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# Predictive regression models for biochemical methane potential tests of biomass samples: Pitfalls and challenges of laboratory measurements

Raposo, F.[1,*], Borja, R.[1], Ibelli-Bianco, C.[2]

1 Instituto de la Grasa-Consejo Superior de Investigaciones Científicas (IG-CSIC). Campus Universidad Pablo de Olavide, Building 46, Carretera de Utrera km 1, 41013 Seville, Spain.

2 Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo. Avenida Trabalhador Sãocarlense, 400, Centro – São Carlos - SP - CEP: 13566-590 - Brazil

[*] To whom correspondence should be addressed:
(Tel.: +34 954611550; E-mail: fraposo@cica.es)

## Abstract

This paper is a compilation of some experimental results published in peer-reviewed articles dealing with predictive regression models between biochemical methane potential tests and different chemical parameters characterizing the organic content of biomass samples. Results reviewed were focused on laboratory measurements with the main objective of bringing together the existing experience to evaluate pitfalls and challenges that could be generalized for future research using this kind of substrates. Firstly, BMP test measurements were briefly described for experimental approaches according to different factors such as inoculum, physical and chemical experimental conditions, inoculum to substrate ratio and gas measurement systems. A lot of information necessary when reporting BMP studies was not included in the description of most articles. It is also unexpectedly the lack of positive control tests as a way to check the reliability of the experimental results obtained. As consequence, BMP test results from different laboratories are normally inconsistent and irreproducible. Secondly, chemical parameters analysed in experimental research works such as moisture/dry matter, total chemical oxygen demand, carbohydrates, lipids, proteins and lignin were also reported in a comparative way. In fact, 70% of analytical determinations were covered in some degree, but the presence of a correct reference description was only occasional. Finally, general regression models were summarized. However, the development of one overall model that applies to all kind of samples is difficult to achieve. In order to be reliable and widely applicable, predictive regression models for methane production of biomass samples should be based on accurate laboratory measurements.

**Keywords:** Anaerobic digestion; Biochemical methane potential; Biomass; Chemical analysis; Laboratory measurements; Methane yield; Predictive regression models

**List of Abbreviations:** ***AD***-Anaerobic Digestion; ***ADF***-Acid Detergent Fibre; ***ADL***-Acid Detergent Lignin; ***AnBD***-Anaerobic Biodegradability; ***APHA-AWWA-WPCF***-American Public Health Association, American Water Works Association and Water Pollution Control Federation; ***BOD***-Biological Oxygen Demand; ***BMP***-Biochemical Methane Potential; ***C***-Carbon; ***CARBs***-Carbohydrates; ***$CH_4$***-methane gas; ***Cl***-Chloride; ***COD***-Chemical Oxygen Demand; ***DFS***-Detergent Fibre System; ***DM***- Dry Matter; ***FC***-Fixed carbon; ***HBu***-Butyric Acid; ***HPLC***-High Performance Liquid Chromatography; ***ISR***-Inoculum to Substrate Ratio; ***KL***-Klason Lignin; ***LCFAs***-Long-Chain Fatty Acids; ***LIG***-Lignin; ***LIPs***-Lipids; ***MW***-Microwave; ***MY***-Methane Yield; ***N***-Nitrogen; ***$NaHCO_3$***-sodium bicarbonate; ***NaOH***-sodium hydroxide; ***NDF***-Neutral Detergent Fibre; ***NDS***-Neutral Detergent Soluble; ***NfE***-Nitrogen free Extracts; ***$N_2$-$CO_2$***-nitrogen-carbon dioxide gases; ***NIR spectroscopy***-Near Infrared spectroscopy; ***NMR spectroscopy***-Nuclear

Magnetic Resonance spectroscopy; ***NREL***-National Renewable Energy Laboratory; ***NtPCF***-Nitrogen to Protein Conversion Factor; ***O***-Oxygen; ***OFMSW***-Organic Fraction of Municipal Solid Waste; ***PROTs***-Proteins; ***S***-Sulphur; ***SAH***-Sulphuric Acid Hydrolysis; ***TAN***-Total Ammonia Nitrogen; ***TDN***-Total Dumas Nitrogen; ***TKN***-Total Kjeldahl Nitrogen; ***TS***-Total Solids; ***USDA***-United States Department of Agriculture; ***VFAs***- Volatile Fatty Acids; ***VM***-Volatile Matter; ***VS***-Volatile Solids; ***3D***-Three-Dimensional; ***w/w***-weight to weight.

## CONTENTS

## 1. Introduction

Anaerobic digestion (AD) plants represent a very successful and promising option for the energy recovery of biomass. Estimation of the potential of particular biomass substrates to produce methane is essential for successful plant operation and optimization. Many biomass samples have been evaluated as potential substrates for renewable energy through anaerobic reactors [1-2]. Obtaining a high methane yield is always desirable but to achieve a maximum value it is important to select the appropriate substrates to be managed through full-scale AD plants [3]. Therefore, it is mandatory to carry out an economically feasible biochemical procedure for some substrates that could have production cost [4].

Anaerobic biodegradability (AnBD) is normally evaluated in the laboratory through biochemical methane potential (BMP) measurements, which are batch experiments where defined amounts of seeding sludge (inoculum), substrate and water are mixed and incubated for a certain period of time [5]. However, it is important to note that AnBD results cannot be directly related to BMP results. This is because AnBD is related to the amount of organic matter degraded by anaerobic microorganisms to produce biogas. Similarly, BMP is the methane yield of the organic substrate that depends on the amount of organic matter removed, but more specifically on its biochemical nature (in terms of carbohydrate, fat, fibre and protein contents), which varies widely [6]. In addition, the lignocellulosic matrix of the substrate can also affect the methane potential of biomass samples. Then, the chemical composition of biomass samples and the three dimensional structure are both key factors for the methane potential obtained [7].

Despite the important information provided by BMP test measurements, there are two main problems with these assays. Firstly, bioassays are relatively difficult to conduct under reliable and reproducible experimental conditions. BMP results can be influenced by several factors such as inoculum, operating conditions and biogas/methane measurement system [8]. Secondly, these bioassays are time consuming [9]. Thus, BMP results cannot be used for evaluating processes in the short-term to inform decisions in industrial plants and consulting companies [10]. Unlike BMP, analytical methods for biomass characterization can be applied in a cost-effective way in the short time [11]. Therefore, linking “less laborious” chemical measurements with BMP results can be useful to establish a firm decision tool in order to design and apply strategies that can be used for process optimization in full-scale applications. To date, different chemical parameters have been used to develop regression models for predicting the specific methane potential of biomass samples [12]. Since these methods only depend on the functional behaviour and typically do

not respect fundamental biochemical dependencies, they are only valid for specific substrates [13]. Not surprisingly, attempts to validate these models using a wide spectrum of types substrates were less successful. In addition, it appears that methane production cannot be predicted by a single chemical parameter. Alternatively, a combination of many variables might be necessary to allow a better understanding of the interactions among them [14].

The main objective of this study was to compile previous research works published in the literature in which diverse predictive regression models were proposed for different biomass samples [15–40]. These models were assessed by focusing on laboratory measurements such as the factors affecting BMP tests and the specific analytical methods reported for the different chemical measurements. Finally, predictive regression models were compared in relation to their suitability for overall applications.

## 2. Summary of reviewed articles

A detailed bibliographic study of selected articles (n=26) is included in **appendix A.** The characteristics of the articles reviewed can be summarized as follows: i) The articles have been published during a period of 12 years (2007-2019), with the highest number of publications (n=4) in 2018. That means that currently there is a lot of interest in this research topic. ii) The information was published in 11 different journals, with nearly 40% of articles from Bioresource Technology journal. iii) The affiliation of the authors of these articles include 14 countries, with Denmark (n=5), Germany (n=7) and France (n=8) in the top positions.

## 3. Laboratory measurements for biomass samples in AD research field

There are different laboratory measurement methods relating to biomass samples required to design and operate AD plants for efficient biogas production. These methods are used in order to describe the quality of the initial substrates as well as to predict reactor performance. The methods can be classified into two main groups: biological/biochemical and chemical measurements. The principal laboratory parameters that influence the biogas production for biomass samples are carbohydrates, lipids and proteins as well as chemical oxygen demand (COD), lignin and BMP tests. Certain parameters are very simple to monitor, therefore their determination take very little time and they have a relatively low cost. On the contrary, other analyses are more complex and require extensive time to obtain the experimental results. This latter group of tests usually requires specific equipment, higher experimental costs, and longer periods of time than conventional analyses.

Despite the high number of laboratories involved in biogas research, laboratory measurements are faced with a multitude of pitfalls and challenges. These are related to the very special characteristics (heterogeneity and matrix/nature) of the substrates and the specific techniques for the whole spectrum of biogas research. On the other hand, since the equipment and experimental procedures have not been completely standardized yet, comparison of results from different laboratories is normally not easy to perform. In the following, the different laboratory measurements will be described.

