates under both mesophilic and thermophilic conditions was  
273 further investigated (Fig.3). SMPR showed similar behaviour for all substrates under  
274 thermophilic conditions (Fig.3): very high rates were observed at the start of the BMP assay  
275 compared to the same substrates tested under mesophilic condition (indicated by arrow A) and  
276 they decreased rapidly after reaching their maximum values (indicated by arrow B). Under  
277 mesophilic conditions, the assessed SMPRs varied more over time and were different for the  
278 different substrates. They were always lower than the thermophilic rates and showed lag  
279 phases after an initial peak at the start of the experiment. These first peaks are probably due to  
280 the degradation of easily biodegradable compounds in the substrate, whereafter a lag phase is  
281 observed due to a delay in degradation of the fibrous material. As it was mentioned earlier,  
282 FSF did not show any lag phase, likely due to the long adaptation period of the inoculum to  
283 FSF substrate and presence of easily degradable matters in the FSF, like fat and proteins.

284 The high SMPR under the thermophilic conditions compared to the mesophilic conditions are  
285 likely associated with the more rapid hydrolysis of cellulose fibers and probably more rapid  
286 digestion of readily degradable compounds such as filling materials (e.g., starch) at elevated  
287 temperatures. The observed fluctuations in the methane production rate might indicate  
288 hydrolyses of different types of biopolymers in the degradation of substrates. Maximum and  
289 minimum amount of SMPR for all components under both conditions are presented in  
290 Table 4.

### 291 3.3. Apparent hydrolysis rate ( $K_h$ )

292 Apparent hydrolysis rates ( $K_h$ ) were calculated using the cumulative methane production  
293 curves from the BMP tests. Such mathematical approach is only warranted when no  
294 intermediates accumulate (see also section 2.6), thus, when acetogenesis and methanogenesis  
295 is not rate limiting. Owing to the set-up of the BMP batch assays, daily VFA measurements  
296 were not performed. However, by employing well-adapted inoculums and applying  $R_{I/S}$  ratios  
297 of 3 in the BMP tests, we assumed that intermediates were not accumulating during the BMP  
298 tests. The applied  $R_{I/S}$  of 3 in the BMP tests coincides with most literature values as reviewed  
299 by (Raposo et al., 2012). At this ratio, a high amount of active inoculum generally avoids any  
300 VFA accumulation. Similar to the SMPR results, higher apparent hydrolysis rates were found  
301 under thermophilic conditions compared to mesophilic conditions for all tested substrates  
302 (Table 4). Maximum and minimum apparent  $K_h$  values were found for VTP, i.e.  $1.90 \pm 0.03$   
303 and  $0.19 \pm 0.03$  (1/d), under thermophilic and mesophilic conditions, respectively. The reason  
304 for this order of magnitude difference is not fully clear. Considering the relatively stable  
305 SMPR (Fig. 3), the accumulation of (inhibitory) intermediates is not very likely. Speculatively,  
306 VTP may contain a higher amount of inhibitory paper chemicals. However, in the latter case,  
307 also the thermophilic batch test would have been impacted. Nonetheless, it is of interest to  
308 note that VTP obtained the lowest  $SMPR_{max}$  value compared to other fiber-based cellulose,  
309 four times less than that under the thermophilic condition (Table 4). Unexpected inhibition  
310 phenomena have been previously observed with paper and pulp wastewaters (Van Ginkel et  
311 al., 2007).

312 Although the inoculum was highly adapted to the FSF, resulting in absence of lag phases, the  
313 apparent  $K_h$  under thermophilic conditions was still the lowest for this material compared to  
314 the other substrates ( $0.85 \pm 0.05$  1/d). Under mesophilic conditions the apparent  $K_h$  for FSF



315 was comparable to the other substrates, except for the lower value of VTP.

316 Another factor characterizing the substrate biodegradability (Parameswaran and Rittmann,  
317 2012) is the time required for achieving 90% of the BMP ( $t_{90\%CH_4}$ ); results are shown in  
318 Table 4 as well. Shortest and longest  $t_{90\%CH_4}$  under the thermophilic conditions were  
319 recorded at 2 and 4.3 days for VTP and MCC, whereas under mesophilic conditions FSF and  
320 MCC achieved the shortest  $t_{90\%CH_4}$  of 5 days and VPPP obtained the longest  $t_{90\%CH_4}$  of 7.6  
321 days.

