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# Impact of microwave pretreatment on manure and endogenous microorganisms prior to acidogenic fermentation

Korantin Pipereau\*, Eric Trably, Gaelle Santa-Catalina, Diana Garcia Bernet, H el ene Carrere\*\*

\*INRAE, Universit e de Montpellier - LBE Av. des Etangs 11100 Narbonne, korantin.pipereau@inrae.fr

\*\* INRAE, Universit e de Montpellier - LBE Av. des Etangs 11100 Narbonne, helene.carrere@inrae.fr

## Abstract:

The production of bio-based Volatile Fatty Acids (VFA) from waste could be a solution for reducing greenhouse gas emissions of fossil fuels-based industry. This paper focuses on the use of a lignocellulosic substrate with indigenous flora for acidogenic fermentation. The impact of microwave pretreatment on VFA production and the effects of pretreatment on microorganisms were investigated. Fermentation tests were conducted using different microwave pretreatment conditions and compared with a conventional thermal treatment and an un-pretreated condition. Results showed no significant enhancement of total metabolite production after manure pretreatment. Moreover, irradiation contribution in inoculum pretreatment showed no major effect on microbial pathways, at least for 200W pretreatment with temperature limited to 40 C.

**Keywords:** pretreatment; fermentation; volatile fatty acids

## Introduction

Volatile Fatty Acids (VFA) are widely used in several industries such as chemicals, pharmaceuticals, food, energy etc. So far, a vast majority of them are produced by the oil industry (Ramos-Suarez *et al*, 2021) but many microorganisms produce these compounds while fermenting substrates (Bhatia and Yang, 2017). This study focuses on VFA production from horse manure. In addition to wheat straw, it contains horse dung which provides a high load of endogenous microorganisms. However, biodegradable polysaccharides of straw are difficult to be used due to the presence of lignin, and a pretreatment step is mostly recommended. The objective of this work was to investigate the effects of microwave and thermal pretreatments to increase horse manure biodegradability and elucidate their impact on dung endogenous microorganisms.

## Material and Methods

Fermentation tests were conducted in quadruplicates using pretreated and raw horse manure with no external inoculum addition. To understand more deeply the impact of pretreatment on dung microorganisms, further tests were carried out with xylose or wheat straw as substrates and pretreated or raw dung as inoculum. Detailed protocol of fermentation tests can be found in Dauptain *et al*, 2020. Microwave pretreatments were performed using Minilabotron, SAIREM with a maximum temperature set up of 90 C using different conditions: 200W 15min; 500W 8min; 1000 W 3min for manure and 200W 15min at max 90 C or 40 C for dung as inoculum in order to differentiate the impacts of temperature and irradiation. In all cases, they were compared with a conventional thermal treatment of 90 C for 15min and an un-pretreated condition.

A severity factor (Overend and Chornet, 1987) was calculated to compare thermal impact between pretreatments:

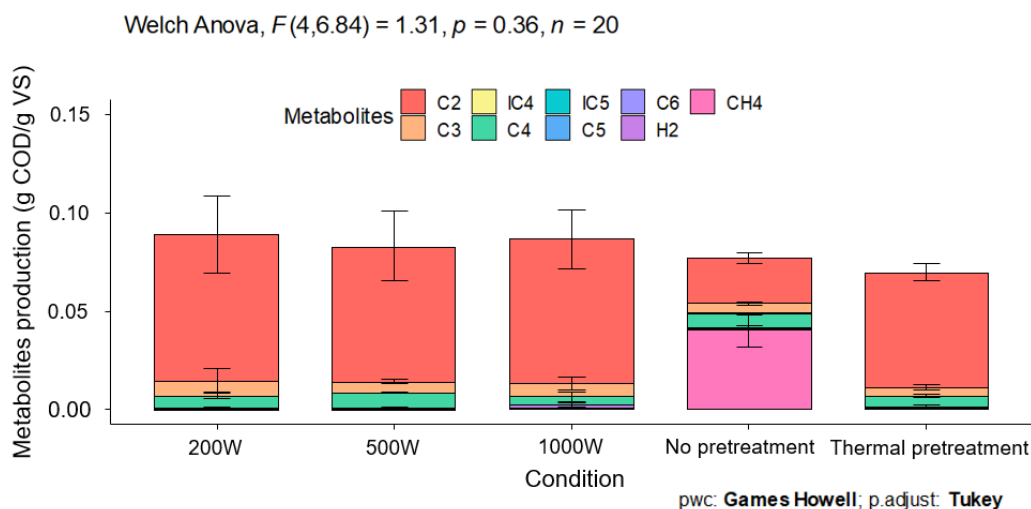
$$R_0 = \int_0^{tf} \exp\left[\frac{(T(t) - 20)}{6.1}\right].dt$$