## 4. BMP tests: Factors influencing measurements

BMP is a powerful and simple technique established to determine the maximum methane production from the organic matter present in pure or mixed substrates such as sludges, wastes and bio-products by AD. This biological determination has proved to be quite a simple method to obtain the extent and rate of organic matter transformation to methane by anaerobic digestion processes. The test is relatively simple to carry out. To start, a substrate is weighed and mixed with a fresh anaerobic inoculum (taken from an active reactor) in a vessel. The mixture is incubated at constant temperature. The methane produced during biodegradation is measured and the methane potential calculated after subtracting the methane production from blank reactors that contain only inoculum. Therefore, the literature includes numerous BMP research studies. However, it is important to note that this test often suffers from a lack of standardization in experimental procedures, parameters and data reporting [5] Consequently, BMP test results carried out by different researchers lead to inconsistent and irreproducible results [8]. Many BMP procedures have been proposed to serve as guidelines at national and international levels. In the reviewed articles, 5 documents (19%) did not include any referenced methodology. The most popular reference methods cited were two German guidelines, VDI 4630 [41] and DIN 38414 [42] and one harmonization paper published by Angelidaki and co-workers [43]. The suggested harmonized paper recently published by Holliger and co-workers [44] was cited once. To carry out an accurate BMP test it is important to consider all the factors required to develop a reliable determination. In this review, different factors influencing BMP results are studied, but only briefly, because previous articles were devoted to study the common factors affecting BMP test measurements [5, 45]. In addition, two recent published articles reviewed in detail previous research studies relating to BMP measurements [46, 47]. As summary, 14 factors should be included in the description of any BMP test measurement belonging to inoculum, physical and chemical experimental conditions, inoculum to substrate ratio and gas measurement systems characteristics. In addition, the quality control of any BMP test measurement should be demonstrated by using a positive control substrate with a known theoretical methane yield [5, 41, 43, 44]. The lack of such information in a research study could create limited comparability in the experimental results reported.

### 4.1. Inoculum

Inoculum is one of the most important BMP factors because it supplies the microorganisms responsible for conducting the AD process. This means that variation in the microbial composition, including diverse trophic groups of symbiotic

microorganisms responsible for the sequential fermentation stages, could affect BMP. However, previous reported results remain inconclusive on inoculum effects, probably due to the associated effect of the biodegradability of substrates [48]. There is a general agreement that anaerobic sludge should be as fresh as possible to avoid a decrease in microbial activity [49]. In any case, the main characteristics of any inoculum that should be considered for BMP assays are origin/source, solid contents, start-up concentration and pre-incubation. These factors are described briefly in the following.

4.1.1. Origin/source

Ideal inoculum should be adapted to the substrate to be degraded but this situation is not very common. The acclimation of sludge to fibrous substrate was proposed as a way to increase the methane production of paragrass samples [50]. Frequently, the inoculum source for BMP tests is variable because referenced guidelines do not suggest the use of special types of digesters for sampling selection. Neither are there suggestions to the specific nature of inoculum, as flocculent or granular sludge. Therefore, inoculum can be collected from a wide range of anaerobic reactors, and is most commonly taken from biogas plants (fed with agricultural or bio-wastes) or municipal wastewater treatment plants. The use of inoculum from pilot plants or small laboratory reactors is also popular.

4.1.2. Solid contents

The inoculum used for BMP tests should be characterized in terms of total solids (TS) and volatile solids (VS). Normally, the content of VS is assumed to be related to the content of active microbial biomass, and, is needed to calculate the quantity of inoculum to be used.

4.1.3. Concentration at the start-up of experiment

The VS concentration of inoculum is a very important factor to be considered individually and when planning the inoculum to substrate ratio (ISR) of the BMP measurements. Although more research has been considered necessary to study the impact of inoculum (and substrate) at fixed ISR, preliminary results showed that dilution of the inoculum or substrate may underestimate the methane yields [51].

4.1.4. Pre-incubation or pre-digesting

Harmonization guidelines suggested the pre-incubation at the same BMP temperature that the inoculum will operate but at different intervals of time, ranging from 2-5 days, 5-7 days and up to 7 days [41, 43, 44]. This procedure has been advised as a way to reduce the gas volume values of blank reactors and improve the accuracy of net methane production. However, the reduction of residual methane production should not be necessary to obtain accurate methane yields.

4.2. Physical operational conditions

Information about the following physical factors should be included in BMP reports.

4.2.1. Reactor capacity: Both total and working volumes should be considered. The total volume defines the reactor headspace as the difference in the working volume. Headspace volume can vary widely depending on the gas measurement system selected. The working volume of the reactor is a key factor to consider the total amount

of substrate (and inoculum) added to the system and also to calculate its initial concentration. In addition, BMP tests are usually carried out in reactors where volume depends on the features of the substrate. Smaller volumes should be used for homogeneous substrates while large volumes are more suitable for substrates of heterogeneous nature. In any case, smaller volumes are considered as less efficient than larger ones [52]

4.2.2. Temperature

The temperature selected for BMP measurements is a very important factor because it is well known that AD process is temperature dependent. Also, the reactor temperature affects the biogas transfer rates. Therefore, it should be necessary to avoid severe changes in the operational temperature selected during a trial. The majority of anaerobic reactors are usually operated at either mesophilic or thermophilic ranges of temperature.

4.2.3. Stirring

Mixing ensures the transfer of substrate to microorganisms. Also, mixing is important to provide uniform temperature in the reactors and for releasing the biogas into the headspace. Different mixing approaches can be found in the literature: no mixing, shaking manually and automatic mixing. In addition, no mixing or manually shaking once per day may be sufficient for reactors treating diluted or easily biodegradable substrates. It was reported that stirring is more important to kinetics than extent of BMP results [53].

4.2.4. Test duration

The time selected to carry out the BMP test should be long enough to ensure the maximum degradation of organic matter. Literature reported incubation times ranging from 30 to 100 days. The recommended incubation time should be used as a guideline, low (lower 1% of cumulative) or null net daily methane production during three consecutive days suggested as a rule to end BMP measurement [44].

4.3. Chemical operational conditions

Information on following chemical factors should be included in experimental reports.

4.3.1. Headspace gas

When performing a BMP test, eliminating the oxygen in the headspace is sometimes assumed to be critical. However, it remains to be demonstrated that the residual oxygen in the headspace has adverse impact on the anaerobic digestion process. Besides, in relation to the question of whether flushing the headspace is really required, mainly two types of flush gas are used: pure nitrogen ($N_2$) and different mixtures of nitrogen and carbon dioxide ($N_2$-$CO_2$) (normally in the proportions 70/30 or 80/20) [54].

4.3.2. pH/alkalinity adjustment

pH and alkalinity are routinely used as indicators for imbalance and failure of anaerobic reactors. Therefore, it is relevant to ensure adequate pH (7-8) and buffering capacity (above 3 g $CaCO_3$/L) [44].

4.3.3. Mineral medium: Optimal microorganism metabolism and microbial growth requires balanced concentrations of macronutrients, micronutrients and some trace metals. Sometimes, it is unclear whether a BMP test will have sufficient nutrients

available considering the content present in the inoculum and the substrate, or alternatively, additional supplements are necessary [55]. Anyway, mostly referenced BMP guidelines suggested the use of additional nutrients [41, 43, 44].

### 4.4. Inoculum to substrate ratio (ISR)

It has been demonstrated that ISR is an important design parameter for achieving accurate BMP results [56]. In order to find the maximum methane potential and methane production rate, a right balance between the substrate and microorganisms is necessary. For routine BMP tests, an ISR ≥2 has been suggested as a default [44].

### 4.5. Gas measurement systems

Since biogas production is the key factor to determine the methane potential and biodegradability of a substrate, accurate measurements of biogas and methane quantity without significant losses or error is important. Techniques for measuring the rate and volume of biogas produced from AnBD assays include mainly volume displacement devices or pressure manometers. Volumetric methodologies are more popular among scientific community due to easy usage and the variety of systems that can be used such as liquid displacements, lubricated glass syringes and gas meters. Manometric methods are more sensitive but may have large systematic errors [57]. In addition, headspace pressure is known to affect biogas composition due to effects on $CO_2$ partitioning between biogas and the liquid portion of reactors [54]. Regardless of the gas measurement system selected, it is very important to apply correction factors to convert the observed methane potential to standard temperature and pressure conditions for obtaining standardized results [58].

### 4.6. Positive control substrate: cellulose

BMP tests measurements can be faulty due to the inadequacy of selected inoculum or experimental conditions. Additionally, the presence of leakage through septa or in any other tubing connections could lead to underestimation of BMP results. Therefore, the use of positive control substrates with theoretical known methane potential can be considered as obligatory to check the quality of BMP results reported. Harmonization guidelines consider the use of standard compounds as a compulsory element to obtain reliable BMP results. Cellulose, with a theoretical methane potential of 414 mL $CH_4$/g VS, has frequently been suggested as the most common choice of standard compound for quality control of BMP measurements [41, 43, 44]. Some reasons to support this choice are its full biodegradability, low price, high quality and worldwide distribution. It is important to note that this theoretical value is not totally reached because around 10-15% of degradable substrate components are used for microbial growth and cell maintenance.

