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Research article

Effect of thermal pretreatment at 70 °C for one hour (EU hygienization conditions) of various organic wastes on methane production under mesophilic anaerobic digestion

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Abstract: The impact of hygienization as mild thermal pretreatment on the methane production of various organic wastes was investigated, including digestate issued from hydrolysis tank, thickened sludge from a municipal wastewater treatment plant (MWWTP sludge) and from a mixed domestic-industrial wastewater treatment plant (D-I WWTP sludge), sludge from a meat-processing plant (MP sludge), sieving rejection from a pork slaughterhouse, pork liver, cattle slurry, cattle scraping slurry and date seeds. They were thermally pretreated at 70 °C for one hour and subsequently put into AD digesters incubated at 37 °C for individual methane potential test. The modified Gompertz model was employed to evaluate the kinetic parameters of methane production curves (R^2 = 0.944–0.999). The results were compared with the untreated samples. Significant enhancement of methane potentials induced by thermal treatment ($p < 0.05$) was observed when it comes to the pork liver (+8.6%), the slaughterhouse sieving rejection (+11.1%), the thickened MWWTP sludge (+12.5%) and the digestate issued from hydrolysis tank (+18.0%). The maximum methane production rates of the 4 substrates mentioned above were increased by thermal pretreatment as well (from 13.5% to 64%, $p < 0.05$). The lag time of the methane production was shortened for the digestate from hydrolysis tank and the MWWTP sludge (by 48.6% and 62.2%

respectively, $p < 0.05$). No significant enhancement was obtained for the cattle slurry, the cattle scraping slurry and the D-I WWTP sludge. Additionally, the maximum methane production rate and the methane potential were reduced by thermal pretreatment for the MP sludge and the date seeds respectively ($p < 0.05$). In this paper, possible mechanisms were discussed to explain the different methane production behaviors of substrates after the mild thermal pretreatment.

Keywords: mesophilic anaerobic digestion; hygienization; mild thermal pretreatment; experimental study; modified Gompertz model

1. Introduction

Faced up with the challenge of global warming and the increasing demand of cleaner energy consumption, the European Union is engaged to transfer its 20% total energy needs into renewable energy by 2020 [1]. Anaerobic digestion (AD) for biogas production, as a solution to the ambition of the European Union, is now drawing a growing attention because of its advantage of performing energy generation and biomass valorization at the same time. Organic wastes, among which we can find municipal solid wastes (MSW), sewage sludge, animal by-products (ABP) from food-processing industries as well as agricultural wastes including waste from livestock raising and crops cultivation, serve as substrates of AD process. These wastes may harbor various infectious pathogens like *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Clostridium perfringens*. Without any preventive measures, these infectious agents may remain active during the following treatment processes and final disposal of the wastes, which may contaminate natural environment like soil, surface water and atmosphere, presenting a high risk for foodborne outbreaks [2].

EU Regulation No 142/2011 regulates the transformation of ABP into biogas by setting a hygienization of feedstock (70 °C for one hour) prior to the anaerobic digestion (AD) process in order to prevent the living pathogens from spreading into environment during the final disposal of AD digestate [3]. This regulation requires that the thermal hygienization should achieve a reduction of 5 log10 of *Enterococcus faecalis* or *Salmonella Senftenberg* W775 as well as a reduction of 3 log10 of infectivity titre of thermoresistant viruses such as parvovirus. Pasteurization for 60 min at 70 °C is stated efficient for the inactivation of the most of no spore forming bacteria [4]. Elving et al. (2014) reported that a lower pasteurisation temperature other than 70 °C for longer treatment time could also be sufficient for an inactivation of *Salmonella Senftenberg* and *Enterococcus faecalis* in dairy cow feces (for example a thermal treatment at 55 °C for 2.24 and 3.29 hours for a 5 log10 reduction respectively) [5]. Sanitation of organic wastes could be attained through the anaerobic digestion as well, especially when it comes to the thermophilic AD process at 50–55 °C, as reviewed by Franke-Whittle and Insam (2013) [4]. In addition, the thermal pasteurisation did not affect the spore forming bacteria like *Clostridium* spp. [6]. Depending on the operational scale, the experimental parameters and the target microorganisms (*Clostridium* spp. and *Bacillus* spp.), contradictory results could be obtained for the survival of bacteria spores during the thermophilic AD treatment of ABP [4,7].