322 In general, the required incubation periods observed in our BMP experiments were  
323 considerably shorter than the ones described in the literature, which may range between 30-50  
324 days (Owen et al., 1979; Hansen et al., 2004; Lesteur et al., 2010). Very likely, the use of well  
325 adapted inoculum is crucial for these substrates (Ghasimi et al., 2015), resulting in an  
326 extremely rapid conversion.

#### 327 *3.4. Anaerobic biodegradability (AnBD) of the different substrates*

328 Figure 4 shows a similar anaerobic biodegradation for the tested substrates under both  
329 temperature conditions. Degradation of easily biodegradable compounds (e.g., lipids and  
330 proteins) might have directly contributed to the higher AnBD (>50%) for FSF under both  
331 conditions compared to VPPP, VTP and RTP that mainly consist of cellulose fibers. However,  
332 MCC, probably due to its physical and chemical structure and manufacturing conditions  
333 (Landin et al., 1993), obtained the highest biodegradation percentage of 91% and 86% under  
334 thermophilic and mesophilic conditions, respectively, also resulting in the highest BMP values  
335 among the tested substrates. The observed differences possibly reflect the influence of  
336 physicochemical properties, used paper chemicals, and applied processing conditions, such as

337 pretreatment and delignification, for the cellululosic fibers and MCC. Pommier et al. (2010)  
338 showed a high heterogeneity in degree of biodegradation of different types of paper and  
339 cardboards (28-58%), which was ascribed to the differences in lignin content. In general, none  
340 of the employed cellulose fiber-based substrates had a higher biodegradation percentage than  
341 the 50% observed in our experiments. The aerobic biodegradation (45 days controlled  
342 aeration) of different paper wastes, including tissue paper (paper handkerchiefs, serviettes  
343 50%, table cloths) were studied by Alvarez et al. (2009). Results of their experiments  
344 indicated 50% biodegradation for the tissue paper compared to the theoretical biodegradable  
345 fraction of the paper volatile solids ( $\approx 63\%$ ), excluding 7% of lignin content. Firstly, the  
346 observed low biodegradability could have been related to the organic additives dosed in the  
347 manufacturing or finishing process. Secondly, the particles of the tissue paper tended to form  
348 “balls” in the test containers due to absorption of humidity and swelling of fibers. This likely  
349 reduced the surface contact with enzymes lowering the final biodegradability determined  
350 (Alvarez et al., 2009).

### 351 *3.5. Overall discussion*

352 Previous and current results showed a limited FSF biodegradability between 57%-62% under  
353 both mesophilic and thermophilic conditions. In order to elucidate the reason for this limited  
354 biodegradability a range of BMP tests were conducted using different types of toilet paper as  
355 well virgin paper fibres. Results showed distinct differences between the tested cellulose  
356 fiber-based substrates and MCC as a fiberless reference material. MCC achieved the highest  
357 BMP value under both temperature conditions amongst all examined substrates. A remarkably  
358 high COD/VS ratio of 1.84 was measured for the VPPP, possibly indicating the presence of  
359 either lignin compounds and/or aromatic paper chemicals which were added during the paper

360 production process. Aromatic or phenolic compounds are characterized by a high COD/mass  
361 ratio, reaching 3.1 and 2.4 g COD/g compound, respectively. The presence of a lag phase  
362 when cellulose fiber-based substrates were used under mesophilic and thermophilic  
363 conditions indicates that hydrolysis is not apparent at the start of the experiments, but requires  
364 an acclimation period. The observed lag phases were somewhat longer under mesophilic  
365 conditions, especially when VPPP was used as the substrate. The absence of lag phases when  
366 FSF was used as the substrate suggests the presence of well adapted inoculums under both  
367 mesophilic and thermophilic conditions. The SMPR was similar for all substrates under  
368 thermophilic conditions showing very high rates compared to the same substrates tested under  
369 mesophilic conditions. Apparent  $K_h$  values describe the velocity of bioconversion of the solid  
370 biomass. Thermophilic digestion of fibrous and non-fibrous substrates showed the highest  $K_h$   
371 values compared to mesophilic digestion. Remarkably, the biodegradability of toilet paper  
372 was found lower than 50% under both conditions. The poor biodegradability might be due to  
373 i) the characteristics of the employed fibers (short or long) during paper making, ii) the degree  
374 of crystallinity of the fibers, iii) the types of pulping applied and the presence of poorly  
375 biodegradable lignin material, iv) the formation of toxic and refractory compounds during the  
376 paper making process, which hampers the anaerobic conversion. Particularly regarding the  
377 latter, more detailed research is needed on the impact of additive chemicals i.e., resins,  
378 binders, wax, anti-foaming agents, cleaning agents, creping chemicals, dyes, etc., in order to  
379 maximize the FSF bioconversion potential.