## 5. Chemical measurements

Biomass samples are complex heterogeneous mixtures of mainly organic matter and to a lesser extent inorganic matter. The composition of natural biomass depends on various factors: type of biomass, plant species or plant parts; growing conditions (geographic location, climate, sunlight, soil, water); age of plants and harvesting time [59]. To get a better understanding of the AD process for biomass samples, the determination of the bulk chemical composition should be the first step and common approach before sample digestion. These chemical characteristics are used mainly to describe the quality of the initial solid substrates, which determines their AD potential application. However, these measurements can also be applied to the end products as well as process states during the fermentation process. The chemical composition of a biomass sample can be stated using different analytical terms [60, 61]. Firstly, proximate analysis gives the composition of the biomass in terms of gross components and is therefore of fundamental importance for biomass energy. The parameters included are moisture, volatile matter (VM) (burn in gaseous state), fixed carbon (*FC*) (burn in solid state) and ash (inorganic material). Secondly, the objective of ultimate analysis is to determine the elemental composition of a solid biomass sample in terms of carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O), but for some solid biomass chloride (Cl) and other elements may also be of interest. Thirdly, compositional analysis of biomass is used to determine feedstock compositions in terms of carbohydrates, lipids and proteins as well as mass balances and product yields from conversion processes [62]. Unfortunately, these organic fractions are not easy to determine due to the heterogeneous nature of samples and also to some analytical limitations. In fact, normally the measurement methods reported for AD research fields were taken from the characterization of wastewater, food, soil and wood samples. As a result, variations and modifications of analytical methods were created which often make comparison of results among different laboratories difficult.
The next sub-sections will explain the most important analytical parameters to take into account for biomass substrates characterization feeding in anaerobic digesters.

### 5.1. Moisture/Dry Matter (DM)

Water is present in all biomass samples. The water content has a great connotation because it determines physical characteristics, microbiological stability and sensory properties. It also has a major effect on technical processes and substrate energy density (hence the cost of transportation and storage). For this and other reasons practical and legal limits exist.
For processing biomass samples in AD processes it is indispensable to be able to measure moisture accurately because the substrates treated are generally based on

DM content. Obviously, the determination of water content is a very important topic and it can be considered as one of the most common analyses performed for solid samples.
Nevertheless, water content determination is not as trivial an analysis as it might be expected. There are several methods to determine the water content, which can be classified as direct and indirect methods [63]. Direct methods are those that can determine water itself. They can be based on physical separation of water (distillation, oven-drying, halogen, microwave and infrared drying). An alternative are the methods based on a selective chemical reaction of the water in the sample (calcium carbide and calcium hydride methods or Karl Fisher titration). Indirect methods may either determine a macroscopic property of the sample which depends on its water content (densimetry, polarimetry, refractometry), or measure the response of the water molecules in the sample to a physical influence such as Nuclear Magnetic Resonance (NMR), Near Infrared (NIR) and Microwave (MW) spectroscopies.

Although not totally reliable for some biomass samples, oven-drying methods estimating the moisture by evaporation, are routinely used because they are simple, inexpensive and relatively fast. These are empirical methods in which the moisture results obtained are defined by the drying conditions. The two most critical factors affecting this measurement are temperature and time. It is very important to define them correctly to avoid misinterpretation because different drying temperatures and times are reported in the literature [64]. Specifically, some laboratories used identical temperature and time for all types of samples while other laboratories changed both parameters depending upon the matrix. There are others factors influencing the moisture determination such as the oven air (forced draft or mechanical convection), size of drying containers (small, medium and large) and type (capsules or crucible) and also usage of suitable desiccators containing effective desiccant material [65].
It must be clearly stated that oven-drying methods cannot distinguish between water and other volatile substances. This means that during oven-drying, volatile substances other than water are lost. For this reason, the term moisture should be replaced by “loss on drying” whenever a drying operation is conducted to determine the water content [66]. In addition, side chemical reactions could be present while the heating process takes place.
In general, for biomass materials, oven-drying methods tend to overestimate dry matter content for untreated material. On the other hand, oven-drying methods generally underestimate dry matter content in pre-treated material due to loss of volatile organic compounds [67]. Some challenging measurements for loss on drying in the AD research field are silage samples. This is due to the formation of volatile fatty acids (VFAs), ammonia and alcohols in the course of the ensiling process [68]. Similarly, samples coming from two-stage AD processes have a lot of volatile compounds, principally VFAs [69]. Ensiling and two-stages processes have been proposed as the way to obtain higher methane yields, although the explanation of this improvement has always been unclear [70, 71]. To avoid inaccurate results for this kind of samples, it is necessary to correct the results using volatility coefficients for organic compounds lost

if their specific chemical structures are identified [72]. Alternatively, Karl Fisher titration is considered as a more accurate method taking into account that only water molecules react with titration solvent [67].

### 5.2. Total Chemical Oxygen Demand (CODt)

The determination of CODt for biomass samples is significant for evaluating the energetic balance of the AD processes. This is due to the ratio between methane produced and COD removed that is a constant theoretical factor equal to 350 mL $CH_4$/g $COD_{removed}$. The reference method used to determine the COD parameter is based on the APHA–AWWA–WPCF standard methods for the analysis of water and wastewater [73]. Therefore, the measurements of biomass samples can be considered as a problematic task because the majority of reported methods rely on liquid samples and should be adapted specifically for solid samples. An interlaboratory study on COD measurement was carried out for different laboratories dealing with the AD research field [74]. This study included four samples, two solid samples as raw materials, and two liquid samples to be prepared with high concentration of suspended solids. Unfortunately, the overall analytical data evaluation showed that only 36 % of results could be considered as acceptable. A second interlaboratory study showed that the overall analytical performance was improved by around 30%, demonstrating the positive effect of a regular participation in proficiency testing studies [75].

Some specific methodologies were reported in the literature trying to characterize accurately the organic matter of solid substrates in terms of COD. Raposo and co-workers proposed an adapted titrimetric approach using concentrate oxidant and open reflux approach [76]. The method was proposed as a way to obtain maximum recovery values for both solid samples and solutions with high suspended solid contents. More recently, some attempts have been carried out using commercial kits based on conventional closed reflux colorimetric method. Firstly, Noguerol-Arias and co-workers proposed a method based on a preceding solid dilution using magnesium sulphate as an analytical inert material [77]. Secondly, Andre and co-workers reported the use of commercial tubes without previous solid sample dilution [78]. In their proposed methodology a homemade plastic support is required to transfer the sample weighing directly into COD tubes. Thirdly, Cazaudehore and co-workers proposed a prior double acid hydrolysis of the solid sample from which, after cooling, a liquid supernatant sample is analysed in the commercial tubes [79].

### 5.3. Lipids

The terms lipids, fats and oils are frequently considered as synonymous and thus used interchangeably. The term lipid normally refers to the whole collection of food molecules that are insoluble in water and soluble in organic solvents. Fats and oils are more specific terms used according to the solid or liquid state of the lipid material at room temperature, respectively.

The lipid content is highly variable for solid samples, for example lignocellulosic samples have low content while organic fraction of municipal solid waste (OFMSW)

and algal samples have high content. The knowledge of lipid content is very important because they are energy-dense organic compounds anaerobically biodegradable and considered as a source of higher methane yield potential when compared to proteins and carbohydrates. During AD processes, the presence of intermediate long chain fatty acids (LCFAs) as inhibitory compounds become the main reason of biological process instability [80].

Several analytical methods have been developed for total lipid extraction. Most of them use liquid-liquid extraction procedures, which are time consuming, and require a lot of manual operations. Nevertheless, lipids are normally characterized by the Soxhlet method. This is a procedure developed by semi-continuous solvent extraction and where fat content is measured by the weight of fat removed from the sample. This is a routine method due to its simplicity and relative safety at laboratory level. Other advantage is its potential for upscaling results to industrial plant level [81]. A very important decision is the solvent selected because the extractive characteristics of different solvents are different and naturally the extraction yield can affect the results obtained. For example, Ramlucka and co-workers studied the effect of 13 solvents in the extraction of lipids from algal biomass. Results reported showed that ethanol, chloroform and hexane were generally more efficient in the extraction of lipids than the other solvents studied [82].

Other alternative technique for the analysis of lipids is the NMR technique, which is based on the measurement of the resonance frequency of the atomic nuclei under the influence of a magnetic field [83]. In any case, the equipment is not easily accessible and the results are difficult to interpret.

### 5.4. Proteins

Proteins play a major role in the AD processes. They are hydrolyzed into amino acids by extracellular enzymes secreted by different bacteria. Later, the amino-acids are de-ammonified by anaerobic oxidation, yielding volatile fatty acids, hydrogen and ammonia. The main disadvantage of protein rich biomass during AD is the high amount of nitrogen released in form of ammonia that can inhibit methane formation [84].