Besides the effect of thermal hygienization on the pathogen inactivation of organic wastes, studies show that this hygienization process, as a mild thermal pretreatment, favors the methane production during the AD by an increase of 50% for secondary sewage sludge and food-processing wastes pre-treated at 70 °C in terms of either the bio-methane potential (BMP) or the kinetics of methane production [8–10]. Nevertheless, no significant effect was observed for mixed pork wastes, livestock manure and a mixture of MSW and ABP [11,12]. These contradictory results could be explained by the physical-chemical reactions of substrates taking place during the thermal pretreatment, including the particle size reduction, the formation of the complex compounds and the inhibitory processes like the accumulation of NH_3 derived from proteins, volatile fatty acids (VFA) degraded from long chain fatty acid (LCFA) and the competition of H_2/sulphate-consuming bacteria with the methanogenic bacteria [13].

The research on the effect of hygienization on the methane production of wastes remains scarce, particularly regarding the operational parameters regulated by EU (70 °C for 1 hour). Only a few kinds of organic wastes were examined by the previous studies and the available data are not sufficient to draw a sound conclusion of whether this pretreatment does affect the methane production of biowastes and to what extent. This paper aims to enlarge the variety of tested substrates in order to have more knowledge about the possible impact of hygienization on different categories of organic wastes. For this purpose, 9 kinds of organic wastes, including digestate issued from hydrolysis tank, thickened sludge from a municipal wastewater treatment plant (MWWTP sludge) and from a mixed domestic-industrial wastewater treatment plant (D-I WWTP sludge), sludge from a meat-processing plant (MP sludge), sieving rejection from a pork slaughterhouse, pork liver, cattle slurry, cattle scraping slurry and date seeds, were thermally pretreated at 70 °C for one hour and subsequently put into AD digesters for individual methane potential test at 37 °C. Their methane yield curves were compared with those of the untreated samples. The kinetic parameters were extracted by fitting the curves of the methane production using the modified Gompertz model. Possible mechanisms were given to explain the different behavior of substrates induced by this thermal pretreatment.

2. Materials and methods

2.1. Inoculum and substrates preparation

Fresh anaerobic inoculum and the digestate of hydrolysis tank were obtained from a local biogas plant (LIGER, Locminé, France) that runs the anaerobic digesters treating mixed agricultural and industrial organic wastes. Cattle scrapping slurry and cattle slurry were collected from local cattle farms. Date seeds were separated from the mixed varieties of dates of secondary class originated from Tozeur (Tunisia). MWWTP sludge and D-I WWTP sludge were collected from 2 wastewater treatment plants of different communities. MP sludge and Sieving rejection of slaughterhouse were obtained from one commercial meat-processing plant in Brittany (France). Ordinary commercialized pork liver was bought in a local supermarket. The inoculum was filtrated through an 800-μm sieve. All of the samples collected were homogenized using a kitchen mixer separately. The total solids (TS) were measured in an oven at 105 °C. The volatile solids (VS) were determined using a muffle furnace at 550 °C. The Chemical Oxygen Demand (COD) were measured using Spectroquant COD test (Merck KGaA, Darmstadt, Germany) analogous to EPA 410.4, APHA 5220 D and ASTM D1252-06 B. All of the characteristic results of the inoculum and 9 organic wastes tested were resumed in Table 1.

Table 1. Characteristics of inoculum and nine different substrates.

	TS %	VS/TS %	COD g COD·kg substrate^{-1}
Inoculum	5.67 ± 0.03	70.5 ± 1.9	58.8 ± 3.4
Cattle scraping slurry	20.5 ± 0.6	55.7 ± 1.1	150.5 ± 0.6
Cattle slurry	11.2 ± 0.2	82.8 ± 1.4	134.2 ± 1.2
Date seeds	98.2 ± 0.0	98.7 ± 0.0	1237.2 ± 7.9
D-I WWTP sludge	11.1 ± 0.1	85.9 ± 0.5	137.8 ± 2.1
Digestate of hydrolysis tank	10.4 ± 0.3	84.0 ± 0.5	148.5 ± 2.5
MP sludge	21.1 ± 0.1	86.3 ± 0.2	332.2 ± 11.8
MWWTP sludge	11.9 ± 0.1	87.3 ± 2.0	144.3 ± 1.6
Pork liver	30.4 ± 0.5	89.2 ± 0.7	750.0 ± 0.0
Sieving rejection	26.7 ± 1.9	98.0 ± 0.9	334.2 ± 6.8

The mild thermal pretreatment was performed using a hotplate-stirrer (Squart Co., Stone, UK) at the same day of the manipulations. Non-liquid samples were at first diluted with distilled water before the pretreatment to facilitate the stirring. Once pretreated, the substrates were left cooled to room temperature and subsequently put into anaerobic digesters for BMP test.