380

381

#### 382 **4. Conclusions**

383 Based on the results of this study the following conclusions were drawn:

384 • Thermophilic and mesophilic digestion of different cellulose fiber-based substrates  
385 (VTP, VPPP and RTP) showed different conversion characteristics, as characterised by  
386 BMP, SMPR, AnBD, apparent  $K_h$  as well as  $t_{90\%CH_4}$ . However, the variations in BMP  
387 ranged from 5% to 12% and their anaerobic biodegradation percentage was, more or  
388 less, in the same range (38%-50%),

389

390 • The non-fibrous MCC obtained the highest BMP and biodegradation percentage under  
391 both thermophilic and mesophilic conditions compared to all employed substrates.

392

393 • The second most biodegradable substrate was FSF. The applied long adaptation period  
394 of the used inoculates and the assumed presence of more readily biodegradable  
395 compounds (e.g., proteins and lipids) in the FSF might have contributed to the higher  
396 BMP and biodegradation percentage compared to the fiber-based substrates.

397

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402

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503



504 **Figure captions**

505

506 Figure 1. Microscopy images of VPPP (A), VTP (B), RTP (C), MCC (D) and FSF (E) in  
507 dried form using Leica Stereo Explorer 3D Microscope at 200  $\mu\text{m}$  magnitude  
508 (first row: A-E) and after blending and mixing with water (except MCC and  
509 FSF) before conducting the BMP tests (second row: A-E)

510

511 Figure 2. Biomethane potential (BMP) tests of VPPP, VTP, RTP, MCC and FSF under  
512 thermophilic and mesophilic conditions at  $R_{I/S}=3$

513

514 Figure 3. Specific methane production rate (SMPR) for VPPP, VTP, RTP, MCC and FSF  
515 under thermophilic and mesophilic conditions at  $R_{I/S}=3$

516

517 Figure 4. Biodegradation percentage of VPPP, VTP, RTP, MCC and FSF under  
518 thermophilic and mesophilic conditions at  $R_{I/S}$  of 3

519

520 **Table 1. Types of additive compounds used in the papermaking process (Bos et al., 1995)**

<b>Kind/sort</b>	<b>Example</b>	<b>Purpose</b>	<b>Main effect</b>
<b>Defoamers</b>	Alcohol derivatives	Process	Suppress foaming during processing and in the paper itself
<b>Binders</b>	Starch, Carboxymethylcellulose	product	Increase of the strength of paper
<b>Bleaching</b>	Sodium peroxide	product	Increase whiteness of the paper
<b>Dispersants</b>	Alcohol ethoxylate	Process	Prevention of coagulation or precipitation of pigments
<b>Fixers</b>	Various polymers	Process	Adhesion of several additives to the fibers
<b>Dyes</b>	Methyl red, violet	product	Colouring or shading of the paper
<b>Adhesives</b>	Resin Adhesive	product	Reduction of water absorption of paper
<b>Wet strength agents</b>	Urea formaldehyde resin	product	Improving the wet strength of paper
<b>pH-regulators</b>	Caustic soda	Process	Changing the acidity of pulp or paper
<b>Cleaning agents</b>	Solvents, acid, base	Process	Cleaning of machinery, piping, sieves and such during process interruption
<b>Retention means</b>	Polyamidoamide	Process	Reduction of fiber and filler fall-through in the sheet forming process
<b>Slimicides</b>	Methylene bis(thiocyanate)	Process	Inhibition of bacterial growth in pulp and process water
<b>Felt detergents</b>	Ethylene oxide	Process	Cleaning of machine clothing
<b>Flocculants</b>	Poly acrylate	Process	Promoting dewatering of rejects and sludge
<b>Fillers</b>	China clay	product	Opacities to improve printability of paper
<b>Water treatment</b>	Polyphosphate	Process	Preventing deposition of dissolved salts