Different methods were reported in the literature to determine the protein content of solid substrates. They are based on diverse analytical principles, providing the protein content either indirectly or directly [85]. Indirect protein determination is habitually used and it is based on the analysis of the organic nitrogen content in the samples. Normally, the organic nitrogen is liberated at high temperature, by combustion in the Dumas method (TDN) [86] or by sulphuric acid hydrolysis and distillation in the Kjeldahl method (TKN) [87]. It is important to note that for indirect protein determination other organic (urea, nucleic acids) or inorganic (nitrates, nitrites and ammonia) nitrogen compounds can also be measured. On the other hand, direct protein determination is calculated based specifically on the analysis of amino acids. To obtain the amino acids profile the sample must be previously extracted and later hydrolysed (110ºC during 24 h). Then, the analysis is carried out by high performance liquid chromatography (HPLC) and the different amino acids can be classified as essential and non-essential.

The first pitfall related to protein content is the difference obtained when results of TKN and TDN are compared [88]. The reason is that TKN measures organic nitrogen and

ammonia while TDN also determines the inorganic fraction in form of nitrites and nitrates. Because of this, Dumas and Kjeldahl methods will lead to different results depending on the inorganic nitrogen content of the sample and also to what degree the organic nitrogen is recovered by Kjeldahl method. Therefore, to avoid conflicts and misinterpretations in official situations it is very important to clearly specify the method used for crude protein determination. There is a clear tendency towards Dumas method as it offers shorter analysis time, ease operation and improved safety when compared to Kjeldahl method. Normally, TDN values are higher than TKN values [89]. Therefore, both methodologies could be interchanged only when differences in results are insignificant.

The second pitfall for protein content measurement is the selection of the nitrogen to protein conversion factor (NtPCF) established to relate the measured nitrogen to the quantity of protein. Historically, the NtPCF for the traditional TKN method has been established considering that 16% of the overall nitrogen content corresponds to proteins for all types of samples and that all organic nitrogen is protein-bound [87]. For that reason, a general conversion factor of 6.25 has been stated. However, the nitrogen content of different proteins varies within a rather large range (13-19%) [90]. Throughout the years, evidence has accumulated that the common conversion factor of 6.25 in most cases overestimates the proteins content because a fraction of nitrogen should not be associated with proteins [91, 92]. In order to adjust for these variations, some conversion factors have been proposed for specific substrates, making the conversion from nitrogen to protein more reliable [93].

### 5.5. Carbohydrates

There is a considerable inconsistency in the terminology used for this kind of organic compounds. Most sources consider an oligosaccharide to be a carbohydrate composed from 2 to 10 sugar (saccharide) units. A polysaccharide usually contains from 30 to at least 60,000 monosaccharide units. There is no official definition of an oligosaccharide. Two monosaccharides (sometimes called simple sugars), D-glucose and D-fructose, are found in significant amounts and can be used directly for anaerobic microorganisms. Higher saccharides (oligo- and polysaccharides) must first be hydrolyzed to monosaccharides before cell absorption and metabolism utilization. From the analytical point of view, determination of both total/soluble carbohydrates and also their structural fractions are useful.

#### 5.5.1. Total/soluble carbohydrates

This determination applies to various types of carbohydrates (CARBs) including monomeric, oligomeric and polymeric compounds. It is important to note that CARBs fraction is the only component in the compositional analysis which may be calculated by difference. In this sense, total amounts of CARBs have been calculated theoretically deducting lipids and protein contents taking into account the VS quantity. Similarly, it is possible to find in the literature the nitrogen free extract (NfE) fraction. This is a very inaccurate name indeed because this fraction has nothing to do with nitrogen and it's not an extract either. NfE hypothetically represents the soluble carbohydrates of the

sample, such as starch and sugars. Unfortunately, these methods have very low accuracy since CARBs fraction accumulates all of the errors existent in the analysis of the other organic constituents.

Experimentally, the most rapid, simple and cheap approach for the determination of total carbohydrate content is based on colorimetric approach. The spectrophotometric determination of CARBs has two main drawbacks [94]. Firstly, measurement results are expressed in terms of glucose-equivalent concentrations. Therefore, the absorbance responses to different saccharides may not be directly applicable for calibration curves using glucose as calibration standard. Secondly, there is a problem with interferences because many organic compounds can react with colour-developing reagents, mainly in presence of concentrated sulphuric acid, and consequently the results of carbohydrate content are usually overestimated. In any case, to measure CARBs spectrophotometrically prior acidification of samples by sulphuric acid is necessary. There are two principal methodologies with different reagents for developing a chromophore. One methodology is based on the use of anthrone with maximum absorbance at 625 nm [95]. The other, Dubois methodology, used a solution of phenol with the maximum absorbance at 490 nm [96]. These assays are generally accepted because they are simple, requiring only chemicals, tubes and a spectrophotometer. However, critical comparisons of both methods were reported in the literature with contradictory conclusions about their accuracy. It seems that the phenol-sulphuric method recovered more carbohydrates than the anthrone method [97].

5.5.2. Structural carbohydrates

This fraction is made of mainly two types of structural carbohydrates such as cellulose and hemicellulose that provide the structural and hold functions of the cell wall. Cellulose is the predominant polymer constituted by D-glucose units linked by β-1,4 glycosidic bonds. Hemicellulose is a mixture of polysaccharides that are grouped together due to their similar structure and chemical properties. Their structural backbone and side chains differ according to the biomass sample selected. Then, the specific composition of sugars (pentosans and hexosans) differs and typically produces a mixture of glucose, galactose, mannose, arabinose, xylose and rhamnose. Usually, hemicellulose is degraded to sugar more easily than cellulose because its structure is mostly an amorphous form. Other residual polysaccharides components include pectins, glucans, fructans and mannans. Two principal analytical methods have been proposed to determine these compounds.

5.5.2.1. Detergent fibre system (DFS)

The detergents system analysis was developed by Van Soest and co-workers at the United States Department of Agricultural (USDA) in the 1960´s for feed assays in ruminant nutrition although it has been widely used for non-ruminant research [98, 99]. The concept behind detergent system is that plant cells can be divided into soluble and insoluble components by using two detergents. Firstly, a neutral detergent gives the neutral detergent fibre (NDF) fraction as an indicator of structural carbohydrates intake.

Following, an acid detergent reagent is used to obtain the acid detergent fibre (ADF) fraction as a measure of digestibility and, thus, the energy intake.
A number of procedural variations have been developed out of attempts to overcome certain difficulties during this gravimetric determination [100]. The first decision is the apparatus to be used, being possible to select manual, semi-automatic or totally automatic systems. The second decision is the selection of the filtration technique between traditional crucible or filter bag [101, 102]. For crucible system it is necessary to apply vacuum to separate the fibre components while for bag an easy self-filtration is carried out. The third choice is related to the use of two reagents to avoid the positive interferences of some organic components: sodium sulphite and alpha-amylase have been proposed to remove residual structural proteins and starch, respectively [103, 104]. The fourth decision is related to the analytical procedure manner. It is possible to carry out the determination using a sequential analysis when NDF and ADF fractions are estimated using the same sample, being necessary to measure ADF after finishing with the NDF fraction. In the non-sequential way, NDF and ADF fractions can be estimated using different samples and, therefore, they should be analysed both at the same time [105]. Finally, to avoid mistakes, it is very important to consider the correction for remaining ash expressing results per volatile solids content [104].

5.5.2.2. Two steps sulphuric acid hydrolysis (SAH)
This method was developed by Sluiter and co-workers at the National Renewable Energy Laboratory (NREL) [106, 107]. The method is labour intensive and requires attention to some details because is empirical. Then, differences in technique could affect the final results. The procedure utilizes a strong sulphuric acid solution (typically 72 % w/w) in a primary hydrolysis, followed by dilution with water and a secondary high temperature (100-125ºC) hydrolysis. This procedure hydrolyses the polymeric carbohydrates into free sugars that can be analysed individually by HPLC to quantify the carbohydrate fraction of the sample. The main advantage of this method is the possibility to carry out a quantitative compositional analysis by adding the individual components.

