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

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**Abstract**

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

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

## 1. Introduction

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

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

Toilet paper is either made from virgin pulp, which is mainly extracted from wood and partly from non-wood cellulose (e.g., bamboo) and is called virgin fibers based toilet paper (VTP), or it is made from recycled paper fibers, which is known as recycled fibers based toilet paper (RTP). The type of pulp and paper chemicals used has an influence on the final quality of the tissue paper, e.g. softness, strength, absorbency and appearance. In the process of making virgin pulp as a raw material for paper production (VPPP), one type of wood is generally usually used, i.e. either soft or hard wood. However, in the production of VTP a combination of soft (long fiber for strength) and hard wood (short fiber for softness) is employed. Depending on the required specifications, paper makers choose their fiber source (long fibers, short fibers and combinations). RTP, which completely or partially consists of recycled fibers, may originate from different sources, such as mixed office waste, or old newsprints. Paper production using recycled fibers in the paper mill follows various process steps such as pulping, screening and de-inking stages (Kamali and Khodaparast, 2014). The majority of paper tissue used in the Netherlands is recycled fibers based. The ratio virgin fibers relative to recycled fibers determines the level of softness of the end product. However, application of specific chemicals and process steps can improve the strength, softness, brightness, etc., of any tissue product, regardless the fibers used (WRAP, 2005). During pulp making, pulp processing and paper-making, certain types of chemicals are used as presented in Table 1. However, every papermaking factory deviates according to their applied raw materials, desired products and process optimization. Generally speaking, these additives can be divided in two categories: (1) additives used during the process (2) additives for product improvement (Table 1). Theoretically, both could end up within the product, which however, is more likely for the ‘product additives’ (Bos et al., 1995). Therefore, there is no standard composition of toilet paper and very likely, also the biodegradability will vary with its composition.

Cellulose is the main constituent of toilet paper and its biodegradability likely depends on its fibrous content and its crystallinity. Maximum biodegradability is expected when no fibers are present, i.e. when the cellulose consists of powdered cellulose (PC) or microcrystalline cellulose (MCC). The chemical composition and physical structure of MCC fully depend on the characteristics of the virgin material from which the cellulose is obtained as well as on the manufacturing conditions (Landin et al., 1993). As a result, several grades of MCC are available on the market with different physicochemical and thermal properties, exhibiting different functional parameters and applications (Azubuike and Okhamafe, 2012). MCCs are prepared by acid hydrolysis under mild conditions of native cellulose to a critical degree of polymerization (DP) (Shcherbakova et al., 2012).

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

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

($t_{90\%}CH_4$) as well as anaerobic biodegradability (AnBD) of designated cellulose fiber-based substrates including VPPP, VTP, RTP and MCC as a fiberless reference material. The results were compared with FSF digestion results from previous studies.

## 2.Materials and Methods

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

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

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

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

### *2.3. Inoculum*

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

*2.4. Biomethane potential (BMP) assays*

The anaerobic biodegradation of the FSF was performed using the anaerobic methane potential test (AMPTS-II), (Lund, Sweden), applying adopted protocols as suggested by Angelidaki et al. (2006, 2009). The 250 and 650mL batch flasks containing thermophilic and mesophilic inoculum, respectively, and designated substrates were incubated in a temperature controlled rotational shaker (New Brunswick™ Biological Shakers Innova® 44/44R, USA) at 150 rpm, instead of using the AMPTS-II individual mixers. The gases $CO_2$ and $H_2S$ were stripped from the biogas by leading the biogas through 100 mL bottles containing a 3M NaOH solution. Hereafter the remaining gas, containing methane, flows into a gas flow cell with a calibrated volume. When the gas volume equals the calibrated volume of the flow cell, the gas was released and recorded as one normalized volume at time t. The test is finished at the moment gas production stops. Biodegradation experiments were performed in triplicate for all inoculum to substrate ratios ($R_{I/S}$) and every batch flask contained the same amount of inoculum. After adding the required amounts of inoculum and substrate, each bottle was filled with a medium including macro-nutrients, micro-nutrients and buffer solution following the protocols of Angelidaki et al. (2006, 2009), and liquid volumes were adjusted accordingly.

The BMP is the net methane production per gram substrate VS added during the entire incubation period (subtracting the blank methane production) at standard temperature and pressure, which has the unit of mL $CH_4/gVS_{added}$.

