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Impact of interactions between substrates on the methane production of codigestion systems: an experimental approach.

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Abstract
To design co-digestion systems, most of the designers consider that the methane production will be equal to 80% of the weighted sum of the biochemichal methanogenic potentials (BMPs) of substrates. However, interactions between substrates can influence the methane production and are generally not taken into account. The objectives of this paper are: (1) the identification of the effect of interactions between substrates on the methane production of co-digestion systems and (2) the identification of the control factors of these phenomena. For this purpose, BMP tests were processed with 30 binary mixtures of substrates and the obtained results were compared with the theoretical BMP values of each mixture obtained by the weighted sum of the BMP value of each substrate in monodigestion. No antagonism was identified. However, synergies were identified for co-digestion tests including porcine slurry and bovine slurry. A statistic analysis highlights that these interaction phenomena are correlated with the biochemical characteristics of co-substrates which can induce enzymatic activation phenomena.

Keywords
Anaerobic digestion, co-digestion, synergy, antagonism, interaction

INTRODUCTION

Anaerobic digestion of substrates like livestock slurries or sludges from wastewater treatment plants allows the reduction of the environmental impact of their management. However, the methanogenic potentials of these substrates are generally low. Hence, to optimize the economical balance of anaerobic digestion plants, co-digestion with highly methanogenic co-substrates is applied. Hence, a mixture of wastes from agriculture, agro-industries and municipalities can be digested in the same co-digestion reactor.

To design co-digestion systems, most of the designers consider that the methane production will be equal to 80% of the weighted sum of Biochemichal Methanogenic Potentials (BMPs) of each substrate. However, some authors have demonstrated with specific mixture of substrates that the BMP of the mixture can be different of the weighted sum of the BMPs of the substrates (Labatut et al., 2011; Mshandete et al., 2004): it can be superior (synergism) or inferior (antagonism).

Different hypotheses can explain these interactions : (i) the modification of the nutrient equilibrium due to the mixture of substrates which can enhance anaerobic biomass growth (Callaghan et al., 2002) ; (ii) the dilution of inhibitors due to the addition of co-substrates ; (iii) the bioaugmentation effects due to the addition of a specific substrate (bovine rumen content which contain cellulolytic enzymes for example) (Ganesh et al., 2011) ; (iv) the modification of the equilibrium between the biochemical fractions of biodegradable organic matter due to the mixture which can induce enzymatic activation (Ponsa et al. (2011) ; (v) the modification of the physical and chemical properties of the reaction medium. Pereira et al. (2005) have demonstrated that this phenomenon is responsible of antagonisms observed for co-digestion with greasy substrates (sequestration of biomass aggregates by hydrophobic capsules). The two first phenomena cannot explain interactions between substrates in BMP tests where conditions for anaerobic degradation are theoretically optimal (no nutrient deficiency, no inhibition).

Hence, if synergisms and antagonisms are often mentioned to explain specific experimental results,

only some references have investigated these phenomena. Then, scientific results are needed (i) to identify if these interactions are significant and to quantify their effect on the BMP value of a mixture of substrates and (ii) to understand the control factors of these phenomena.

This paper focuses on the identification of synergies and antagonisms in 30 binary co-digestion BMP tests. To understand the reason of the interactions between substrates observed, nutrient and organic matter analysis were performed and analyzed regarding to BMP results.

MATERIALS & METHODS

Substrates

Two kinds of substrates were used: (i) the “major substrates”: substrates with low BMP value which constituted the major volume of substrate introduced in the digester but a slight proportion of the produced methane; (ii) the “co-substrates”: substrates with high BMP value, which constitutes a slight proportion of the volume introduced in the digester but which are responsible of the major part of the methane produced. 3 current “major substrates” are considered: porcine slurry, bovine slurry and waste activated sludge. 10 current co-substrates from agro-industry are considered: 3 wastes from a bovine slaughterhouse (bovine blood, greasy sludge from flotation process, rumen content), 3 wastes from a porcine slaughterhouse (screening refusals, greasy sludge from flotation process, waste activated sludge), 2 wastes from vegetable industry (carrot pulp, onion pulp), 2 wastes from meat industry (meat wastes, greasy sludge from flotation process).

BMP tests

Two series of BMP tests were performed. The first was processed in mono-digestion for each “major substrate” and each “co-substrate”. The second was processed in co-digestion: each “major substrate” was tested with each “co-substrate”. The “co-substrate” ratio in the mixture was calculated to represent 30% of the biodegradable COD of the mixture. The BMP of each substrate or mixture of substrates was determined using a previously described method (Vedrenne et al., 2008). Samples were incubated at 38 °C for 40 days. The substrate/inoculum ratio was $1gCOD_{biodegradable\ substrate}/gCOD_{inoculum}$. For all the tests, the inoculum was sampled in an anaerobic digester processing pig slurry and horse feed.