5.6. Lignin (LIG)
This is a three-dimensional macromolecule biosynthesized by the polymerization of three types of phenyl-propanoid or monolignol units such as p-coumaryl, coniferyl and sinapyl alcohol units. It is mainly water-insoluble and a major component of the plant cell wall because it provides the structural support and prevents microbial attacks and decomposition of the structure. Lignin is considered as “cellular glue” because is both physically and chemically connected to structural CARBs in lignocellulosic samples. There are several analytical procedures for determining the lignin content of cell walls [108]. The most commonly described procedures for biomass samples are methods that remove cell wall constituents except lignin. These procedures are directly connected to the previously explained DFS and SAH methods for the determination of structural carbohydrates. In this sense for DFS method, the ADF fraction is treated with concentrated sulphuric acid solution which removes cellulose from lignin components that are gravimetrically measured and named as acid detergent lignin (ADL) [109]. In

the case of the SAH methodology, the non-lignin structural components are removed during the two hydrolysis stages from the solid structure leaving a lignin-rich residue named as Klason lignin (KL) which is vacuum filtered and measured gravimetrically [110,111]. It should be noted that the main difference between both methodologies is the sequence in which acid concentration and temperature are employed to effect the hydrolysis of polysaccharides. Relating to inter-method variability between DFS and SAH, it can be indicated that the lignin components are either: insoluble and soluble. However, the Van Soest methodology has a negative bias because the soluble components of lignin are not determined [112, 113]. In any case, independently of the methodology used, the lignin content should be corrected for protein-like compounds to avoid being significantly overestimated [114].

## 6. Predictive regression models versus BMP tests measurements

BMP test measurements collected on this manuscript have been evaluated thoroughly focusing on the description of the different experimental factors affecting the assay. A detailed summary of BMP assays from selected articles is included in **table 1**. Unfortunately, a lot of information necessary when reporting BMP studies was not included in the description of most articles. Specifically, 50% of the possible information that should be reported in the reviewed articles was omitted. This situation makes it difficult to compare the experimental results obtained.

### 6.1. Description of inoculum

#### 6.1.1. Origin/Source

Different sources of inoculum were reported in the reviewed articles. BMP studies mainly reported the use of an inoculum from a biogas plant (35%). Other options for inoculum origin were municipal wastewater treatment plants (15%), laboratory reactors (12%) and pilot plants (8%). It is important to note that this relevant information was missing in four of the articles reviewed.

#### 6.1.2. VS content

Unfortunately, this information was absent in the majority of reviewed articles (77%). In the articles containing this information, the organic content reported ranged between 60-70% of DM.

#### 6.1.3. Initial concentration.

For this factor, the information reported in the reviewed articles was infrequent (23%). In addition, some articles included this information incorrectly by using the percentage of inoculum volume. Then, only three articles (12%) included the information in appropriate units, ranging from 2-5 g VS/L.

#### 6.1.4. Pre-digestion.

In spite of this procedure being suggested in the harmonization guidelines, only 19% of reviewed articles included a previous step of incubation for the inoculum. In addition, the time selected ranged between 7-14 days, a period of time higher that the suggested one (2-7 days).

### 6.2. Description of physical operational conditions

#### 6.2.1. Total and working volumes.

Conveniently, all the selected articles included information on this factor. However, the information regarding both volumes was included in only 38% of the selected articles. The total volume was reported more times (81%) than the working volume (58%). In any case, both volumes ranged widely, including small (250 mL), medium (250-500 mL), large (500-1000 mL) and extra-large (>1000 mL) volumes. One article included a total volume of 20 L. It is important to note that this high volume is not suitable at all for BMP test measurements from the laboratory point of view considering that a minimum of nine reactors are necessary (3 blanks, 3 replicates/sample and 3 controls).

6.2.2. Temperature.

Fortunately, only one experiment excluded this information from the report. The majority of experiments were carried out at the mesophilic range of temperature. The incubation temperature was achieved mainly by using thermostat-controlled water baths.

6.2.3. Stirring.

The information for this factor was again missing in the majority of reviewed articles (69%). In the occasional information found regarding this factor, manual mixing was mainly used, being followed by automatic shakers working continuously or intermittently.

6.2.4. Duration (incubation time)

Unfortunately, 27% of articles omitted this information. The time elapsed to finish the measurements ranged from 30 to 100 or more days. The majority of batch tests (80%) were carried out over more than 35 days.

6.3. Description of chemical operational conditions

6.3.1. Headspace flushing gas.

This information was not present in half of the selected articles. In the rest of articles, they described the removing of oxygen before start BMP tests using mainly $N_2$ (27%) or $N_2$-$CO_2$ mixtures (23%).

6.3.2. pH/alkalinity adjustments.

This information was not reported in 88% of selected articles. The use of additional buffering, provided by sodium bicarbonate ($NaHCO_3$), was reported in only 3 articles.

6.3.3. Mineral medium.

More than half of selected articles (58%) have no information about the use or no use of mineral medium for batch tests. 38% of articles included the use of nutrients while only one article (4%) reported that nutrients were not employed.

6.4. Description of ISR

In spite that ISR is considered as one of most important parameters in batch tests, 31% of articles left out this information. The majority of studies included an optimal ISR higher or equal to two. Another important aspect to be considered is the different units selected for defining ISR, such as TS, VS or COD.

6.5. Description of gas measurement systems

This relevant information was omitted in 15% of articles. The most frequent methods for gas measurement were volumetric systems, by liquid displacement (27%), gas counter (23%) or syringe (12%) devices. One article (4%) reported both

methodologies, liquid displacement and syringe. Manometric systems were reported in 15% of selected articles.

### 6.6. Cellulose as positive control

Surprisingly, only six (23%) of selected articles incorporated the study of cellulose to check the usefulness of reported results. In addition, only two articles included the values obtained while one article explained that higher than 80% of theoretical methane production was obtained.

## 7. Predictive regression model versus chemical measurements

A detailed study of chemical measurements from selected articles is included in **appendix B.** Normally, analytical methods reported are based on wet chemistry methodologies although some articles unusually included NIRs measurements. **Figure 1** shows that up to seven possible analytical determinations were used in different degree for the selected articles. In fact, 70% of analytical determinations were covered in some degree. However, the analytical information including a correct reference description was only occasional.

### 7.1. Moisture/Dry matter

This analytical determination was reported in the totality of articles reviewed except one where, in general, the analytical information was uncommon. One aspect to comment is the improper use of some method description references. This situation is more related with the nature of samples analysed than with the presence of errors in the results obtained. In fact, reference to APHA selected in many articles should be reported exclusively for wastewater samples. Therefore, more appropriate references should be related to soils and forage matrix samples.

In general, the most commonly applied methodology was oven-drying until constant weight followed by gravimetric determination of the mass lost. But the important operational factors, such as temperature and time, were not reported in the majority of selected articles. Exceptionally, one of the reported articles using silage samples, included the DM correction for losses of volatile compounds for improving the accuracy of results.

### 7.2. Total Chemical oxygen demand

This determination was included in 8 out of 26 (31%) articles selected. Unfortunately, specific methodology was not included in all the reports. Most references are related to liquid samples. Some references are also related to soil samples, but as a measure of the organic carbon instead of COD. In any case, many articles reported spectrophotometric determination as a measurement technique. It is important to note that to carry out this type of methodology using biomass samples, they should be previously diluted to be measured. Dilution of solid samples has been reported as the main reason for the underestimation of results [74,75].

### 7.3. Lipids

The content of total lipids was included in the 54% of reviewed articles. Similarly to previous analytical parameters, the reference APHA-AWWA-WPCF was included to describe this determination. However, this reference should be used to determine dissolved or emulsified oil and grease from water samples. Unfortunately, only three of the selected articles reported the use of Soxhlet methodology for lipid determination. In addition, only once was the solvent selected (petroleum ether) reported. Another article included the use of one automatic extraction system device (ANKOM XT-10 extractor) where samples are placed into filter bags which prevent sample transfer error. In this way, filter bags serve to simplify handling and enable batch processing up to fifteen samples at a time and up to 100 samples per day.

### 7.4. Proteins

The determination of the protein content was reported in 73% of reviewed articles. From 19 articles including protein determination, 7 of them (37%) omitted the information relating to the specific methodology. Normally, protein in biomass samples is difficult to measure directly, therefore in many cases the nitrogen content of the biomass sample is measured indirectly by Kjeldahl (42%) and Dumas (16%) methods. Specifically, for TKN methodology the interfering total ammonia content (TAN), if not negligible, must be removed to achieve a reliable protein content. Unexpectedly, the Lowry method was described once (5%). It is important to note that this methodology is designed for liquid samples and, therefore, the results must correspond to soluble protein content. In addition, one research article determined both protein contents, total (by Kjeldahl) and soluble (by Lowry, previous extraction with NaOH). Irrespective of the way for the measurement of Nitrogen content, one NtPCF must be selected for protein content calculation. For the reviewed articles, the use of 6.25 as NtPCF was used in all cases. It was previously emphasized (section 5.4) that, in general, this factor may lead to errors when applied to biomass feedstock or process intermediates.

### 7.5. Carbohydrates

It is very important to distinguish carbohydrate compounds between structural and non-structural fractions. Research articles reviewed were more involved in the analytical determination of structural CARBs (24 articles-92%) versus non-structural CARBs (8 articles-31%).

#### 7.5.1. Non-structural carbohydrates

Only 4 out of 8 articles reported the analytical method selected. Specifically, the anthrone methodology was used in the reviewed articles. However, it must be explained that this spectrophotometric method is used for the soluble fraction of CARBs if no previous sample digestion was carried out. A couple of articles explained the determination of water soluble CARBs while other two articles reported the content of reducing sugars.