The BMP tests were conducted at an inoculum to substrate ratio ($R_{I/S}$) of 3 under both conditions. Table 2 shows the dosed inoculum and substrate concentrations for the BMP tests at thermophilic and mesophilic conditions, as well as its VS content per sample. Working volumes of the digestion bottles were 0.2L and 0.4L for the thermophilic and mesophilic digestion series, respectively. The final inoculum concentration in the batch digestion bottles was 21.9 and 7.7 g VS/L and the substrate concentration (VS basis) was 7.3 and 2.6 g VS/L, both for the thermophilic and mesophilic conditions, respectively. It is noted that the TS and VS values of examined substrates were different under both conditions since the experiments were not performed simultaneously and new substrates were made for each condition. Owing to the used different volumes of the serum bottles, the amounts of TS and VS were higher under thermophilic conditions for all substrates except MCC (Table 3), however, the COD/VS ratio was constant under both conditions. The results of the BMP assays using different cellulosic fiber-substances and MCC were compared to the BMP of FSF under both conditions as presented elsewhere (Ghasimi et al., 2016).

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

Specific methane production rate (SMPR) (expressed in mL $CH_4$/g $VS_{inoc}$.d) was obtained by dividing the daily methane volume per gram added VS of inoculum.

### *2.6. Apparent hydrolysis rate (Kh)*

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

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

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

*2.7. Anaerobic biodegradability (AnBD)*

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

$$\mathrm{AnBD} = \frac{BMP(mLCH_4 / gVS)}{350 \times COD_{substrate}(gCOD / gVS)} \tag{2}$$

Giving the conversion 1 $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$, 1 g COD equals 350 mL of $CH_4$ at standard temperature (273 K) and pressure (100 kPa). It is noted that this theoretical approach does not take into account the needs for bacterial cell growth and their maintenance, which has been reported typically 5-10% of organic material degraded (Angelidaki and Sanders, 2004), meaning that not all biodegraded COD is transformed into methane. Moreover, during bioconversion non-methanised biodegradable or non-biodegradable intermediates may occur, lowering the actual methane yield of the substrate. In the latter case $K_h$ must be calculated taking the accumulating intermediates into account.

## 3. Results and Discussion

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

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

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

The BMP, or ultimate methane yield tests, giving the maximum amount of mL $CH_4$/g $VS_{added}$, were conducted under mesophilic and thermophilic conditions for all substrates. Thermophilic and mesophilic digestion presented different substrate degradation characteristics. With respect to the assessed BMP, the values for RTP, MCC and FSF were higher under thermophilic conditions compared to the mesophilic digesters, whereas VPPP and VTP obtained higher BMP values under mesophilic conditions. As expected, the highest BMP was found for MCC (369±5 mL $CH_4$/g VS) and the lowest for VTP (200±10 mL $CH_4$/g VS), both under thermophilic conditions. The second highest BMP was found for FSF with values reaching 338±8 and 309±5 mL $CH_4$/g VS under thermophilic and mesophilic conditions, respectively (Ghasimi et al., 2016) . FSF is more heterogeneous than the tested papers and virgin materials, since other particulate matter originating from the raw sewage, e.g. lipids and proteins will stay behind on the fine sieve. These compounds might have contributed to the overall higher BMP values for FSF (Table 4).

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

Initial lag phases of almost 0.5 day and 1.2-2.0 days were found for all cellulose fiber-based substrates under thermophilic and mesophilic conditions, respectively, followed by a rapid methane production, which was higher in thermophilic assays compared to the mesophilic ones. However, no lag phase was observed during digestion of FSF, likely because of: (1) the long adaptation period of the inoculum to FSF substrate (over 500 days) and (2) the presence of readily degradable matter in the FSF, like fat and proteins, that may have resulted in a steady methane generation from the start, masking any possible lag phase related to refractory fiber degradation. Previous studies achieved varying BMP values under mesophilic conditions for different types of paper: Paper and cardboard ranged between 109-128 mL $CH_4$/g VS (Pommier et al., 2010), whereas paper bags were reported to have a BMP of 250 mL $CH_4$/g VS (Hansen et al., 2004), office printer paper and newsprint paper gave a BMP of 340 and 58 mL$CH_4$/gVS, respectively (Jokela et al., 2005), newspaper (shredded) 92 mL$CH_4$/gVS (Tong et al., 1990) and magazine paper 203 mL$CH_4$/gVS (Owens and Chynoweth, 1993). For the commercial paper or cardboard, the range of lignin content is very wide: between 2% (office paper) and 24% (newspaper) according to Barlaz et al. (1990).