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522

523 **Table 2. Experimental set-up of the thermophilic (T) and mesophilic (M) BMP assays**

Components	Substrate-wet basis (g/bottle=0.2L) (T, 55°C)	gCOD/L (T, 55°C)	Substrate-wet basis(g/bottle=0.4L) (M, 35°C)	gCOD/L (M, 35°C)
VPPP	10.6	12.0	12.2	4.8
VTP	8.9	11.0	9.9	3.8
RTP	9.9	11.8	12.6	5.1
MCC	1.5	8.5	1.1	3.0
FSF	9.1 (V <sub>w</sub> =0.2L)	15.6	8.4 (V <sub>w</sub> =0.4L)	14.3

524

525

526

527 **Table 3. Characteristics of thermophilic (T) and mesophilic (M) inoculum and different**  
 528 **cellulose-based substrates (VPPP, VTP, RTP, MCC and FSF)**

Component	Appearance	COD/VS	TS[g/L]		VS[g/L]		VS/TS[%]
			T	M	T	M	
Inoculum (T)	Brown-darkish	1.54	30.0±0.0	-	24.0±0.0	-	79.6
Inoculum (M)	Brown-darkish	1.58	-	13.0±0.1	-	8.2±0.0	63.1
VPPP	Multi-layer compacted sheet, white	1.84	125.9±1.8	86.5±0.5	124.6±1.7	85.7±1.5	99.0
VTP	Very soft and white, 2-ply	1.50	168.8±3.5	115.0±0.9	166.8±2.0	113.9±1.8	99.0
RTP	Soft with some black spots, white-grey	1.43	168.7±0.9	115.0±1.0	166.0±1.8	112.7±2.0	98.0
MCC	Powder, white	1.17	960.0±1.2	960.0±1.2	960.0±1.2	960.0±1.2	100.0
FSF	Bulky, brownish	1.56	233.0±10.0	233.0±10.0	220.0±1.5	220.0±1.5	94.0

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531

532 **Table 4. Biomethane potential (BMP), maximum specific methane production rate**  
 533 **(SMPR<sub>max</sub>), apparent hydrolysis rate (K<sub>h</sub>) and time to achieve 90% of maximum BMP**  
 534 **(t<sub>90%CH<sub>4</sub></sub>) at R<sub>I/S</sub> of 3 under mesophilic and thermophilic conditions**

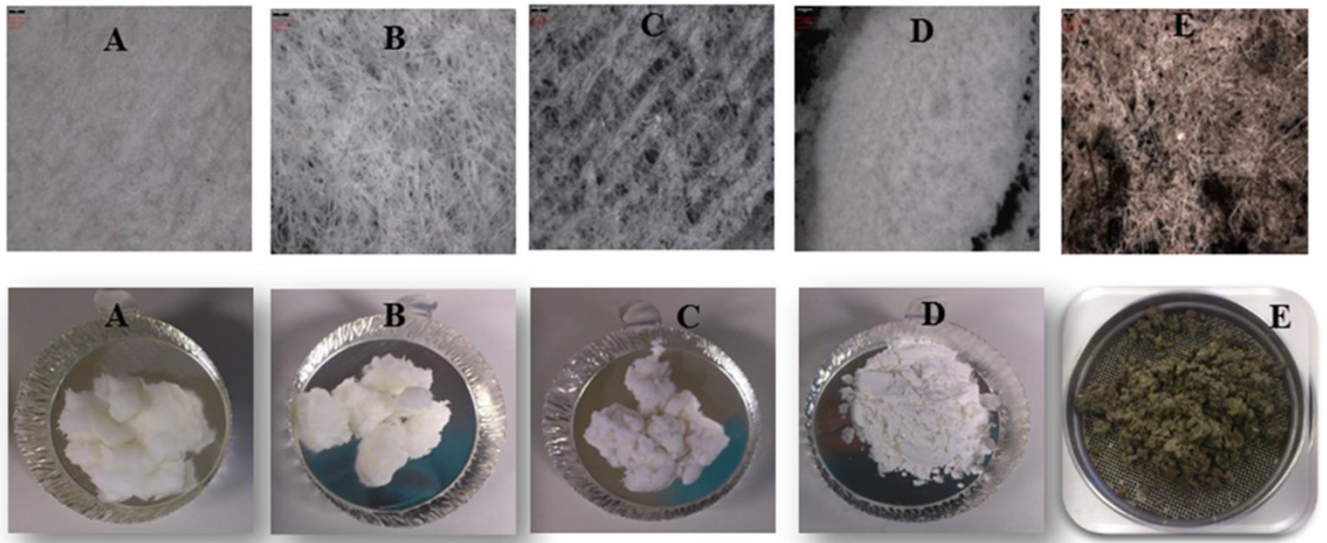
Components	BMP (mL CH <sub>4</sub> /gVS)		SMPR <sub>max</sub> (mL CH <sub>4</sub> /(gVS <sub>in</sub> ·d))		K <sub>h</sub> (1/d)		t <sub>90%CH<sub>4</sub></sub> (day)	
	35°C	55°C	35°C	55°C	35°C	55°C	35°C	55°C
VPPP	274±2	244±4	46.7±3.9	74.5±1.5	0.77±0.01	1.54±0.04	7.6	2.5
VTP	230±15	200±10	17.9±5.0	73.7±9.0	0.19±0.03	1.90±0.03	7.0	2.0
RTP	254±10	285±15	30.8±1.5	99.5±2.0	0.41±0.02	1.34±0.04	6.0	2.6
FSF	309±5	338±8	39.0±2.0	73.0±4.0	0.60±0.05	0.85±0.05	5.0	3.3
MCC	351±5	369±5	45.3±1.0	135.0±1.0	0.77±0.02	1.54±0.02	5.0	4.3

