Determination of lipid fraction from organic wastes using Nuclear Magnetic Resonance (NMR): Comparison to the soxhlet method

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Abstract
During this study, nuclear magnetic resonance (NMR) was compared to the soxhlet extraction for determination of the lipid content of organic waste usually used in anaerobic digestion. Thus, 48 different wastes were analyzed using both methods and three replicates were performed for each. The correlation between Soxhlet methods and NMR was 0.90 and the regression slope was equal to 1.02. A better precision was obtained for NMR method with a coefficient of variation of 5%, while for the Soxhlet method, this coefficient is 14%. In contrast, the NMR method gave overall values below the reference method soxhlet. This difference can be explained by the advanced state of hydrolysis of fats where a large amount of volatile fatty acids, saturated molecules small chains linked would be present, and the degree of saturation of fatty acids long chains. NMR method seems suited to the analysis of lipids with better repetition and has the advantage of not using any solvent, be fast and non-destructive. However, the determination of some lipid matrices seems undervalued using the conditions established by the NMR protocol. Finally, this study also established data on lipid content of organic waste.

Keywords
Waste; anaerobic digestion; lipids; soxhlet; NMR

INTRODUCTION

From several years, the production of organic waste is steadily increasing due to farming practices, industrialization, treatment processes and more intense waste collection. To answer the environmental challenges of organic waste management, biological processes of treatment and recovery are implemented, particularly anaerobic digestion. Livestock manure are relevant substrates for such treatment due to their macro and micronutrient content necessary for the development of micro-organisms and also due to their buffering capacity allowing to stabilize the process. However, the low dry matter content of such substrates (<10% DM) and the limited biodegradability of their organic matter, generate a methane potential quite low relative to their volume. Consequently, the economic rentability of anaerobic digestion facilities treating livestock manure requires the addition of additional substrates as fats with higher methane potential. However, the addition of fat can lead, sometimes, to inhibitions in the digestion process [1]. In fact, the hydrolysis of lipids, which can occur quickly inside digester, generates a production of long chain fatty acids (LCFA). If these LCFA are not consumed simultaneously, they could be adsorbed to bacterial membranes limiting the exchanges between the micro-organisms and external solution, and thus limiting significantly their efficiency [2]. Thus, the knowledge of the lipid content of waste appears essential both to ensure a good production of methane and to prevent the risk of inhibition. For that, literature describes many methods concerning the determination of lipids. The most used is the soxhlet method consisting in extraction of lipids using a solvent and by heating [3]. Such a method has some disadvantages as the use of solvent but also the time of several hours required for

extraction and evaporation after extraction. In this context, an alternative technique for the analysis of lipids like nuclear magnetic resonance (NMR) is interesting. The principle of NMR is based on the measurement of the resonance frequency of the atomic nuclei, under the influence of a magnetic field. NMR was compared with conventional method for analysis of the lipid content of dried fish muscle and the methods correlated very well [4]. In the field of waste, NMR has been used for the characterization of organic matter, especially humic acids, and changes of the material during composting [5], but no reference appears available on the analysis of lipids. The objective of this work was to evaluate the NMR as a technique for analysis of lipids in the organic waste. NMR was compared to Soxhlet gravimetric method commonly used and fifty wastes of different origins were analyzed using both methods.

MATERIAL AND METHODS

Substrates

The substrates used for this study correspond mainly to waste and organic residues used in the anaerobic digestion sectors in France [6]. To cover a wide range of substrates characterized by either their origin or their expected rate of lipid, fifty two substrates were studied from different fields: manure from pigs and cattle, waste from pig and cattle slaughterhouses, viscera of fish, fats from flotation pretreatment of wastewater (domestic or industrial). Additionally, wastes from supermarket and from canteen were also added. Samples of vegetable origin have also been selected namely seaweeds of beach, green waste and lawn mowing. Large diversity of substrates studied allows to compare both methods (Soxhlet and NMR) with very different fat contents. Besides, each method has been verified using controls of known concentration, 99% oleic acid (CAS 112801, Fluka) and 99% commercial triolein (CAS 122327, Fluka). Furthermore, in order to bring the best features of our substrates, two types of mixture containing casein (CAS 9000719, VWR) starch (CAS 9055258, VWR) and trioleic acid for one and oleic acid for another (25:25:50) were analyzed.

Lipids analysis

Sample preparation

Analysis of the lipid content of the substrates by both methods (soxhlet and NMR) requires a drying allowingtoprovide, for NMR, a signal depending only on the lipid, and avoiding, for the soxhlet method, the appearance of two phases. Thus, samples were dried at 105 ° C. Then dried products were manually ground. Triplicate analysis of the lipid content was first carried out by NMR, then using soxhlet method.

NMR analyzes

NMR measurements were performed with a low field operating at a frequency of 10 MHz using a Brucker spectrometer Minispec MQ 10. Approximately 1 to 1.5 g of dried sample was placed in a 30mm-diameter NMR tube with an approximate height of 10±2 mm. The calibration equation of the NMR apparatus was calculated with four reference NMR tubes filled with different heights of colza oil between 1 and 10 mm. (CAS 8002139). For each sample and for each reference tube, the free induction decay (FID) was measured in about 45s using a relaxation delay of 3s and nine scan accumulations. The FID relaxation curves consisted of two distinct parts: the first component which disappeared in less than 70 s, attributed to solid protons related to dry proteins in samples, and the second component with a longer relaxation time, related to lipids in an amorphous state at the measurement temperature. Lipid content was calculated on basis of a simple mono-linear calibration equation [4].