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

*3.2 .Specific methane potential rate (SMPR)*

The methane production rate varied over time, following the batch degradation of the substrate. The variation in SMPR, expressed in (mL $CH_4$/g $VS_{inoc.}$.d), during the digestion of the cellulose fiber-based substrates under both mesophilic and thermophilic conditions was further investigated (Fig.3). SMPR showed similar behaviour for all substrates under thermophilic conditions (Fig.3): very high rates were observed at the start of the BMP assay compared to the same substrates tested under mesophilic condition (indicated by arrow A) and they decreased rapidly after reaching their maximum values (indicated by arrow B). Under mesophilic conditions, the assessed SMPRs varied more over time and were different for the different substrates. They were always lower than the thermophilic rates and showed lag phases after an initial peak at the start of the experiment. These first peaks are probably due to the degradation of easily biodegradable compounds in the substrate, whereafter a lag phase is observed due to a delay in degradation of the fibrous material. As it was mentioned earlier, FSF did not show any lag phase, likely due to the long adaptation period of the inoculum to FSF substrate and presence of easily degradable matters in the FSF, like fat and proteins.

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

*3.3. Apparent hydrolysis rate ($K_h$)*

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

Although the inoculum was highly adapted to the FSF, resulting in absence of lag phases, the apparent $K_h$ under thermophilic conditions was still the lowest for this material compared to the other substrates (0.85±0.05 1/d). Under mesophilic conditions the apparent $K_h$ for FSF was comparable to the other substrates, except for the lower value of VTP.

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

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

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

Figure 4 shows a similar anaerobic biodegradation for the tested substrates under both temperature conditions. Degradation of easily biodegradable compounds (e.g., lipids and proteins) might have directly contributed to the higher AnBD (>50%) for FSF under both conditions compared to VPPP, VTP and RTP that mainly consist of cellulose fibers. However, MCC, probably due to its physical and chemical structure and manufacturing conditions (Landin et al., 1993), obtained the highest biodegradation percentage of 91% and 86% under thermophilic and mesophilic conditions, respectively, also resulting in the highest BMP values among the tested substrates. The observed differences possibly reflect the influence of physicochemical properties, used paper chemicals, and applied processing conditions, such as pretreatment and delignification, for the cellulolosic fibers and MCC. Pommier et al. (2010) showed a high heterogeneity in degree of biodegradation of different types of paper and cardboards (28-58%), which was ascribed to the differences in lignin content. In general, none of the employed cellulose fiber-based substrates had a higher biodegradation percentage than the 50% observed in our experiments. The aerobic biodegradation (45 days controlled aeration) of different paper wastes, including tissue paper (paper handkerchiefs, serviettes 50%, table cloths) were studied by Alvarez et al. (2009). Results of their experiments indicated 50%  biodegradation for the tissue paper compared to the theoretical biodegradable fraction of the paper volatile solids (≈ 63 %.), excluding 7 % of lignin content. Firstly, the observed low biodegradability could have been related to the organic additives dosed in the manufacturing or finishing process. Secondly, the particles of the tissue paper tended to form ‘‘balls” in the test containers due to absorption of humidity and swelling of fibers. This likely reduced the surface contact with enzymes lowering the final biodegradability determined (Alvarez et al., 2009).

### *3.5. Overall discussion*

Previous and current results showed a limited FSF biodegradability between 57%-62% under both mesophilic and thermophilic conditions. In order to elucidate the reason for this limited biodegradability a range of BMP tests were conducted using different types of toilet paper as well virgin paper fibres. Results showed distinct differences between the tested cellulose fiber-based substrates and MCC as a fiberless reference material. MCC achieved the highest BMP value under both temperature conditions amongst all examined substrates. A remarkably high COD/VS ratio of 1.84 was measured for the VPPP, possibly indicating the presence of either lignin compounds and/or aromatic paper chemicals which were added during the paper production process. Aromatic or phenolic compounds are characterized by a high COD/mass ratio, reaching 3.1 and 2.4 g COD/g compound, respectively. The presence of a lag phase when cellulose fiber-based substrates were used under mesophilic and thermophilic conditions indicates that hydrolysis is not apparent at the start of the experiments, but requires an acclimation period. The observed lag phases were somewhat longer under mesophilic conditions, especially when VPPP was used as the substrate. The absence of lag phases when FSF was used as the substrate suggests the presence of well adapted inoculums under both mesophilic and thermophilic conditions. The SMPR was similar for all substrates under thermophilic conditions showing very high rates compared to the same substrates tested under mesophilic conditions. Apparent $K_h$ values describe the velocity of bioconversion of the solid biomass. Thermophilic digestion of fibrous and non-fibrous substrates showed the highest $K_h$ values compared to mesophilic digestion. Remarkably, the biodegradability of toilet paper was found lower than 50% under both conditions. The poor biodegradability might be due to i) the characteristics of the employed fibers (short or long) during paper making, ii) the degree of crystallinity of the fibers, iii) the types of pulping applied and the presence of poorly biodegradable lignin material, iv) the formation of toxic and refractory compounds during the paper making process, which hampers the anaerobic conversion. Particularly regarding the latter, more detailed research is needed on the impact of additive chemicals i.e., resins, binders, wax, anti-foaming agents, cleaning agents, creping chemicals, dyes, etc., in order to maximize the FSF bioconversion potential.