For microbial analysis, DNA was extracted and sent to Genotoul (Toulouse, France) for PCR & Metabarcoding analysis based on 16S rRNA of the *Bacteria* domain.

## Results and Conclusions

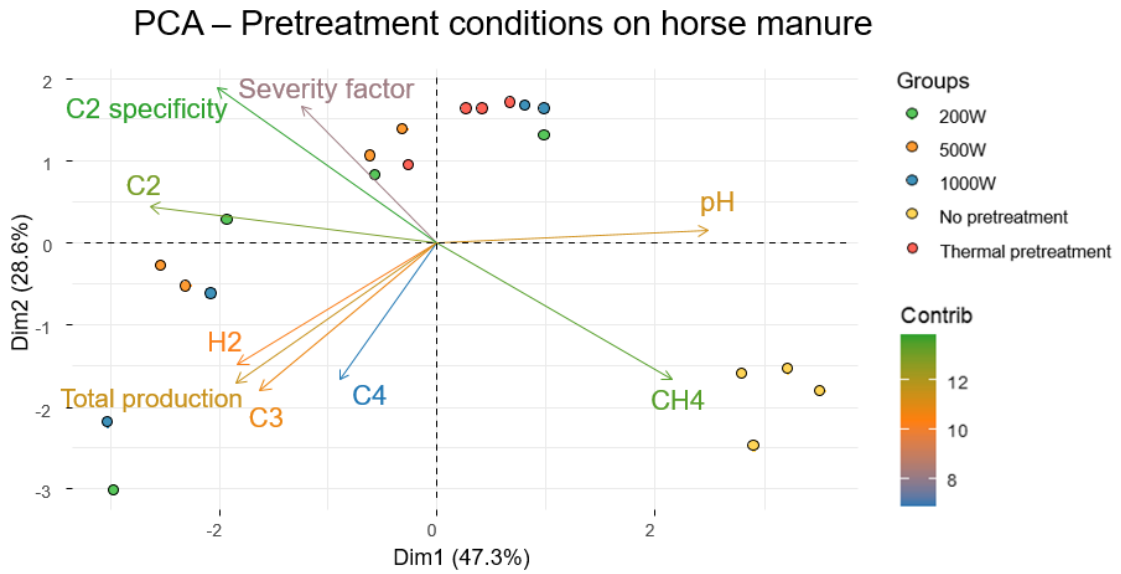
Results of horse manure fermentation (**figure 1.1**) showed no significant enhancement of total metabolite production after pretreatments when compared to untreated control. No impact of microwave pretreatment was also reported on cattle manure methane yield (Jin *et al.*, 2009). Moreover, microwave powers tested here showed no statistical difference. However, a significant difference was observed between microwave and thermal pretreatments with respect to the control in terms of C2 and CH<sub>4</sub> production. This difference regarding methane production confirms the effective inactivation of *Archaea* by both pretreatment types.

The standard deviations of microwave pretreatments showed a higher heterogeneity than other conditions. This could be due to microwave irradiation effect on the endogenous microorganisms, in addition to thermal effect.