Soxhlet analyzes

Approximately 5 g of dry matter corresponding to the quantity introduced into a glass tube for NMR analysis, are put down in a cellulose cartridge of extraction previously weighed, then placed in a Soxhlet extractor cylinder (150mL) [3]. In the distillation flask whose weight is known, 200mL solvent hexane/isopropanol (3/2) are heated for 5 hours [7]. At the end of the extraction, solvent contained in the flask is evaporated under vacuum using a rotary evaporator Büchi RE111 461. Then the remaining content of the flask is dried at 105 ° C until get a constant mass and, finally the flask is weighed. The content is expressed in material extracted with hexane (MEH) as a percentage of dry matter.

RESULTS AND DISCUSSION

Lipid contents

Lipid contents (MEH) from studied wastes and determined by the Soxhlet method (Figure 1) ranged from 0.1% to 94.1%. As expected, the five studied manure have low and quite similar contents close to 8-9%. In contrast, the wastes from slaughterhouses varied from 0.6% (bovine blood) to 83.5%. High values were obtained for fish viscera (79.0%) and fat from flotation process (83.5%). These values are coherent with the origin of waste or specific waste treatment. The eighteen fats issued from processing industries also have very variable contents ranging from 1.3% (fats from vegetable industry) to 94.1% (fat flotation) with a relatively high average of 43.6%. Average rates of lipids for supermarket wastes and wastes from municipality are respectively 34.6% and 12.1%. The average lipid content of seaweeds and lawn mowing are very low while fats from water-treatment are the highest in this category. Besides, the contents of the four substances control, oleic acid and trioléic were in accordance with expected values. Lipid contents obtained over a wide range and can lead to better identify the substrates of interest.

Comparison of methods

The lipid content determined by NMR varied from 1.8% to 89.7% for the 48 substrates studied with an average of 25.3 ± 30.6%. Figure 1 compares all values between the NMR method and the Soxhlet method. The average coefficient of variation was calculated and is equal to 14% for Soxhlet method and 5% for the NMR, indicating greater accuracy for NMR. Moreover, the correlation between both methods was very correct with 102% recovery and a correlation of 0.90. However, some values corresponding mainly to waste from manufacturing industries are quite different between both methods. Overall, the NMR method gave values lower than those obtained by Soxhlet. However, when the lipid content is below 27%, the NMR method is superior to the soxhlet method. Similarly, a slight tendency to higher values in NMR was observed for the reference samples in contrast to the general trend. The value of the limit of quantification of the method sohxlet was studied and is worth 2%, which explains the trend reversal for very low values. When the value from NMR method is lower than the value from Soxhlet method, the underestimation may be influenced by non-selective Soxhlet extraction, a heating temperature insufficient due to the amorphous state of lipids or by the degree of saturation of the fat in different products for NMR. Indeed, if the waste fraction contains saturated fats in large quantities, then the determination of lipids by NMR is intimately related to the state of the lipids. Half of the concentrations lower than 20% gives a deviation greater than 40%. The hexane extraction may have been less effective when the fat is linked to complex molecules.

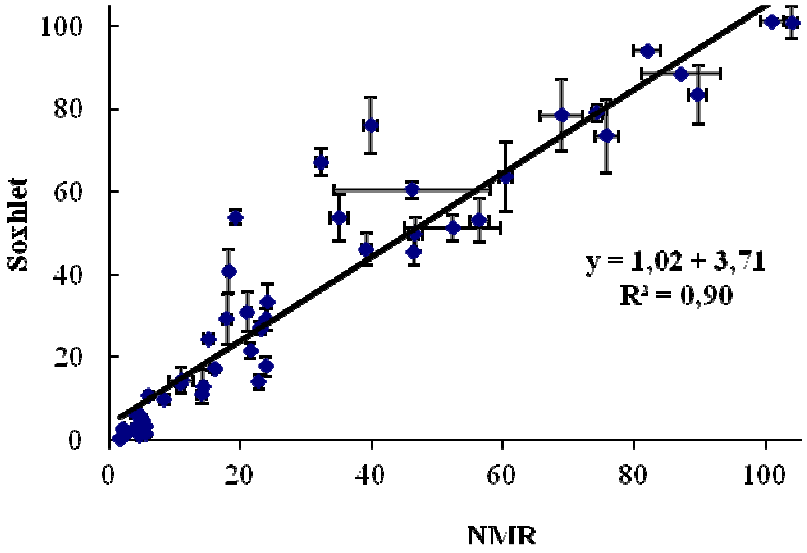

Figure 1: Comparison of the lipid contents obtained using NMR and Soxhlet methods for 52 substrates (expressed in % of DM)

CONCLUSION

In this work, all kind of substrates used in anaerobic digestion was analyzed for lipid content determination using the Soxlhet and NMR methods, giving a wide range ranging from 0.1% to 94%. The comparative analysis between both methods showed a good correlation with a better accuracy for NMR method. On the one hand, the condition of hydrolysis of fats related to storage and the degree of saturation of long-chain fatty acids could influence the lipid measurement by NMR. On the other hand, according to the results obtained, analysis of lipids by NMR seems interesting and promising because the method does not use solvent and is not destructive. In spite of the necessity of drying samples, the NMR technique is very fast (<1 min) compared to soxhet requiring several hours.

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