## 4. Conclusions

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

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

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

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

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## References

Abildgaard, A., Mikkelsen, S.H., Stuer-Lauridsen, F., 2003. Survey of chemical substances in paper handkerchiefs and toilet paper (Danish EPA report).

http://eng.mst.dk/media/mst/69117/34.pdf

Alvarez, J.V.L., Larrucea, M.A., Bermúdez, P.A., Chicote, B.L., 2009. Biodegradation of paper waste under controlled composting conditions. Waste Manag. 29, 1514–9. doi:10.1016/j.wasman.2008.11.025

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–934. doi:10.2166/wst.2009.040

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, L., Guwy, A., Jenícek, P., Kalyuzhnui, S., van Lier, J., 2006. Anaerobic Biodegradation , Activity and Inhibition (ABAI) Task Group Meeting 9 to 10 October 2006, in Prague.

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol. 3, 117–129.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, American Water Works Association, Washington D.C., USA.

Azubuike, C.P., Okhamafe, A.O., 2012. Physicochemical, spectroscopic and thermal properties of microcrystalline cellulose derived from corn cobs. Int. J. Recycl. Org. Waste Agric. 1, 9.

Barlaz, M., Ham, R.K., Shaefer, D.M., 1990. Methane production from municipal refuse: a review of enhancement techniques and microbial dynamics. CRC Crit. Rev. Environ. Control 19, 557–584.

Bos, J.H., Veenstra, P., Verhoeven, H., Ripke, J.C., Sikma, B., 1995. Het papierboek (In English: The paper book). Apeldoorn : VAPA, p. 520.

Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.-P., 2006. Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Sci. Technol. 53, 233–241. doi:10.2166/wst.2006.254

De Baere, L., 2000. Anaerobic digestion of solid waste: State-of-the-art. Water Sci. Technol. 41, 283–290.

dos Santos, A.B., Bisschops, I. a E., Cervantes, F.J., van Lier, J.B., 2005. The transformation and toxicity of anthraquinone dyes during thermophilic (55 degrees C) and mesophilic (30 degrees C) anaerobic treatments. J. Biotechnol. 115, 345–53.

Ghasimi, D.S.M., de Kreuk, M., Maeng, S.K., Zandvoort, M.H., van Lier, J.B., 2016. High-rate thermophilic bio-methanation of the fine sieved fraction from Dutch municipal raw sewage: Cost-effective potentials for on-site energy recovery. Appl. Energy 165, 569–582. doi:10.1016/j.apenergy.2015.12.065

Ghasimi, D.S.M., Tao, Y., de Kreuk, M., Abbas, B., Zandvoort, M.H., van Lier, J.B., 2015. Digester performance and microbial community changes in thermophilic and mesophilic sequencing batch reactors fed with the fine sieved fraction of municipal sewage. Water Res. 87, 483–493. doi:10.1016/j.watres.2015.04.027

Hagelqvist, A., 2013. Batchwise mesophilic anaerobic co-digestion of secondary sludge from pulp and paper industry and municipal sewage sludge. Waste Manag. 33, 820–824. doi:10.1016/j.wasman.2012.11.002

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J.L.C., Mosbæk, H., Christensen, T.H., 2004. Method for determination of methane potentials of solid organic waste. Waste Manag. 24, 393–400.

Holik, H. (Ed.), 2006. Handbook of Paper and Board. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (Federal Republic of Germany).

Jokela, J.P.Y., Vavilin, V. a, Rintala, J. a, 2005. Hydrolysis rates, methane production and nitrogen solubilisation of grey waste components during anaerobic degradation. Bioresour. Technol. 96, 501–508. doi:10.1016/j.biortech.2004.03.009

Kalyuzhnyi, S., Sklyar, V., Mosolova, T., Kucherenko, I., Russkova, J.A., Degtyaryova, N., 2000. Methanogenic biodegradation of aromatic amines. Water Sci. Technol. 42, 363–370.

