Thermal and acidic pre-treatments applied to cow manure: effects on pathogenic bacteria persistence and on biogas production during thermophilic anaerobic digestion

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Introduction

In the last decades, anaerobic digestion has been a windfall process for supporting ecological transition. A lot of waste (domestic, agricultural…) can be used to feed anaerobic digester and recovered them both by biogas and fertilizer (digestate) production. Indeed, the biogas produced during anaerobic digestion of organic waste produce sustainable energy which can be used for electricity generation of for residential heating. The biogas is composed in majority of methane and carbon dioxide. The digestate, which is the solid-liquid residue of anaerobic digestion, is used as a fertilizer for land spreading on agricultural land. However, this digestate may contain pathogens such as micro-organisms responsible for human and animal diseases (Pourcher *et al* (2008), Liu *et al* (2019)). The production of a "safe" digestate, which means a digestate stripped of any pathogen, is an important issue to continue the digestate spreading pathway. Agricultural waste like slurry or manure are known for their pathogenic micro-organisms content. To achieve digestate safety, some specific treatments can be applied to farm waste inputs before being incorporated into the digester. Different parameters can simultaneously influence pathogen survival during anaerobic digestion and methane production (Devlin *et al* (2011), Nazari *et al* (2017)). Among these parameters, temperature and Volatile Fatty Acids (VFA) lead to a decrease of pathogen levels during anaerobic digestion (Salsali *et a*. (2008)). However, consequences of such pre-treatment on biogas production have not been yet investigated. The objective of this work is to apply different pre-treatments (thermic, acid or combined acid and thermic) on farm animal waste (cow manure) to evaluate their ability to reduce pathogen survival and content. In parallel, the impact on biogas production during anaerobic digestion is obviously investigated.

Material and Methods

During these experiments, cow manure from a farm (Limoges, France) was sampled in order to assess pre-treatment effects. Different pre-treatments were applied on farm inputs: acid, thermic and acido-thermic pre-treatments. Acid treatments consisted in adding an equimolar mixture of acetic, propionic and butyric acids at increasing concentrations 1.5, 3 and 6 g/L. Thermic treatment lasted 1 hour in a water bath at 70°C. Acido-thermic treatments were a combination between acid and thermic pre-treatments.

Enumeration of bacteria: the effects of the different pre-treatments on 3 micro-organisms persistence were determined. *C. perfringens* were chosen because of their occurrence and resistance to high temperature conditions and 2 bacteria known to be good indicators of faecal contamination (*Escherichia coli* and *Enterococcus sp*.). Enumeration of *C. perfringens* was performed by suspending 10 g of cow manure into 90 mL of sterile water. To enumerate *C. perfringens* spores, each dilution was placed in a water bath at 80°C for 10 minutes before being inoculated in TSN tubes. Ten-fold dilutions were managed until obtaining $1*10^{-6}$ dilutions. 1 mL of each dilution was inoculated into a TSN tube in duplicate and incubated at 37°C during 24 hours. 96-well microplates MUG (4-methylumbelliferil-β-D-glucuronide, Biokar®) were used to quantify *E. coli*. Microplates MUD (4-methylumbelliferil-β-D-glucoside, Biokar®) were used to detect *Enterococci*.

Biochemical methane potential production assessment: the pre-treated cow manure samples were introduced into 1 L glass bottles to achieve BMP (Biochemical Methane Potential) tests in triplicate. BMP operating conditions consisted in mixing pre-treated manure sample with inoculum in bottles (Inoculum/Substrate ratio of Volatile Solids = 4). The bottles were 40% filled and sealed to preserve anaerobic conditions. Inoculum was sampled from an agricultural anaerobic digester near Limoges (France). The BMP tests' bottles were incubated at 55°C (thermophilic conditions) until the end of biogas production which was daily measured.

Results and discussion

Results obtained during these experiments showed that the kind of pre-treatments differentially influenced concentrations of pathogens (Table 1), and methane production of BMP tests (Table 2). Table 1 summarises influence of thermic, acid and acido-thermic pre-treatments on pathogens concentrations. With acido-thermic pre-treatment (acid mixture at 3 g/L and 1 hour at 70°C), *Escherichia coli* totally disappeared in raw matter (RM). Besides, under those conditions, *Enterococcus sp*. had a 2-log reduction (from 10^6 Colony Forming Unit (CFU) to 10^4 CFU after treatment) but *Clostridium perfringens* spores remained in samples after treatment. Non significative effects on spores were demonstrated with acido-thermic pre-treatment.

Salsali *et al* (2008) showed effects on *C. perfringens* persistence after acid treatments but the origin of the waste was completely different. It was a mixture of primary and thickened waste-activated sludge. Cow manure and sludge structure's and composition's can't definitely be compared. It could be assumed that sludge structure allows better diffusion of acid compared with cow manure. Besides, Salsali *et al.* (2008) did not focus on spores but both vegetative and spore of *C. perfringens*.