Statistic analysis of the results

A statistical analysis of the results was performed with the Statgraphics Centurion XVI© software. The experimental BMP value of each mixture of substrates was compared with its theoretical BMP obtained with the weighted sum of the BMP value of each substrate in mono-digestion. An “interaction” variable was defined as the difference between the experimental BMP value of the mixture and the theoretical BMP value of the mixture. To evaluate if interactions are significant, t tests were performed (the difference is significant if the probability P is inferior to 0.05).
Correspondence analysis and principal component analysis (PCA) were performed to identify correlations between observed interactions and co-substrate properties or experimental conditions.

RESULTS AND DISCUSSION

For each mixture of “major substrate” and “co-substrate”, the experimental BMP value is compared with the theoretical BMP value obtained with the weighted sum of the BMP value of each substrate in mono-digestion. The obtained results are presented in Figure 1. Whatever the mixture, no significant antagonism between a “major substrate” and a “co-substrate” is observed. However,

many synergies are highlighted. Hence, for mixtures with porcine slurry as “major substrate”, a significant excess of methane production in the BMP value of the mixture is observed with 4 “co-substrates”: carrot pulps (+25%), screening refusal (+16%), rumen content (+16%) and meet wastes (+15%). For mixtures with bovine slurry as “major substrate”, a significant excess of methane in the BMP value of the mixture is observed with only 2 “co-substrates”: bovine blood (+12%) and greasy sludge from a bovine slaughterhouse (+12%). For mixtures with waste activated sludge as “major substrate”, no significant synergy is highlighted.