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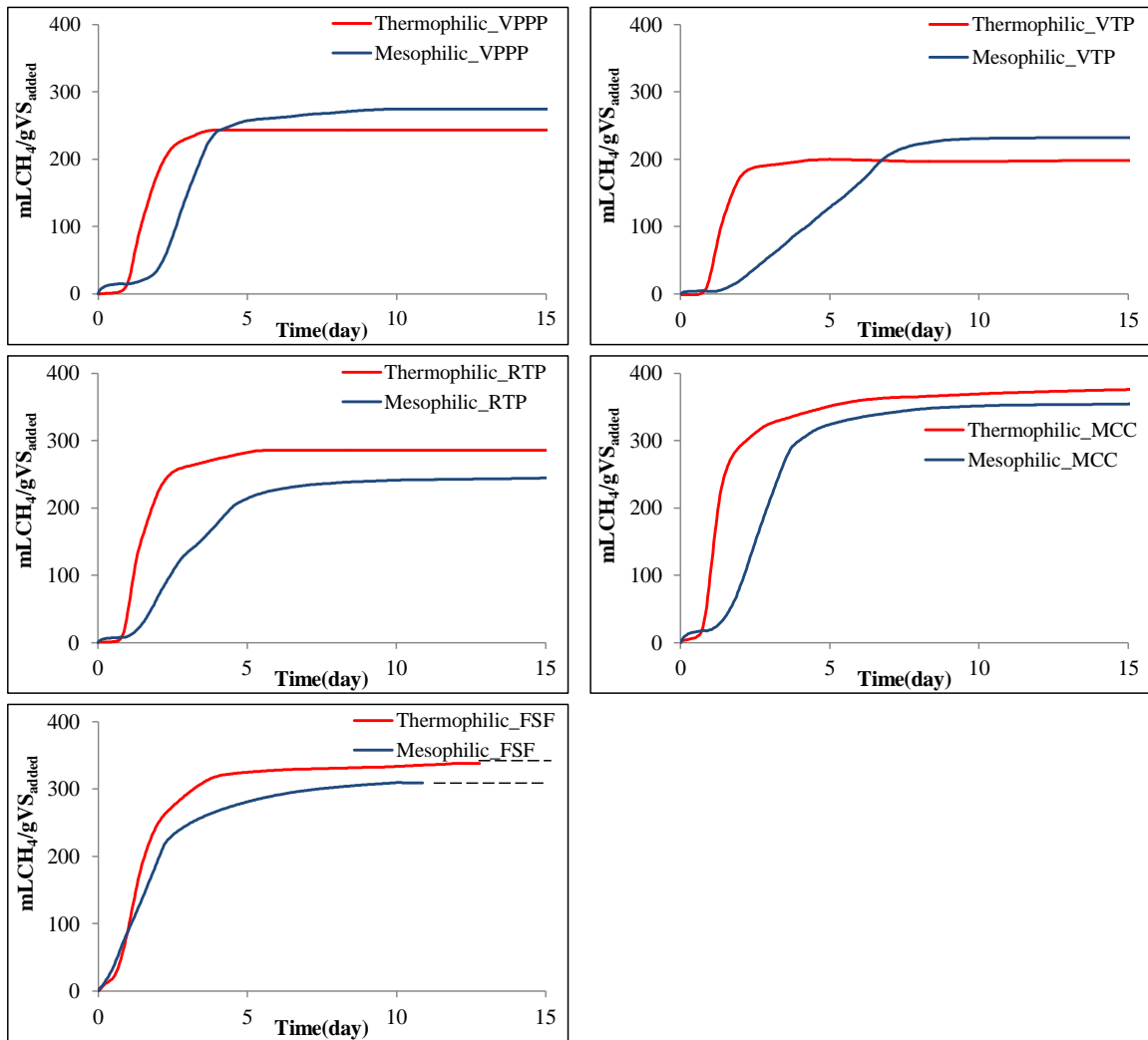
539