2.2. Batch BMP measurement

The batch AD tests of different pretreated or unpretreated substrates were carried out in duplicate using 0.5 L glass bottles incubated at 37 °C with the aid of Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control Co., Sweden). The biogas produced in each vial passed through an individual NaOH absorption unit to retain its acidic gas like CO_2 and H_2S. A measuring device then measured and recorded the volume of CH_4 produced. The quality of the biogas was therefore not studied in this paper. The initial Feed/Inoculum (F/I) ratio was kept between 0.35 and 0.5 based on the VS of the substrates and that of the inoculum. At least 2 controlled bottles filled with inoculum (blank) were prepared for each experiment of AMPTS II. The initial and final pH ranged between 7.5 and 8.3.

2.3. Analytical method and statistical analysis

The modified Gompertz [14] was employed to model the methane production curves of different substrates using R studio (Massachusetts, US), due to its better performance for modeling the curves considering the lag time as compared to the first order kinetics (data of this comparison not shown). It is a 3-parameter model with the formula shown in Eq 1.

$$BMP\ (t) = BMP_0 \cdot \exp\left\{ -\exp\left[\frac{R_m \cdot e}{BMP_0} \left(\lambda - t \right) + 1 \right] \right\} \quad (1)$$

where the BMP(t) is the cumulative methane production at instant t ($Nm^3\ CH_4 \cdot kg\ COD^{-1}$), BMP_0 the methane potential of the substrate ($Nm^3 \cdot kg\ COD^{-1}$), R_m the maximum methane production rate ($Nm^3\ CH_4 \cdot kg\ COD^{-1} \cdot day^{-1}$), λ the lag phase time (day), e the exp(1) and t the anaerobic digestion time (day). The goodness of the modeling was evaluated by the coefficient of determination R^2 and the sum of squared errors (SSE). The data obtained from AMPTS II were at first treated by Microsoft excel (Microsoft office 2016, Washington, US) and the following statistical tests (Student's t-test and Pearson correlation test) were performed at significant level of α = 0.05 using R studio.

3. Results and discussion

3.1. Methane yield enhancement

The methane production data acquired through AMPTS II were exported for further statistical processing. The net methane production of samples was calculated by removing the corresponding methane yield of controlled blank bottles from the initial methane production. The Figure 1 gives the experimental means and the modeled methane production curve of various substrates tested, either pretreated or unpretreated. Each experimental curve was fitted by modified Gompertz in search of kinetic parameters (BMP_0, R_m and λ). The means of experimental and modeled daily methane yield were calculated.