On the other hand, in 7 articles (27%) the CARBs content were calculated theoretically by differences. In these articles different names were used such as nitrogen free extracts (NfE), non-fibre carbohydrates, total carbohydrates and non-lignocellulosic carbohydrates.

7.5.2. Structural carbohydrates

Unfortunately, nearly 30% (7 out of 24) of articles, where these compounds were considered, omitted the information about the methodology selected. From the rest, 58% of articles reported the DFS to calculate gravimetrically the cellulose and hemicellulose fractions. Some of these articles explained the use of modifications proposed by Mertens and co-workers about the importance of using alpha-amylase and sodium sulphite. Only two articles (8%) described the SAH as the methodology to determine the individual sugars corresponding to both CARBs polymers. This situation can be explained considering the specific equipment necessary to obtain individual sugars from biomass samples. Surprisingly, only one article described both analytical methodologies with no clear explanation about the objective of these measurements.

7.6. Lignin

This determination was reported in 88% of reviewed articles emphasizing the interest of this parameter relating to the AnBD of biomass samples. The majority of articles used the ADL fraction to evaluate the lignin content of samples. Only two articles reported the KL as the lignin-like fraction. It is important to remind that ADL values are always lower when compared to KL values because some soluble lignin components are removed in the Van Soest methodology (section 5.6).

## 8. Evaluation of predictive regression models

A bibliographic study of selected articles about predictive regression models is included in **table 2.** In this section, the previously published predictive models were assessed to explore their power and limitations for predicting BMP.

8.1. Lack of one general model

Development of one overall model that applies to all kind of samples is difficult to achieve. This is due to several reasons. First, the number of biomass samples studied and their nature was highly variable. For some studies, the research was very specific including the same biomass samples or some varieties, while other investigations included very different types of biomass. Second, some biomass samples were studied as raw materials and also after pre-treatment procedures. Specifically, some substrates were studied as ensiled forms where the chemical composition varied due to the presence of unstable substances such as volatile fatty acids and alcohols. Thus, the silage fermentation products would have extremely limited applicability in the majority of predictive models. Third and moreover, the variability in the biochemical composition of substrates, other factors such as particle size, porosity and surface area are normally not considered. Fourth, the majority of predictive models were focused on methane potential but a few of them were dedicated to biogas yield. Therefore, to transfer the results from biogas to methane, the biogas composition should be obtained accurately. Fifth, different forms to express the biomass composition in the general equations for predictive models were selected. In this way, %TS, %VS or w/w were reported. Sixth, different analytical methodology was used to calculate the chemical composition of biomass samples.

8.2. Number of variables

Some regression models were initially developed using a single variable predictor but the results obtained showed only weak relationships between the individual components [40]. Although, they were used as an initial estimation approach, it is important to note that no single parameter, usually applied to characterize the biomass sample, is sufficient to predict BMP results of each substrate. Alternatively, chemical composition of biomass samples can be considered as a mixture where the combination of several constituents may be useful to reflect the AnBD process. Therefore, results of multiple regression models can be considered as more reliable for predicting methane potentials [14, 23, 24].

8.3. Contribution to the model: negative versus positive correlation

Biomass samples can be divided into three fractions taking into account their biodegradability. In this sense, biomass elements can be considered as totally digestible, poorly digestible and non-digestible. According to AD theory, the digestible fractions should be positively correlated with methane potential while the non-digestible fraction should be present in predictive models as negative relationship. However, previous reported predictive models have diverse contributions for some significant correlation factors.

8.4. General explanation about significance of chemical parameters

In any case, predictive models published in the literature demonstrated that some parameters linked to the organic fraction of biomass samples can be correlated to methane potentials. Typically, lipids, proteins, carbohydrates and lignin are the key parameters for AD processes. However, other different parameters have been used for predictive calculations about biogas and methane potentials. In the next section the significant chemical parameters present in the 23 regression models reviewed will be evaluated.

8.4.1. Overall organic content (VS and CODt)

The VS and CODt contents are usually denoted as good indicators of the organic matter content in a substrate. In fact, there is a direct relationship between CODt values and the VS content of an organic material. However, taking into account the different nature of VS and their biodegradability, these parameters may not be applicable as reliable indicators of the methane production. Therefore, both parameters were scarcely reported as regressors for BMP modelling. Specifically, VS content was used twice and CODt content three times.

8.4.2. Lipids

From the theoretical point of view, crude fat content is particularly important for biogas production because it exhibits much higher methane potential than proteins and carbohydrates. Therefore, as expected, a positive correlation was always found between BMP results and lipid content. However, their contribution can be considered as minor because only 8 out of 23 predictive models included the lipid content. One possible explanation could be that the lipid’s contents for some biomass samples were low or with limited variation in their concentrations.

8.4.3. Proteins

The crude protein content has been identified as another parameter with positive impact on methane yields. Surprisingly, only 10 out of 23 predictive models considered protein content as a significant parameter for methane yield. Exceptionally, one predictive model included the N content instead of protein content for modelling.

8.4.4 Carbohydrates

They can be divided in two main groups: structural and non-structural. Each group can be correlated to mainly soluble or insoluble fractions, although sometimes there is no way to know if some CARBs are soluble or attached to the solid organic matrix. It is also important to note that CARBs can be separated in different groups according to the analytical methodology carried out. In this way, the Weende method gives two fractions such as crude fibre (insoluble) and NfE (soluble). Alternatively, Van Soest method provides cellulose, hemicellulose and lignin (insoluble) and non-fibre carbohydrates (mainly soluble sugars and starch). In addition, carbohydrates contents (total or non-structural) are sometimes estimated as the missing part from the rest of organic fractions

8.4.4.1. Non-structural carbohydrates

Surprisingly, this parameter was included only in 8 out of 23 predictive models. In addition, different fractions, with different names, were used for BMP modelling. Thus, it is possible to find NfE, total soluble (after extraction), water soluble and reducing sugars as different regressors to be related to BMP potential. In addition, one predictive model used uronic acids, as specific acid sugars, in the equations for BMP modelling. Another particularity of non-structural CARBs is the variability in its relationship with BMP results, sometimes the correlation was presented as positive and sometimes as negative.

8.4.4.2. Structural carbohydrates

There is an agreement about the important energy potential of cellulose and hemicellulose fraction. Therefore, previous predicted models considered these parameters as very relevant for methane potential. In fact, 18 out 23 models included cellulose and/or hemicellulose fractions in the equations. Specifically, hemicellulose was included 10 times, cellulose 9 times and both parameters together 4 times.

On one hand, the contribution of hemicelluloses to the different predictive models was always considered in a positive role. On the other hand, cellulose correlation was different in previous studies described. Their contribution was inconsistent and widely variable from strong or weak to not observable. The variability is also reflected in the positive or negative correlation influence with BMP results. As cellulose is known to be fully biodegradable, the surprisingly negative effect was argued considering the crystallinity of cellulose or its interrelated effect with lignin.

There are other chemical parameters including not exclusively structural carbohydrates that were used in different predictive models. In this sense, the NDF fraction (hemicellulose, cellulose and lignin) was used twice. More frequently, 6 times, was the ADF fraction (cellulose and lignin) used for BMP prediction. In addition, ADF was used sometimes in combination to lignin fraction. More specifically, arabinan fraction was also considered an important regressor for methane potential of lignocellulosic biomass.

8.4.5. Lignin

In general, methane potentials of biomass samples are negatively correlated with parameters that describe fibre fractions. Normally, lignocellulosic substrates with high cellulose and/or hemicellulose contents were generally poor in lignin, and vice versa. There is a general agreement in the previous published results for biomass samples that the lignin content can be considered as the strongest predictor of BMP results. It was recognized decades ago that lignin is not fully degraded anaerobically [115]. More recent studies confirmed that only a limited fraction of lignin could be degraded anaerobically [116,117]. Then, lignin content was presented, without exception, as a negative regressor for methane potential in the 18 out of 23 predictive models. Nevertheless, unexpectedly, some of the reviewed studies did not establish a correlation between lignin and BMP. The only explanation provided for the non-significant effect of lignin was a limited content range used to check their effect on BMP test measurements.

In any case, the comparative analysis of many substrates reported in the literature using different regression models showed the lack of good correlation between lignin content and BMP results [40]. There are some reasons that could be useful to elucidate that the inhibition mechanism of lignin for BMP tests of biomass samples is complex. Firstly, the variability in ADL results obtained according to the specific analytical characteristics of Van Soest methodology. In addition, results obtained for KL are always different to ADL. Secondly, a limitation of the previous studies is relating to the complexity of lignin as an organic polymer. Lignin compounds are difficult to describe from a structural point of view. Thus, AnBD should be discussed in relation to the structural lignin effects [118, 119]. Thirdly, other important subject is the interaction of lignin with other components of the cell wall for biomass samples [119-121]. Therefore, cross-linking lignin with other components of the cell wall can limit the access to other fermentable structural carbohydrates. However, it is very frequent that interactions among chemical compounds are not taken into account for predictive models.