540 **Figure 1. Microscopy images of VPPP (A), VTP (B), RTP (C), MCC (D) and FSF (E) in**  
541 **dried form using Leica Stereo Explorer 3D Microscope at 200  $\mu\text{m}$  magnitude**  
542 **(first row: A-E) and after blending and mixing with water (except MCC and FSF) before**  
543 **conducting the BMP tests (second row: A-E)**

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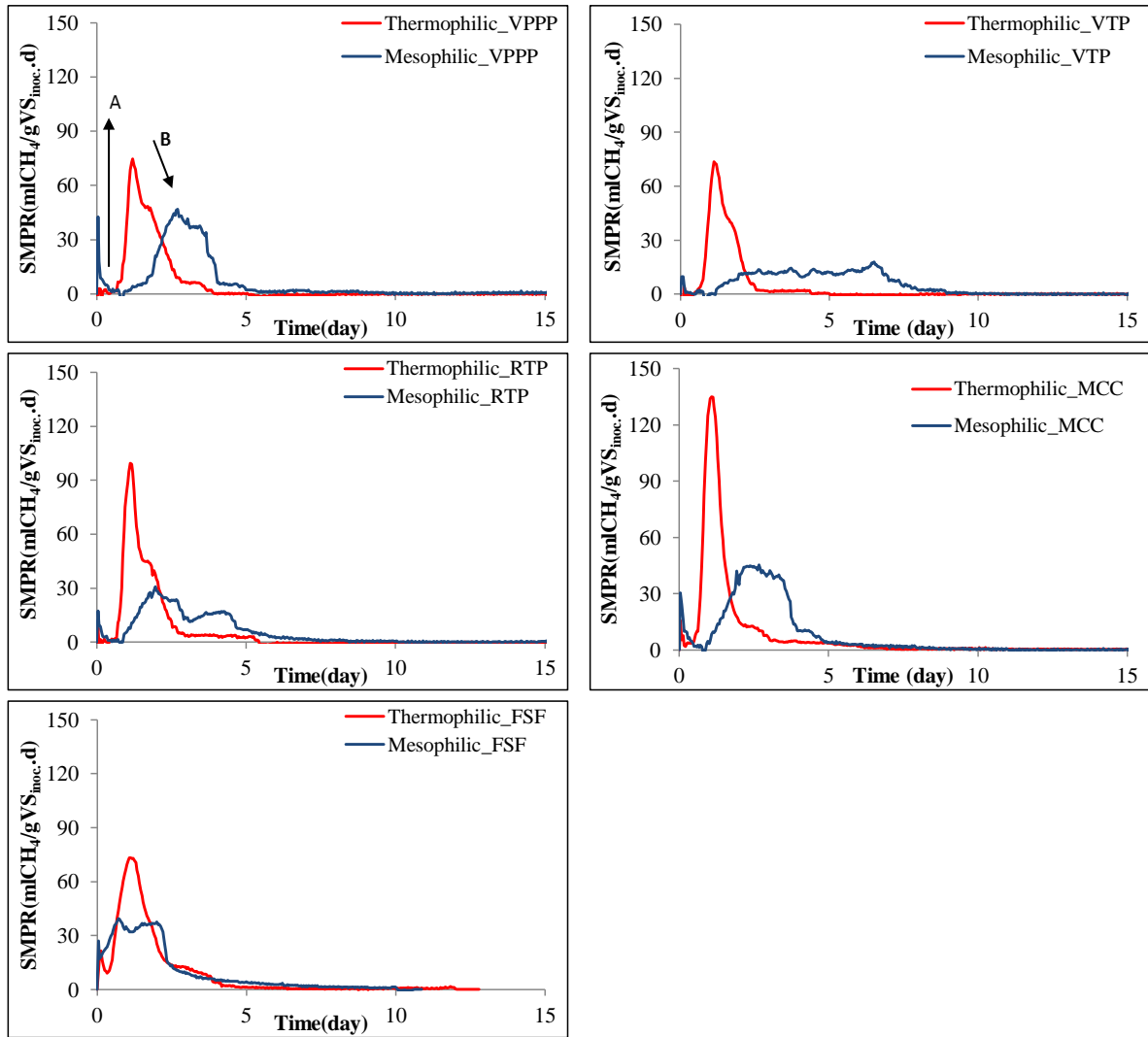
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548 **Figure 2. Biomethane potential (BMP) tests of VPPP, VTP, RTP, MCC and FSF under**