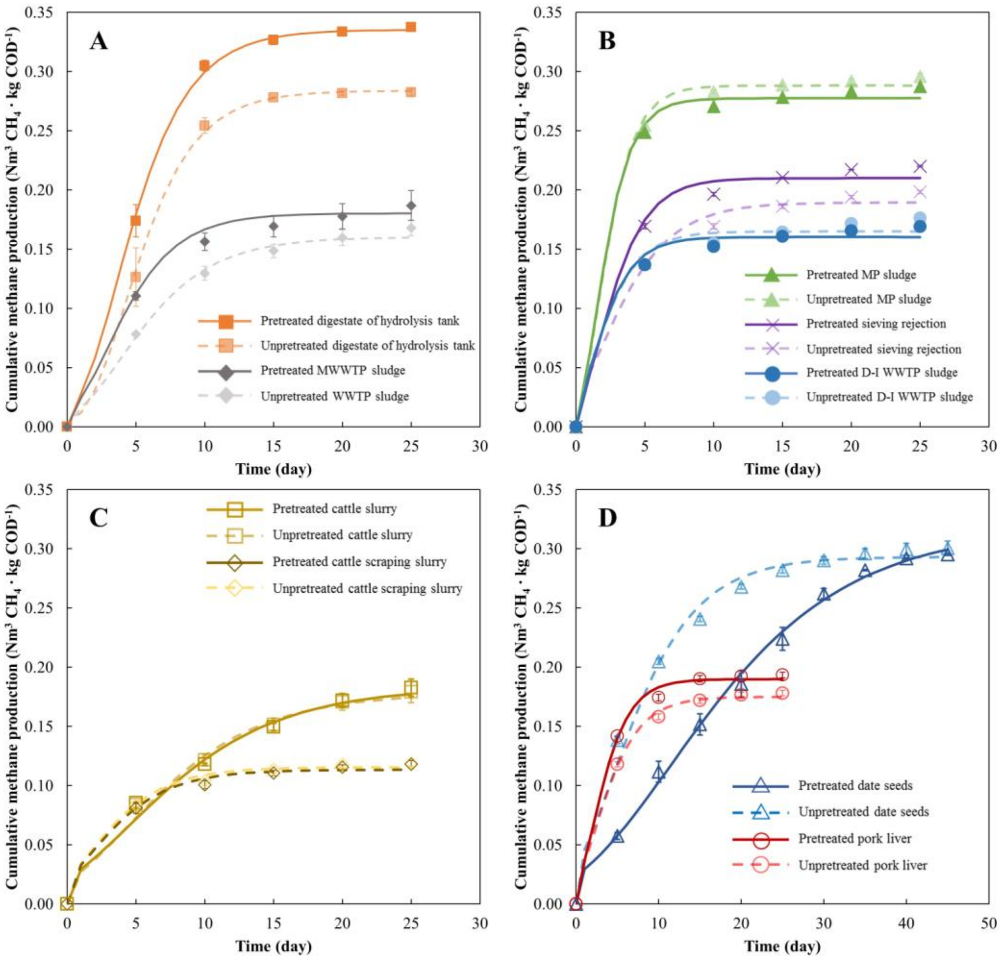

Figure 1. Cumulative methane yield of different substrates with or without mild thermal pretreatment. Marked points are the selected experimental means of methane production of every 5 days with standard deviation (whose values are too small to be visible for certain cases). The solid and dashed lines are the modeled values of modified Gompertz.

The anaerobic degradability of the substrates was calculated using the Eq 2 with BMP_{exp} the methane potential experimentally mesured and 0.35 Nm^3 CH_4·kg COD^{-1} the theoretical maximum methane production supossing the full degradability of COD of the substrates [15].

$$\text{Degradability (\%)} = \frac{BMP_{exp}}{0.35} \times 100 \quad (2)$$

All of the substrates achieved the 95% of the experimental methane potential in 20 days, except for the date seeds which required 27 days. The experimental measured BMP and the degradability of samples were listed in Table 2. The anaerobic degradability of intact samples varied to a large exent (from 33.9% for cattle scraping slurry to 84.5% for MP sludge). In this paper, the duplication of the tested samples seems to be adequate for performing Student's t-test since the standard deviations within each group were found comparatively small. Significant positive effect of mild thermal pretreatment on the BMP was seen for hydrolysis digestate, MWWTP sludge, pork liver and sieving rejection ($p < 0.05$). A negative effect was found for MP sludge ($p < 0.05$) and no significant impact was obtained for the rest of the substrates. The corresponding anaerobic degradability followed the same tendency of the experimental BMP.

Table 2. Experimental methane production BMP_{exp} and the corresponding anaerobic degradability of intact (O) and thermally pretreated (+) substrates. Data in bold mean the significant difference within the group ($p < 0.05$).

	BMP_{exp} Nm^3·kg COD^{-1}		Degradability %	
Pretreatment	O	+	O	+
Cattle scraping slurry	0.119 ± 0.002	0.118 ± 0.003	33.9 ± 0.7	33.7 ± 0.9
Cattle slurry	0.179 ± 0.009	0.183 ± 0.007	51.1 ± 2.6	52.2 ± 2.1
Date seeds	0.300 ± 0.006	0.295 ± 0.001	85.8 ± 1.7	84.3 ± 0.2
D-I WWTP sludge	0.176 ± 0.002	0.169 ± 0.001	50.4 ± 0.5	48.3 ± 0.4
Hydrolysis digestate	**0.283 ± 0.003**	**0.337 ± 0.002**	**80.7 ± 0.9**	**96.4 ± 0.6**
MP sludge	**0.296 ± 0.003**	**0.287 ± 0.000**	**84.5 ± 0.8**	**82.1 ± 0.1**
MWWTP sludge	**0.168 ± 0.007**	**0.187 ± 0.013**	**48.0 ± 1.9**	**53.8 ± 3.6**
Pork liver	**0.178 ± 0.002**	**0.193 ± 0.002**	**50.9 ± 0.7**	**55.3 ± 0.7**
Sieving rejection	**0.198 ± 0.001**	**0.220 ± 0.001**	**56.7 ± 0.3**	**62.9 ± 0.0**

Table 3 gives a summary on the kinetic modeling parameters of modified Gompertz accompanied with the corresponding statistical test results. Besides, the time constant τ of the model was calculated. This time constant signifies the time (day) required for the methane production to reach 36.8%, namely exp(−1), of the predicted methane potential BMP_0.