#### 8.4.6. Other chemical parameters

The different reviewed regression models also included other chemical parameters. In fact, residuals components or everything which is not structural CARBs or lignin were considered as regressors. Similarly, the neutral detergent soluble (NDS) fraction was considered as a positive regressor. Specifically, for silage samples, the content of butyric acid (HBu) and alcohols were considered in the regression model. Finally, a biochemical determination such as biological oxygen demand (BOD) was considered to be correlated to AnBD. This decision can be considered as controversial taking into account that this determination is an aerobic bioassay in which time duration was increased from 5 to 28 days. Therefore, the disadvantages of BMP measurements were doubled.

## 9. Current state, challenges, recommendations and future research work

### 9.1. Current state

The review manuscript shows that many studies have been carried out to try to predict methane production from different biomass samples. This is due to the importance of having a screening tool to compare or maximise methane generated from different organic substrates. However, the critical evaluation of predictive models developed until today showed that they are only valid to specific substrates and under certain

experimental conditions. This lack of reliable predictive regression models can be explained mainly due to the variation of experimental data reported in the literature as consequence of limitations in the laboratory measurements routinely used by scientists working on this research field.

On the one hand, there is an agreement in the scientific community that the information about the BMP value for a new substrate is decisive before designing an industrial plant for its treatment. Unfortunately, current BMP testing procedures result in a lack of comparable experimental results due to the differences in equipment, experimental conditions and procedures. As a consequence, there is a need to promote common rules, which would be recognized as good practices among researchers and practitioners. There are ~~a~~ few guidelines for BMP test protocols published in the last years which describe some criteria that are considered obligatory in order to accept as correct the experimental results from BMP test assays. These documents may be useful to BMP measurements because they permit evolvement and help in the elimination of systematic errors. In any case, a very important factor in BMP measurements that cannot be standardised is the inoculum to be used. It can only be suggested that the presence of an initial appropriate methanogenic community is necessary to obtain reliable BMP results.

On the other hand, the present review document also focuses on the analytical methods used for the characterization and quantitation of biomass substrates. It is important to highlight that the majority of chemical measurements routinely used at laboratory level can be considered as empirical determinations. Therefore, these procedures can only help scientists to know roughly the chemical composition of raw biomass samples. However, due to limitations in the analytical methodology, the results that they provide cannot be considered appropriate as the basis to create an overall predictive regression model for methane productivity.

### 9.2. Challenges

It was a challenge finding predictive regression models in the published literature which could be compared with the same biomass sample where all the laboratory measurements have been recorded appropriately according to BMP studies and analytical determinations. Additionally, it should be noted that the review document shows that the majority of papers published in the literature did not provide the necessary information relating to laboratory measurements. The omission of this information in research papers can be considered as a very problematic issue to science. It is important to consider that this information is necessary for the reproducibility of experiments, and without the possibility to reproduce experimental assays, there is no real science.

### 9.3. Suggestions

To obtain a reliable overall prediction model for methane production based on laboratory measurements, it is really important that the determinations necessary to construct the model can be based on accurate methodologies, and in this way, unquestionably reporting experimental results with low uncertainty. This review can help less experienced researchers to give more importance to laboratory

measurements, identifying problems and finding solutions in their experimental procedure.
Concerning to BMP measurements, the rules included in the guidelines for BMP test protocols should be followed. That means: 1) Provide detailed information about all the experimental conditions to perform the test; 2) Using with care manometric methodologies because there is some evidence that headspace pressure affects measured gas production; 3) Performing the appropriate number of replicates for each sample, including blanks and positive controls; 4) Application of a fresh and robust anaerobic inoculum providing viable microorganisms to convert organic substrate into biogas; 5) Using mandatorily positive controls, such a microcrystalline cellulose, as the only way to validate the experimental BMP results reported.
Concerning to chemical measurements, experimental results should be considered only as a raw characterization of biomass samples. For moisture, oven-dried methods should be carefully applied because volatile substances other than water may be lost. For COD analysis, wastewater methods should not be used for solid samples by their previous water dilution because this is the main reason for the underestimation of results. For lipids, the selection of solvent extraction is a key factor. For proteins, differences between TDM and TKN methodologies should be considered. In addition, the NtPFC should be selected according to substrate characteristics. For total carbohydrates, the method by difference should never be reported. For structural carbohydrates, DFS methodology has limited applicability while two steps SAH methodology is more reliable. For lignin determination, both frequently methodologies used, such as ADL and KL, can provide important differences in the lignin content of a biomass sample. In any case, lignin content cannot be accurately measured by gravimetric determination.
In summary, it should be necessary to understand better the compositional analysis of samples by performing high-quality analytical characterization. These results could be used to determine biomass composition as well as product intermediates and product yields from conversion processes.

### 9.4. Future research work

Upcoming investigations should be focused to evaluate the potential of anaerobic digestion as a promising and effective approach to substitute fossil fuels and mitigate climate change [122]. Thus, accurate BMP predictive models are necessary to evaluate recent biological innovations to improve biogas production [123, 124]. In addition, it is necessary that information on methane production generated through lab-scale investigations can be useful by commercial plants for the right decision-making [125, 126]. As future perspectives, it could be interesting to investigate some ideas arising from all the above information. In general, it would be interesting the scheduled participation of research labs in interlaboratory comparison studies as a way to improve the quality assurance of the experimental results obtained. From BMP tests, future research activities should be conducted to try to develop suitable storable anaerobic inocula for practical and reproducible application in BMP tests. Another proposal is to create a methane yield data base where the authors must report from their investigations all the significant information requested in BMP harmonization guidelines. Thus, the importance of results comparability can be proved in the short term. From analytical characterization, the global parameters used for organic matter

characterization should be replaced by more specific ones. Additionally, there are some evidences that the chemical composition of an organic substrate is not the only key factor for its biodegradability. Other characteristic such as the bio-accessibility according to the structural organization of the organic molecules in the substrates could be probably also a crucial factor to understand the AD process. Finally, methane production of BMP test assays obtained by simple regression models should be compared to soft computing methods [127].

## 10. Conclusion

In order to be accurate and widely applicable, predictive regression models for methane production of biomass samples should be based on correct laboratory measurements. Firstly, relating to BMP measurements, although some variability in experimental conditions is acceptable, the different factors affecting the bioassay should be selected accurately according to suggested recommendations. To help ensure accuracy in BMP measurements, the factors affecting BMP results should be described in detail. In addition, the utilization of positive control samples to check the performance of the complete BMP system should be obligatory. Secondly, the selection of robust analytical methods is essential to obtain reliable regression models. As with BMP measurement, analytical methodology should be described in detail, including a correct descriptive reference. Finally, the selected regression parameters should be in agreement with the AD theory. In this way, specific or particular chemical parameters should not be used for AnBD modelling. In this review it was demonstrated that the most important factor for predicting BMP of biomass samples is lignin content, which affects in three fold through composition (monolingol units), three dimensional structure and cross-linking.

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## References


[1] Labatut RA, Angenent LT, Scott NR. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour Technol 2011;102:2255–64. doi:10.1016/j.biortech.2010.10.035.

[2] Fitamo T, Triolo JM, Boldrin A, Scheutz C. Rapid biochemical methane potential prediction of urban organic waste with near-infrared reflectance spectroscopy. Water Res 2017;119:242–51. doi:10.1016/j.watres.2017.04.051.

[3] Jacobi HF, Ohl S, Thiessen E, Hartung E. NIRS-aided monitoring and prediction of biogas yields from maize silage at a full-scale biogas plant applying lumped kinetics. Bioresour Technol 2012;103:162–72. doi:10.1016/j.biortech.2011.10.012.

[4] Walla C, Schneeberger W. The optimal size for biogas plants. Biomass and Bioenergy 2008;32:551–7. doi:10.1016/j.biombioe.2007.11.009.

[5] Raposo F, De La Rubia MA, Fernández-Cegrí V, Borja R. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. Renew Sustain Energy Rev 2012;16:861–77. doi:10.1016/j.rser.2011.09.008.

[6] Peres CS, Sanchez CR, Matumoto C, Schmidell W. Anaerobic biodegradability of the organic components of municipal solid wastes (OFMSW). Water Sci Technol 1992;25:285–93. doi:10.2166/wst.1992.0160.

[7] Gonzalez-Estrella J, Asato CM, Jerke AC, Stone JJ, Gilcrease PC. Effect of structural carbohydrates and lignin content on the anaerobic digestion of paper and paper board materials by anaerobic granular sludge. Biotechnol Bioeng 2017;114:951–60. doi:10.1002/bit.26228.