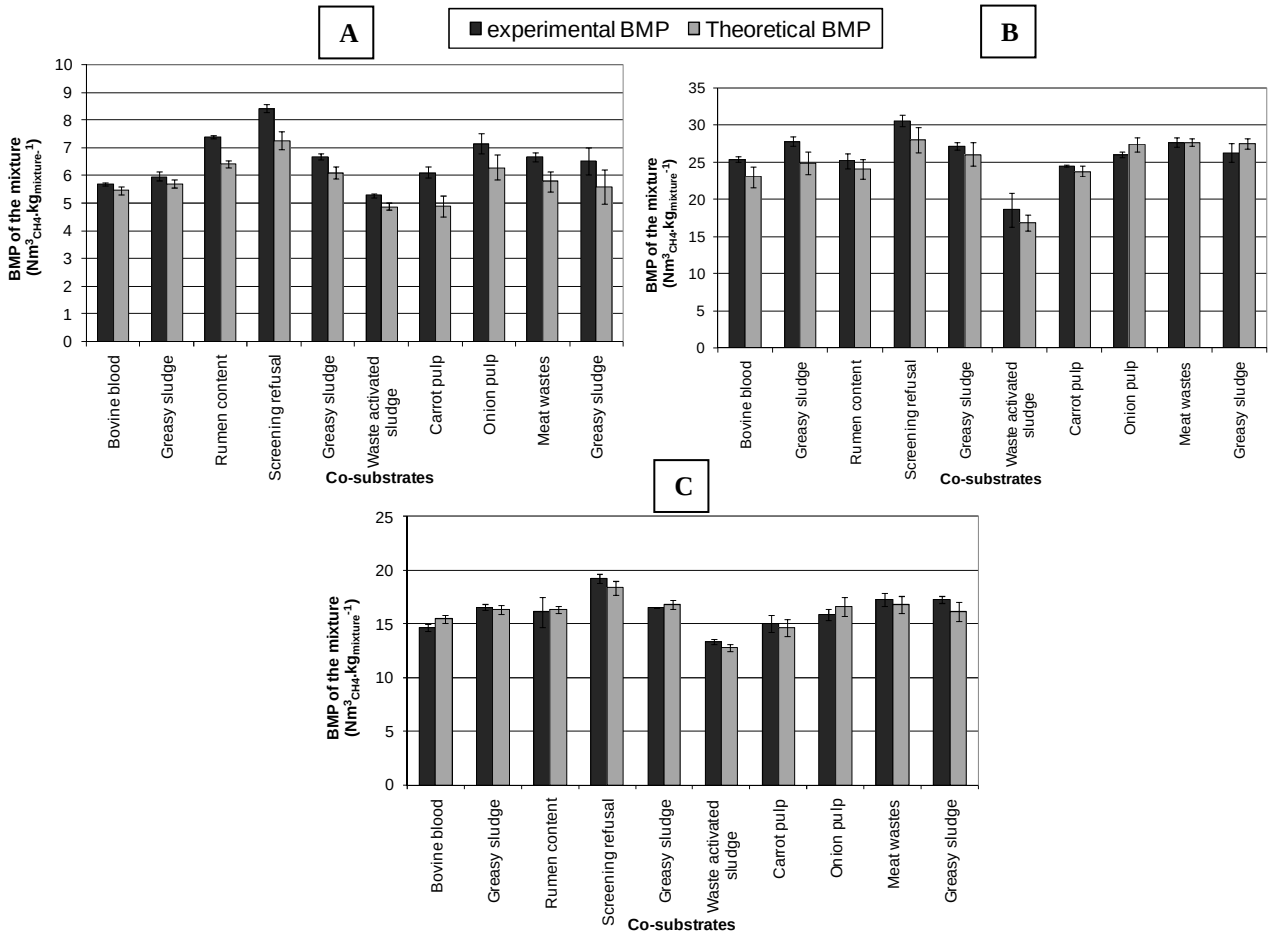

Figure 1. Comparison of the experimental BMP value of each co-digestion system with its theoretical BMP value obtained by the weighted sum of the BMP of each constituent substrate in mono-digestion (A: Co-digestion systems with porcine slurry as “major substrate” ; B: Co-digestion systems with bovine slurry as “major substrate”; C: Co-digestion systems with waste activated sludge as “major substrate”).

A statistic analysis is performed to identify correlation between the excess of methane production for co-digestion BMP test (“interaction” variable) and parameters related to the mixture or “co-substrate” properties. First, the correspondence analysis highlights no significant correlation between the “interaction” variable and the C/N ratio of each mixture. Nevertheless, correlations can be highlighted between the “interaction” variable and the biochemical fractionation (protein, lipids and carbohydrates) of “cosubstrates”. Hence, for the 10 mixtures with porcine slurry as “major substrates”, the PCA highlights a positive correlation between the “interaction” variable and the carbohydrate content of the “co-substrates”. In addition, for the 10 mixtures with bovine slurry as “major substrate”, the PCA highlights a positive correlation between the “interaction” variable with the protein content of the “co-substrates”. However, whatever the “major substrate”, no correlation between the “interaction” variable and Van Soest fractionation of the organic matter of the “co-

substrates" could be identified.

Another question consists on the origin of the excess of methane production in co-digestion tests. Firstly, these synergies can be due to a better degradation of the "major substrate" or of the "co-substrate". However, results show that the biodegradability (calculated from COD analysis and the BMP value) of many "co-substrates" (carrot pulp, screening refusals, etc) is closed to 100%. For example, the biodegradability of carrot pulps is equal to 80% on COD basis. Hence, if the observed synergy for its co-digestion with porcine slurry were only due to a better degradation of the "co-substrate", its biodegradability would be equal to 131%. In conclusion, the major part of the excess of methane in co-digestion tests is due to a better degradation of the "major substrate".

The analysis of the characterization results of each substrate show that the porcine slurry is slightly biodegradable in mono-digestion (biodegradability of 26% on a COD basis) and contains an important fraction of non-biodegradable carbohydrates. Hence, observed synergies can be due to enzymatic activation phenomena. Hence, the addition of "co-substrates" with a high content of carbohydrates (for instance, the organic matter of carrot pulp contains 88% of carbohydrates) can induce an important production of enzymes allowing the hydrolysis of carbohydrates. This increase of the concentration in hydrolytic enzyme in the reaction media can allow the degradation of very slowly biodegradable of carbohydrates, which are considered as non-biodegradable in mono-digestion BMP tests.

CONCLUSION

In this paper, the effect of interactions between substrates (synergies or antagonisms) on the BMP value of 30 mixtures of substrates was investigated. First, no antagonism was indentified in co-digestion BMP tests. However, significant synergies were highlighted for co-digestion BMP tests including porcine slurry and bovine slurry as "major substrates". The maximal synergy was observed for the co-digestion of porcine slurry with carrot pulps (excess of methane production of 25%). For co-digestion tests including porcine slurry as "major substrate", a statistic analysis of the results highlights that synergies are mainly observed for co-substrates containing a high proportion of carbohydrates. However, for co-digestion tests including bovine slurry as "major substrate", synergies are mainly observed for co-subtrates containing a high proportion of proteins. These interactions between substrates can be due to enzymatic activation phenomena.

To complete these results, co-digestion experiments in continuous reactors are needed to identify the effect of these phenomena on the methane production of anaerobic co-digestion plants.

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