When it comes to the maximum methane yield (BMP_0), significant intensification induced by thermal pretreatment ($p < 0.05$) was observed for the pork liver (+8.6%), the slaughterhouse sieving rejection (+11.1%), the thickened MWWTP sludge (+12.5%) and the digestate issued from hydrolysis tank (+18.0%). The maximum methane production rates R_m were increased by thermal pretreatment by 31.8%, 64.0%, 50.0% and 13.5% for the same four substrates as mentioned ($p < 0.05$), which means that the methane production was accelerated by thermal pretreatment. The negative values of lag time λ were not taken into consideration because this signifies that λ serves as a mathematical parameter to which no physical meaning could be addressed. However, positive lag time means the duration of time required by microorganisms to acclimatize to the AD conditions with the possible presence of inhibitive effect [16]. The duration of lag phase was shortened for the digestate from hydrolysis tank and the MWWTP sludge (by 48.6% and 62.2% respectively, $p < 0.05$). The time constant for these four substrates mentioned was also ameliorated (decrease of the value) at the statistical level, corresponding to an increase of methane yield rate. It is interesting to note that the AD kinetics were inhibited by thermal pretreatment for the meat-processing sludge in terms of the methane potential BMP_0 (−3.82%, $p < 0.05$) and the date seeds in terms of the maximum methane yield rate R_m (−47.4%, $p < 0.05$) as well as the corresponding time constant τ. The other 2 kinetics parameters (BMP_0 and λ) of the MP sludge and date seeds were not influenced. No significant enhancement of AD kinetics was obtained for the cattle slurry, the cattle scraping slurry and the D-I WWTP sludge, based on both the visualization of AD curves and modeling results.

The correlation between the increase in BMP_0 and the increase in R_m induced by mild thermal pretreatment was studied in Figure 2. The point belonging to the digestate of hydrolysis tank was removed from the linear regression and the correlation test because apparently it does not follow the general tendency with regard to the other substrates. This phenomenon can be explained by the fact that the digestate from hydrolysis tank had already undergone a period of hydrolysis step to improve the methane production rate and as a result, the mild thermal pretreatment had little effect on the improvement of R_m as compared to the other substrates that were not biologically pretreated as such. A linear regression was conducted with the equation shown in the Figure 2 (R^2 = 0.714). Despite the limited number of samples, the Pearson correlation test was performed and a correlation coefficient 0.829 was obtained (p = 0.011, <0.05), indicating a significant positive correlation between the increase in R_m and BMP_0. However, this correlation remains to be verified by enlarging the variety of tested samples.

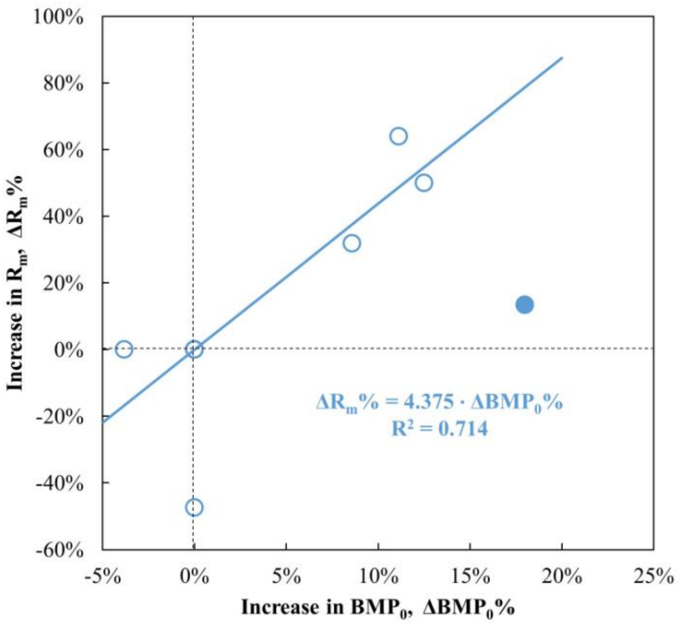

Figure 2. Correlation between the increase in BMP_0 and the increase in R_m induced by mild thermal pretreatment (the solid circle is related to the digestate from hydrolysis tank, excluded from linear regression).