549 **thermophilic and mesophilic conditions at  $R_{1/s}=3$**

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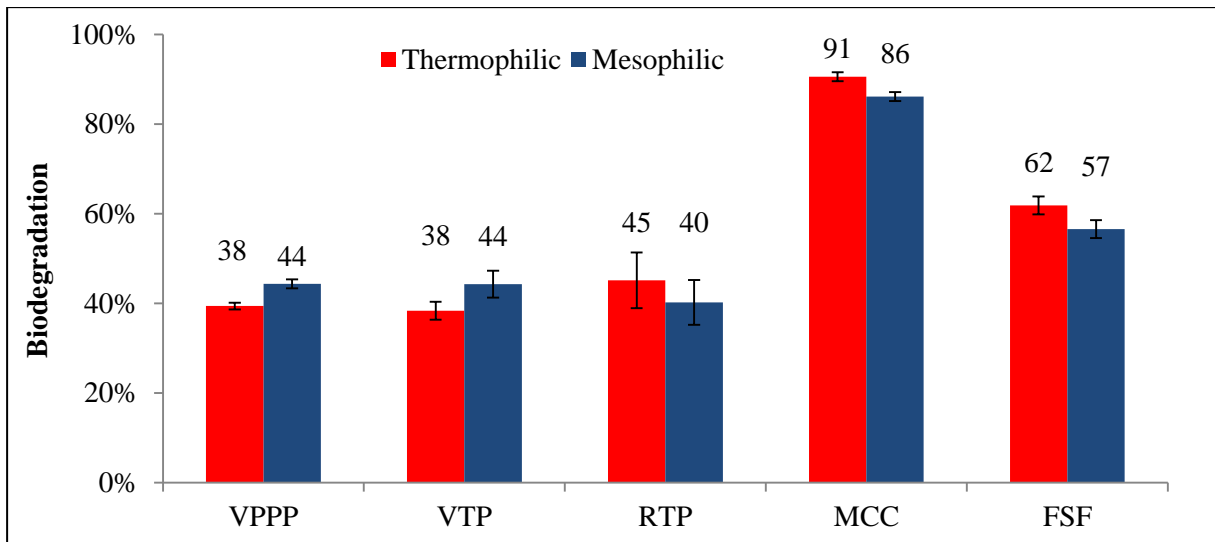
552

553 **Figure 3. Specific methane production rate (SMPR) for VPPP, VTP, RTP, MCC and FSF**  
 554 **under thermophilic and mesophilic conditions at  $R_{I/S}=3$**

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558 **Figure 4. Biodegradation percentage of VPPP, VTP, RTP, MCC and FSF under**  
 559 **thermophilic and mesophilic conditions at  $R_{1/S}$  of 3**

560