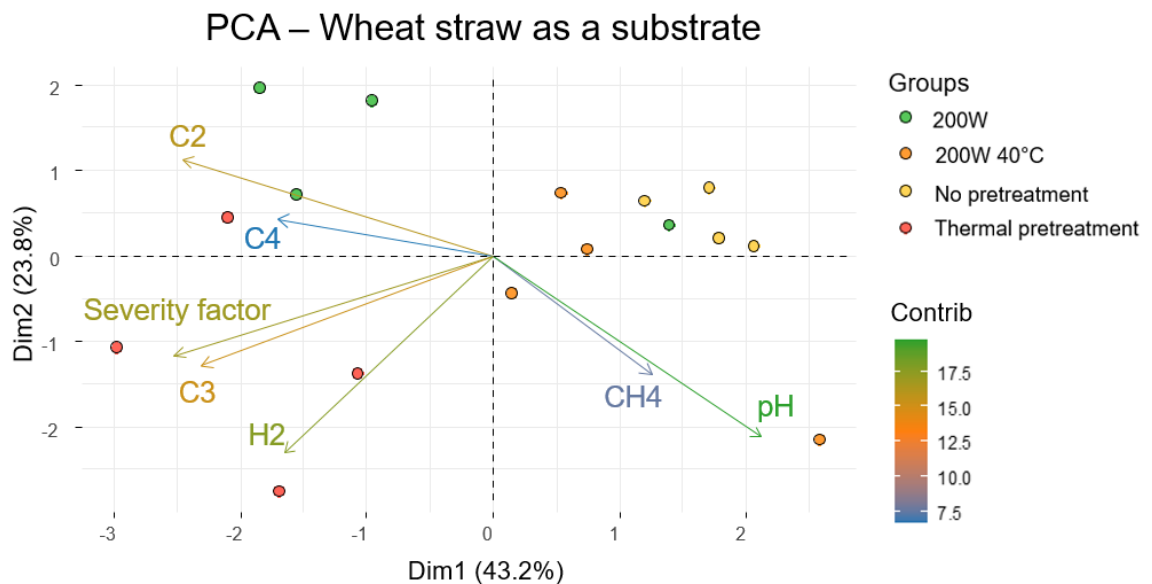
**Figure 1.1** Metabolites production from horse manure after different pretreatments conditions

These conclusions are strengthened by **figure 1.2** showing the PCA with metabolites and experimental data (pH, severity factor). The thermal and microwave pretreatments are relatively close compared to the condition without pretreatment, mainly due to the methane production. A higher heterogeneity linked to microwave pretreatment is also observed. Here, severity factor is inversely correlated with CH<sub>4</sub> production, due to the inactivation of *Archaea* by both pretreatment types. Moreover, this factor is correlated with C2 specificity, corresponding to the share of acetate within total metabolites.



**Figure 1.2** Principal components analysis focusing on metabolites production from horse manure at day seven and parameters linked to pretreatments.

Results of xylose (not shown) and wheat straw (**figure 1.3**) fermentation by pretreated dung show that thermal and 200W micro-wave pretreatment follow the same trend thanks to the thermal effect of both pretreatments. In opposition, 200W 40°C micro-wave and no pretreatment are closer on the projection, suggesting that there is no major microwave irradiation effect.



**Figure 1.3** Principal components analysis focusing on metabolites and pretreatment parameters production from wheat straw inoculated with pretreated or un-pretreated horse dung at day seven.

In addition, a microbial analysis shows some differences between microwave pretreatment (200W; 500W and 1000W) and un-pretreated condition on *Bacteria* domain (not shown).

Results of this study showed no significant enhancement of total metabolite production but a higher C2 specificity for both thermal and micro-wave manure pretreatments. Moreover, irradiation contribution in inoculum pretreatment showed no major effect on microbial pathways at least between 200W 40°C and un-pretreated conditions, which will be verified by metabarcoding analysis.

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