Table 3. Summary of modeling results of kinetic study using modified Gompertz. "O" and "+" represent non-pretreated and pretreated samples respectively. Data in bold mean the significant difference within the group (p < 0.05).

	BMP_0 $Nm^3 \cdot kg\ COD^{-1}$		R_m $Nm^3 \cdot kg\ COD^{-1} \cdot day^{-1}$		λ day		$\tau = \lambda + \frac{BMP_0}{R_m \cdot e}$ day		R^2 -	SSE 10^{-4}
Pretreatment	O	+	O	+	O	+	O	+		
Cattle scraping slurry	0.115 ± 0.003	0.113 ± 0.002	0.014 ± 0.000	0.014 ± 0.001	−1.359 ± 0.112	−1.430 ± 0.095	1.595 ± 0.024	1.664 ± 0.188	0.952 − 0.964	7.38 − 9.89
Cattle slurry	0.178 ± 0.008	0.183 ± 0.009	0.012 ± 0.000	0.011 ± 0.000	−0.985 ± 0.100	0.100 ± 0.049	4.398 ± 0.122	4.626 ± 0.366	0.981 − 0.988	7.97 − 12.8
Date seeds	0.293 ± 0.004	0.310 ± 0.008	**0.019 ± 0.000**	**0.010 ± 0.001**	−1.115 ± 0.185	−0.564 ± 0.100	**4.457 ± 0.052**	**11.48 ± 1.14**	0.982 − 0.995	11.1 − 42.4
D-I WWTP sludge	0.165 ± 0.000	0.160 ± 0.001	0.034 ± 0.001	0.037 ± 0.002	−0.512 ± 0.061	−0.336 ± 0.057	1.283 ± 0.018	1.272 ± 0.038	0.944 − 0.966	1.25 − 2.27
Hydrolysis digestate	**0.284 ± 0.001**	**0.335 ± 0.001**	**0.037 ± 0.000**	**0.042 ± 0.003**	**1.425 ± 0.623**	**0.733 ± 0.049**	**4.246 ± 0.640**	**3.652 ± 0.178**	0.998 − 0.999	1.94 − 6.98
MP sludge	**0.288 ± 0.002**	**0.277 ± 0.000**	0.073 ± 0.001	0.074 ± 0.001	0.195 ± 0.022	0.162 ± 0.040	**1.639 ± 0.056**	**1.541 ± 0.022**	0.989 − 0.994	8.18 − 13.4
MWWTP sludge	**0.160 ± 0.007**	**0.180 ± 0.011**	**0.016 ± 0.001**	**0.024 ± 0.000**	**0.386 ± 0.118**	**0.146 ± 0.011**	**3.983 ± 0.171**	**2.923 ± 0.125**	0.991 − 0.995	3.21 − 5.61
Pork liver	**0.175 ± 0.002**	**0.190 ± 0.003**	**0.022 ± 0.000**	**0.029 ± 0.001**	−0.541 ± 0.006	−0.181 ± 0.065	**2.379 ± 0.021**	**2.190 ± 0.019**	0.990 − 0.991	5.39 − 6.29
Sieving rejection	**0.189 ± 0.001**	**0.210 ± 0.001**	**0.025 ± 0.002**	**0.041 ± 0.000**	−0.807 ± 0.158	−0.082 ± 0.092	**1.951 ± 0.062**	**1.806 ± 0.083**	0.965 − 0.973	2.11 − 2.29