Kamali, M., Khodaparast, Z., 2014. Review on recent developments on pulp and paper mill wastewater treatment. Ecotoxicol. Environ. Saf. doi:10.1016/j.ecoenv.2014.05.005

Koch, K., Drewes, J.E., 2014. Alternative approach to estimate the hydrolysis rate constant of particulate material from batch data. Appl. Energy 120, 11–15. doi:10.1016/j.apenergy.2014.01.050

Landin, M., Martinez-Pacheco, R., Gomez-Amoza, J.L., Souto, C., Concheiro, A., Rowe, R.C., 1993. Effect of country of origin on the properties of microcrystalline cellulose. Int. J. Pharm. 91, 123–131.

Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J.M., Junqua, G., Steyer, J.P., 2010. Alternative methods for determining anaerobic biodegradability: A review.

Process Biochem. 45, 431–440. doi:10.1016/j.procbio.2009.11.018

Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y., McCarty, P.L., 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. 13, 485–492. doi:10.1016/0043-1354(79)90043-5

Owens, J.M., Chynoweth, D.P., 1993. Biochemical methane potential of municipal solid waste (MSW) components, in: Water Science and Technology. pp. 1–14.

Parameswaran, P., Rittmann, B.E., 2012. Feasibility of anaerobic co-digestion of pig waste and paper sludge. Bioresour. Technol. 124, 163–168. doi:10.1016/j.biortech.2012.07.116

Pfeffer, J.T., 1974. Temperature effects on anaerobic fermentation of domestic refuse. Biotechnol. Bioeng. 16, 771–787.

Pommier, S., Llamas, A.M., Lefebvre, X., 2010. Analysis of the outcome of shredding pretreatment on the anaerobic biodegradability of paper and cardboard materials. Bioresour. Technol. 101, 463–468. doi:10.1016/j.biortech.2009.07.034

Raposo, F., De La Rubia, M.A., Fernández-Cegrí, V., Borja, R., 2012. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. Renew. Sustain. Energy Rev. 16, 861–877. doi:10.1016/j.rser.2011.09.008

Ruiken, C.J., Breuer, G., Klaversma, E., Santiago, T., van Loosdrecht, M.C.M., 2013. Sieving wastewater - Cellulose recovery, economic and energy evaluation. Water Res. 47, 43–48. doi:DOI 10.1016/j.watres.2012.08.023

Shcherbakova, T.P., Kotelnikova, N.E., Bykhovtseva, Y. V., 2012. Comparative study of powdered and microcrystalline cellulose samples of a various natural origins: Physical and chemical characteristics. Russ. J. Bioorganic Chem. 38, 689–696. doi:10.1134/S1068162012070187

STOWA, 2010. NEWs: The Dutch Roadmap for the WWTP of 2030. Utrecht, The Netherlands.

Tong, X., Smith, L.L.H., McCarty, P.P.L., 1990. Methane fermentation of selected lignocellulosic materials. Biomass 21, 239–255. doi:10.1016/0144-4565(90)90075-U

Van Ginkel, S.W., Kortekaas, S.J.M., Van Lier, J.B., 2007. The chronic toxicity of alcohol alkoxylate surfactants on anaerobic granular sludge in the pulp and paper industry. Environ. Sci. Technol. 41, 4711–4714.

WRAP, 2005. Specifying recycled content in tissue paper for your organisation. http://www.wrap.org.uk/sites/files/wrap/Tissue%20paper.pdf.

## Figure captions

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

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

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

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

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

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

**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 ($V_w$=0.2L) | 15.6 | 8.4 ($V_w$=0.4L) | 14.3 |

**Table 3. Characteristics of thermophilic (T) and mesophilic (M) inoculum and different 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 |

**Table 4. Biomethane potential (BMP), maximum specific methane production rate ($SMPR_{max}$), apparent hydrolysis rate ($K_h$) and time to achieve 90% of maximum BMP ($t_{90\%}CH_4$) at $R_{I/S}$ of 3 under mesophilic and thermophilic conditions**

| Components | BMP (mL $CH_4$/gVS) | | $SMPR_{max}$ (mL $CH_4$/($gVS_{in}$·d)) | | $K_h$ (1/d) | | $t_{90\%}CH_4$ (day) | |
|---|---|---|---|---|---|---|---|---|
| | 35ºC | 55ºC | 35ºC | 55ºC | 35ºC | 55ºC | 35ºC | 55ºC |
| 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 |