Table 1. Pathogens concentrations of raw or thermic or acid (3 g/L) or acido-thermic pre-treated cow manure

Pathogens	Raw cow manure (Control) (CFU/g of RM)	Thermic treated cow manure (CFU/g of RM)	Acid treated cow manure (CFU/g of RM)	Acido-thermic treated cow manure (CFU/g of RM)
Escherichia coli	$2.3*10^3 \pm 1.1*10^3$	$8.2*10^1 \pm 3.8*10^1$	$2.3*10^3 \pm 1.1*10^3$	0
Enterococcus sp.	$6.8*10^6 \pm 3.3*10^6$	$1.28*10^4 \pm 5.4*10^3$	$8.01*10^6 \pm 3.9*10^6$	$2.77*10^4 \pm 1.3*10^4$
Clostridium perfringens spores	$2.5*10^2 \pm 2.1*10^2$	$1*10^2 \pm 0$	$3.5*10^2 \pm 7.07*10^1$	$5.5*10^2 \pm 3.54*10^2$

Table 2 described methane production of BMP tests with pre-treated cow manure with a thermic treatment 1 hour at 70°C, an acid concentration at 3 g/L and a combination of these two pre-treatments. On the one hand, acid (3 g/L) or thermic (1 hour at 70°C) pre-treated cow manure showed a higher methane production compared with the control. Indeed, after 32 days at 55°C, the methane production was to 293.9 mL CH4/g Volatile Solids (VS) for thermic treated cow manure compared with 270.4 mL CH4/g VS for raw cow manure. Furthermore, acid treated cow manure led to 317.6 mL CH4/g VS compared with 270.4 mL CH4/g VS for raw cow manure. On the other hand, acido-thermic treated cow manure could be described as a factor inhibiting methane production. After 32 days at 55°C, acido-thermic treated cow manure led to 163.7 mL CH4/g VS compared with 270.4 mL CH4/g VS for raw input.

Table 2. Methane production of BMP tests with raw or thermic or acid (3 g/L) or acido-thermic pre-treated cow manure

	Raw cow manure (Control)	Thermic treated cow manure	Acid treated cow manure	Acido-thermic treated cow manure
Methane production (mL CH4/g of VS)	270.4 ± 5.8	293.9 ± 0.9	317.6 ± 46.8	163.7 ± 23.2

Thermic pre-treatment methane production increase (around 9%) could be attributed to an enhanced biodegradability due to the solubilization of organic particles and the stimulation of thermophilic bacteria (Nazari *et al* (2017)). Concerning acid pre-treatment, methane production increased by 17% which is corroborated by other published studies (Devlin *et al* (2011)). Acido-thermic pre-treatment led to an important decrease in methane production by 39%. In other studies, this treatment was used as a post-treatment and showed better benefits than in our analysis (Takashima *et al* (2014)). Other results obtained with acid concentrations at 6 g/L confirmed these results cited above. However, acid treatments at 1.5 g/L did not show the same effects.

Conclusion

To conclude, pre-treatments led to a decrease of main studied pathogens excepting for spores of *C. perfringens*. *Clostridium* spores were such resistant that they could survive despite of the treatments applied to cow manure. Moreover, acido-thermic pre-treatment led to disappointing results concerning of biogas production. It should be interesting to apply these pre-treatments to pure culture of *C. perfringens*. Then, an understanding of the mechanism, thanks to flow cytometry or flow imaging, should be of interest and shed new light.

References

Devlin DC, Esteves SRR, Dinsdale RM, Guwy AJ. The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresource Technology. 2011 Mar;102(5):4076–82.

Liu X, Lendormi T, Lanoisellé J-L. Overview of hygienization pretreatment for pasteurization and methane potential enhancement of biowaste: Challenges, state of the art and alternative technologies. Journal of Cleaner Production. 2019 Nov;236:117525.

Nazari L, Yuan Z, Santoro D, Sarathy S, Ho D, Batstone D, et al. Low-temperature thermal pre-treatment of municipal wastewater sludge: Process optimization and effects on solubilization and anaerobic degradation. Water Research. 2017 Apr;113:111–23.

Pourcher A-M, Burton C, Ziebal C, De-Guardia A. Impact of temperature-time combinations on enteric bacteria in separated solids from pig manure. 2008. :5

Salsali H, Parker WJ, Sattar SA. The effect of volatile fatty acids on the inactivation of Clostridium perfringens in anaerobic digestion. World J Microbiol Biotechnol. 2008 May;24(5):659–65.

Takashima M, Tanaka Y. Acidic thermal post-treatment for enhancing anaerobic digestion of sewage sludge. Journal of Environmental Chemical Engineering. 2014 Jun;2(2):773–9.