3.2. Possible mechanisms analysis

The positive effect of hygienization on the methane yield was achieved for slaughterhouse-related wastes, namely pork liver and sieving rejection, and for less complex substrates derived from biological treatment process, like digestate from hydrolysis tank and MWWTP sludge. This observation was also reported by Luste et al. (2009) [17], Luste and Luostarinen (2010) [9] for the hygienization of ABP, particularly slaughterhouse sieving wastes, and by Edström et al. (2003) [18], Gavala et al. (2003) [8] and Climent (2007) [10] for municipal waste activated sludge thermally pretreated at 70 °C. The enhancement of the methane potential was explained by the fact that the pretreated substrates presented a higher soluble COD than untreated samples, indicating a solubilization of the organic substances that convert the complex chemical compounds (e.g., LCFA, proteins) into simpler ones (e.g., VFA, ammonia) [17]. This soluble COD is much more accessible to the bacteria and therefore facilitates the fermentative biological activities in the anaerobic digesters. Additionally, it was also reported that a morphological difference could be observed in terms of the particle size reduction of the samples. This particle transformation favors the hydrolysis step, the limiting phase during the degradation of organic matters [9]. An equilibrium between the processes mentioned above makes the methane production more efficient and the possibility of the accumulation of intermediates (VFAs, sulfate, ammonia and hydrogen) is thus reduced [19]. All of these factors gave rise to the enhancement of methane production of slaughterhouse wastes and biological process wastes by increasing the degradable organic matters (for BMP), facilitating the accessibility of substrates to the microorganisms (for methane production rate) and avoiding the accumulation of inhibitors (for lag time).

The similar reduction of the methane potential of meat-processing sludge (MP sludge) induced by mild thermal pretreatment could be found in the studies of Hejnfelt and Angelidaki (2009) [11] and Luste et al. (2009) [17] whose substrates were meat-processing industry wastes rich in grease, hair and blood as well. This negative effect was perhaps due to the formation of complex chemical compounds that are toxic to the methanogenic process during the pretreatment, usually with high concentration in ammonia and lipid. However, this phenomenon remains unclear and requires further study [20].

We obtained a contradictory result concerning the cattle slurry as compared to Paavola et al. (2006) and Luste and Luostarinen (2011). They achieved 180–290 Nm^3 CH_4·t VS^{-1} for the untreated and 210–340 Nm^3 CH_4·t VS^{-1} for the hygienized, i.e. an enhancement of around 20% [21,22] while we obtained 154 Nm^3 CH_4·t VS^{-1} for cattle scraping slurry and 247 Nm^3 CH_4·t VS^{-1} for ordinary cattle slurry, no significant enhancement observed after hygienization. It is worth noting that the cattle slurry and scraping slurry in the present study were more concentrated than that studied by the authors mentioned in terms of the TS and COD. This difference could be possibly due to the fact that in our study, the cattle slurry was collected fresh from the farm and was put into digesters right after the determination of TS, VS and COD. Though the 2 studies mentioned did not specify their collection protocol of the cattle slurry, it is a common practice that the tested samples could be collected from a manure storage facility of the farms where the easily biodegradable organic content was reduced during the storage and therefore the TS and COD of the samples decreased [23]. Consequently, the remaining organic matters in the cattle slurry were not easily degradable and the mild thermal pretreatment might have a more significant effect on them than the fresh cattle slurry containing more easily degradable organic matters. It could also be possible that our substrates contained many more straw fibers than the substrates of those studies and as a result, the thermal pretreatment at low temperature had almost no effect on such substances rich in cellulose as straw fibers.

It is the same reason for the no effect of hygienization on the BMP of date seeds which contain more complex organic compounds like the family of cellulose and lignin [24]. These substances require a more intensive pretreatment to break the long-chain molecules. The reduction of methane yield rate of date seeds pretreated could result from the formation of complex compounds during the pretreatment considering that date seeds possess large amount of antioxidant substances like polyphenol, lipid, sugar, tannin and pigments [25].

The presence of non-biodegradable organic matters that could not be thermally broken at ease possibly resulted in no effect on the BMP intensification of the mixed domestic-industrial waste activated sludge (D-I WWTP sludge) that treated the effluent issued from mixed chemical industries.

4. Conclusion

Nine different organic wastes were tested concerning the impact of hygienization (serving as mild thermal pretreatment) on the methane yield kinetics. Results show that the slaughterhouse-related wastes and biological process wastes received a significant effect on their methane potential. The heating of the wastes with too much lipids and ammonia such as meat-processing sludge could form toxic and non-biodegradable organic matters that reduced the methane potential to some extent. Mild thermal pretreatment at 70 °C for one hour had little influence on the solubilization and the particle reduction of the substrates rich in complex compounds like lignin, hemicellulose, cellulose and the chemical compounds issued from chemical industries. More studies should be concentrated on the hygienization of different kinds of AD feedstock to have a global idea concerning the impact of this pretreatment on both the pathogen reduction and the subsequent methane production. Studies on the the chemical composition changes of substrates after hygienization should be paid attention to as well.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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