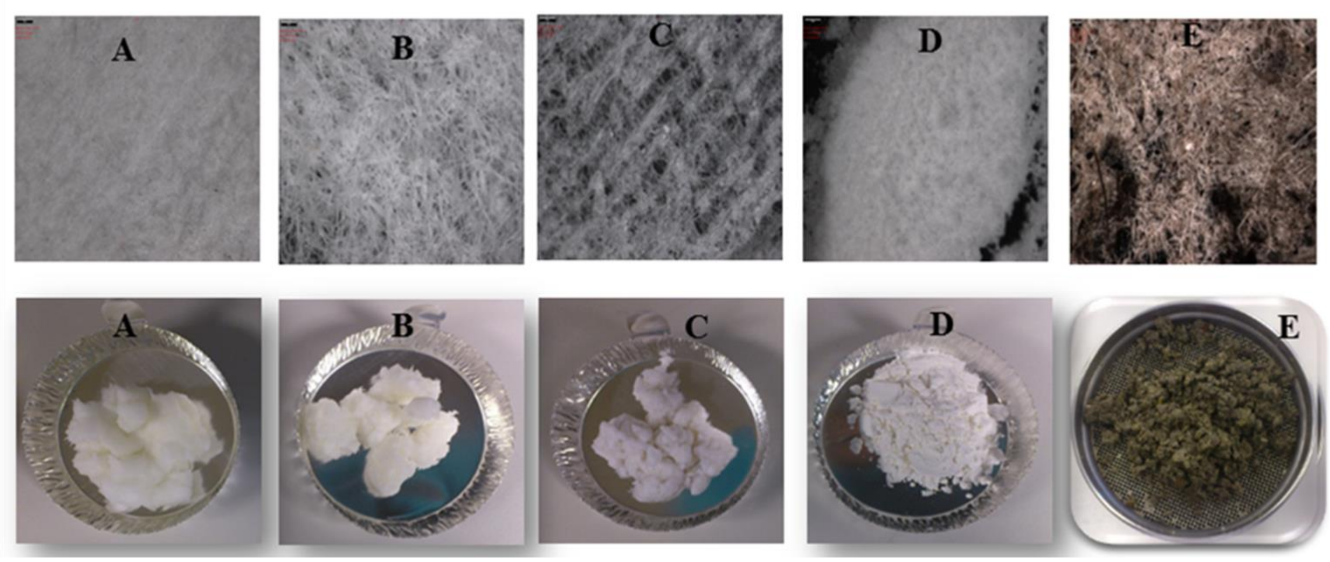


**Figure 1. Microscopy images of VPPP (A), VTP (B), RTP (C), MCC (D) and FSF (E) in dried form using Leica Stereo Explorer 3D Microscope at 200 µm magnitude (first row: A-E) and after blending and mixing with water (except MCC and FSF) before conducting the BMP tests (second row: A-E)**

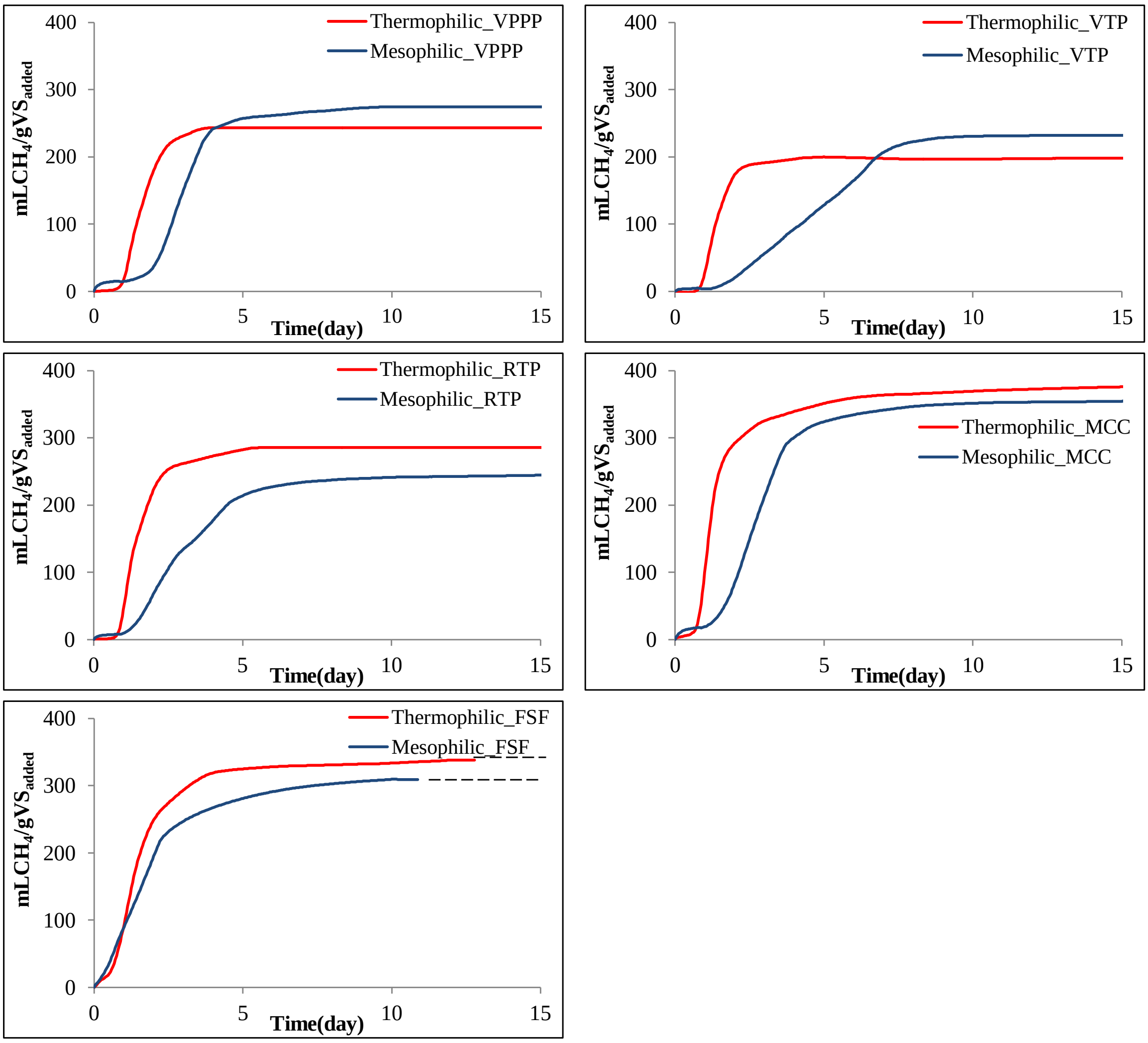


**Figure 2. Biomethane potential (BMP) tests of VPPP, VTP, RTP, MCC and FSF under thermophilic and mesophilic conditions at $R_{I/S}$=3**

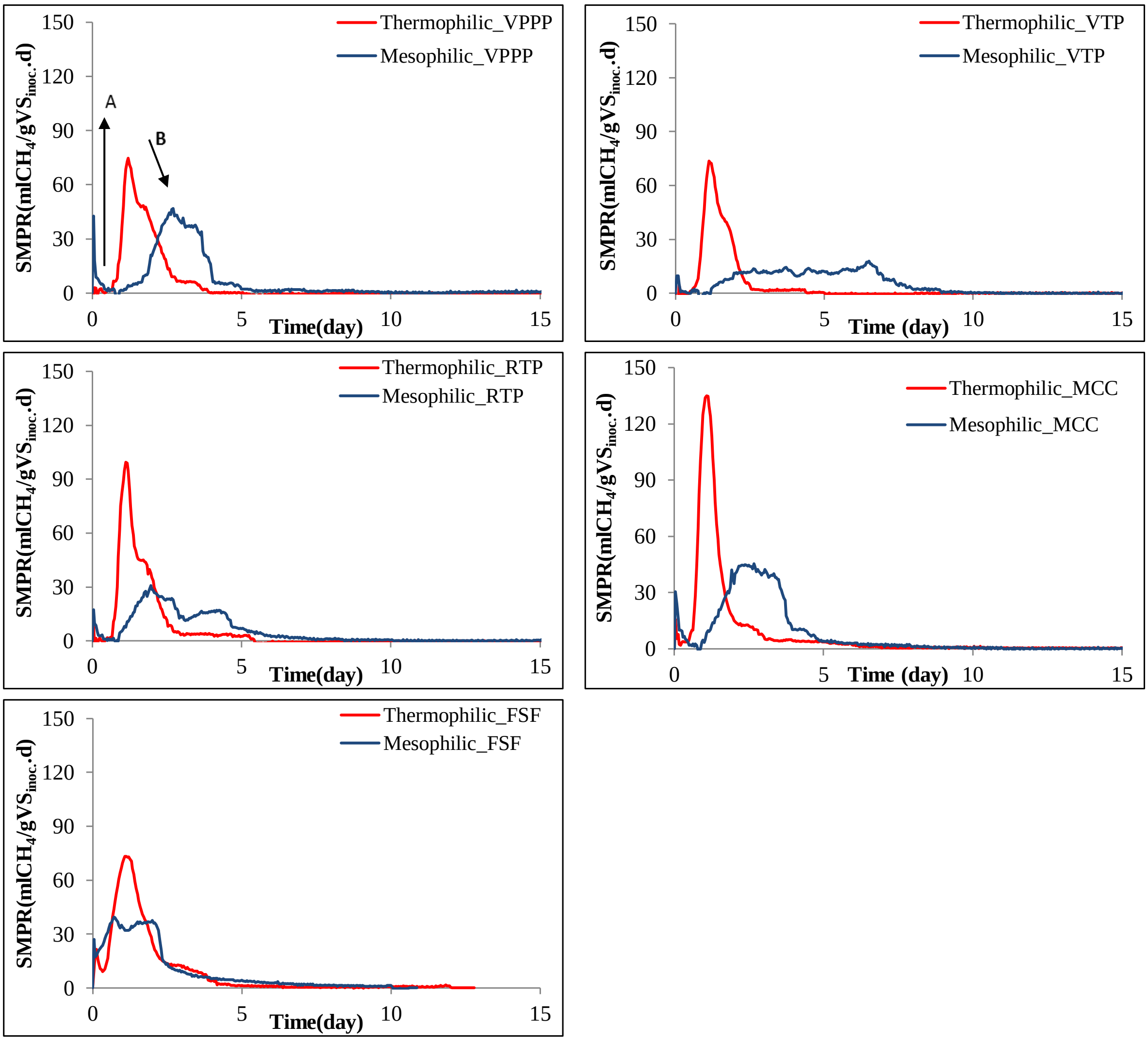


**Figure 3. Specific methane production rate (SMPR) for VPPP, VTP, RTP, MCC and FSF under thermophilic and mesophilic conditions at $R_{I/S}$=3**

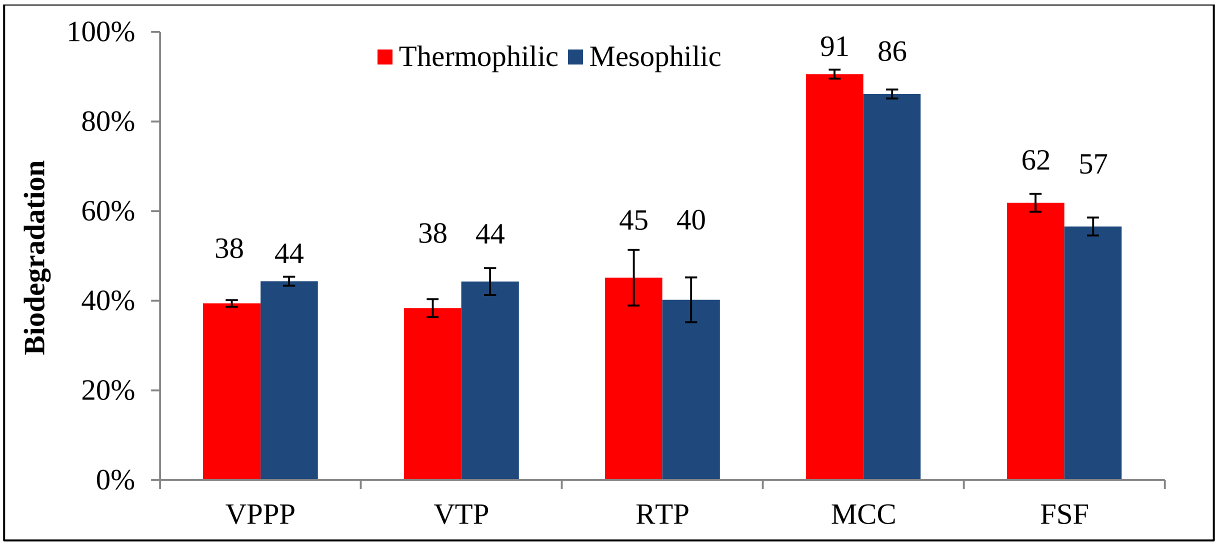


**Figure 4. Biodegradation percentage of VPPP, VTP, RTP, MCC and FSF under thermophilic and mesophilic conditions at $R_{I/S}$ of 3**