[8] Raposo F, Fernández-Cegrí V, de la Rubia MA, Borja R, Béline F, Cavinato C, et al. Biochemical methane potential (BMP) of solid organic substrates: Evaluation of anaerobic biodegradability using data from an international interlaboratory study. J Chem Technol Biotechnol 2011;86:1088–98. doi:10.1002/jctb.2622.

[9] Lesteur M, Bellon-Maurel V, Gonzalez C, Latrille E, Roger JM, Junqua G, et al. Alternative methods for determining anaerobic biodegradability: A review. Process Biochem 2010;45:431–40. doi:10.1016/j.procbio.2009.11.018.

[10] Doublet J, Boulanger A, Ponthieux A, Laroche C, Poitrenaud M, Cacho Rivero JA. Predicting the biochemical methane potential of wide range of organic

substrates by near infrared spectroscopy. Bioresour Technol 2013;128:252–8. doi:10.1016/j.biortech.2012.10.044.

[11] Raju CS, Ward AJ, Nielsen L, Møller HB. Comparison of near infra-red spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility assay for rapid determination of the biochemical methane potential of meadow grasses. Bioresour Technol 2011;102:7835–9. doi:10.1016/j.biortech.2011.05.049.

[12] Godin B, Mayer F, Agneessens R, Gerin P, Dardenne P, Delfosse P, et al. Biochemical methane potential prediction of plant biomasses: Comparing chemical composition versus near infrared methods and linear versus non-linear models. Bioresour Technol 2015;175:382–90. doi:10.1016/j.biortech.2014.10.115.

[13] Herrmann A, Rath J. Biogas production from maize: current state, challenges, and prospects. 1. Methane Yield Potential. Bioenergy Res 2012;5:1027–42. doi:10.1007/s12155-012-9202-6.

[14] Xu F, Wang ZW, Li Y. Predicting the methane yield of lignocellulosic biomass in mesophilic solid-state anaerobic digestion based on feedstock characteristics and process parameters. Bioresour Technol 2014;173:168–76. doi:10.1016/j.biortech.2014.09.090.

[15] Amon T, Amon B, Kryvoruchko V, Zollitsch W, Mayer K, Gruber L. Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. Agric Ecosyst Environ 2007;118:173–82. doi:10.1016/j.agee.2006.05.007.

[16] Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, et al. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 2007;98:3204–12. doi:10.1016/j.biortech.2006.07.007.

[17] Gunaseelan VN. Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour Technol 2007;98:1270–7. doi:10.1016/j.biortech.2006.05.014.

[18] Buffiere P, Delgadillo Mirquez L, Steyer JP, Bernet N, Delgenes JP. Anaerobic Digestion of Solid Wastes Needs Research to Face an Increasing Industrial Success. Int J Chem React Eng 2008;6. doi:10.2202/1542-6580.1619.

[19] Gunaseelan VN. Predicting ultimate methane yields of Jatropha curcus and Morus indica from their chemical composition. Bioresour Technol 2009;100:3426–9. doi:10.1016/j.biortech.2009.02.005.

[20] Triolo JM, Sommer SG, Møller HB, Weisbjerg MR, Jiang XY. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresour Technol 2011;102:9395–402. doi:10.1016/j.biortech.2011.07.026.

[21] Monlau F, Sambusiti C, Barakat A, Guo XM, Latrille E, Trably E, et al. Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. Environ Sci Technol 2012;46:12217–25. doi:10.1021/es303132t.

[22] Triolo JM, Pedersen L, Qu H, Sommer SG. Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. Bioresour Technol 2012;125:226–32. doi:10.1016/j.biortech.2012.08.079.

[23] Rath J, Heuwinkel H, Herrmann A. Specific Biogas Yield of Maize Can Be Predicted by the Interaction of Four Biochemical Constituents. Bioenergy Res 2013;6:939–52. doi:10.1007/s12155-013-9318-3.

[24] Dandikas V, Heuwinkel H, Lichti F, Drewes JE, Koch K. Correlation between biogas yield and chemical composition of energy crops. Bioresour Technol 2014;174:316–20. doi:10.1016/j.biortech.2014.10.019.

[25] Thomsen ST, Spliid H, Østergård H. Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass. Bioresour Technol 2014;154:80–6. doi:10.1016/j.biortech.2013.12.029.

[26] Dandikas V, Heuwinkel H, Lichti F, Drewes JE, Koch K. Correlation between Biogas Yield and Chemical Composition of Grassland Plant Species. Energy and Fuels 2015;29:7221–9. doi:10.1021/acs.energyfuels.5b01257.

[27] Liu X, Bayard R, Benbelkacem H, Buffière P, Gourdon R. Evaluation of the correlations between biodegradability of lignocellulosic feedstocks in anaerobic digestion process and their biochemical characteristics. Biomass and Bioenergy 2015;81:534–43. doi:10.1016/j.biombioe.2015.06.021.

[28] Rath J, Heuwinkel H, Taube F, Herrmann A. Predicting Specific Biogas Yield of Maize-Validation of Different Model Approaches. Bioenergy Res 2015;8:832–42. doi:10.1007/s12155-014-9562-1.

[29] Bayard R, Liu X, Benbelkacem H, Buffiere P, Gourdon R. Can Biomethane Potential (BMP) Be Predicted from Other Variables Such As Biochemical Composition in Lignocellulosic Biomass and Related Organic Residues? Bioenergy Res 2016;9:610–23. doi:10.1007/s12155-015-9701-3.

[30] Herrmann C, Idler C, Heiermann M. Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. Bioresour Technol 2016;206:23–35. doi:10.1016/j.biortech.2016.01.058.

[31] Nurk L, Bühle L, Wachendorf M. Degradation of Fibre and Non-fibre Fractions During Anaerobic Digestion in Silages of Maize, Sunflower and Sorghum-Sudangrass of Different Maturities. Bioenergy Res 2016;9:720–30. doi:10.1007/s12155-016-9717-3.

[32] El Achkar JH, Lendormi T, Hobaika Z, Salameh D, Louka N, Maroun RG, et al. Anaerobic digestion of nine varieties of grape pomace: Correlation between biochemical composition and methane production. Biomass and Bioenergy 2017;107:335–44. doi:10.1016/j.biombioe.2017.10.030.

[33] Fisgativa H, Tremier A, Le Roux S, Bureau C, Dabert P. Understanding the anaerobic biodegradability of food waste: Relationship between the typological, biochemical and microbial characteristics. J Environ Manage 2017;188:95–107. doi:10.1016/j.jenvman.2016.11.058.

[34] Bayard R, Benbelkacem H, Gourdon R, Buffière P. Characterization of selected municipal solid waste components to estimate their biodegradability. J Environ Manage 2018;216:4–12. doi:10.1016/j.jenvman.2017.04.087.

[35] Dandikas V, Heuwinkel H, Lichti F, Drewes JE, Koch K. Predicting methane yield by linear regression models: A validation study for grassland biomass. Bioresour Technol 2018;265:372–9. doi:10.1016/j.biortech.2018.06.030.

[36] Edwiges T, Frare L, Mayer B, Lins L, Mi Triolo J, Flotats X, et al. Influence of chemical composition on biochemical methane potential of fruit and vegetable waste. Waste Manag 2018;71:618–25. doi:10.1016/j.wasman.2017.05.030.

[37] Tsapekos P, Kougias PG, Angelidaki I. Mechanical pretreatment for increased biogas production from lignocellulosic biomass; predicting the methane yield from structural plant components. Waste Manag 2018;78:903–10. doi:10.1016/j.wasman.2018.07.017.

[38] Hirunsupachote S, Chavalparit O. Predicting the Biomethanation Potential of Some Lignocellulosic Feedstocks using Linear Regression Models: The Effect of Pretreatment. KSCE J Civ Eng 2019:1–12. doi:10.1007/s12205-019-1589-6.

[39] Perendeci NA, Yılmaz V, Ertit Taştan B, Gökgöl S, Fardinpoor M, Namlı A, et al. Correlations between biochemical composition and biogas production during anaerobic digestion of microalgae and cyanobacteria isolated from different sources of Turkey. Bioresour Technol 2019;281:209–16. doi:10.1016/j.biortech.2019.02.086.

[40] Rodrigues RP, Rodrigues DP, Klepacz-Smolka A, Martins RC, Quina MJ. Comparative analysis of methods and models for predicting biochemical methane potential of various organic substrates. Sci Total Environ 2019;649:1599–608. doi:10.1016/j.scitotenv.2018.08.270.

[41] VDI 4630. 2016. Fermentation of organic materials - characterization of the substrate, sampling, collection of material data, fermentation tests. Verein Deutsch er Ingenieure e.V., Düsseldorf, Germany. 2016.

[42] DIN 38414-8. 1985 Determination of the amenability to anaerobic digestion (S8). German standard methods for the examination os water, waste water and sludge. Sludge and sediments (group S). Deutsches Institut für Normung (Germany Institut for Normative), 1985.

[43] Angelidaki I, Alves M, Bolzonella D, Borzacconi L, Campos JL, Guwy AJ, et al. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Sci Technol 2009;59:927–34. doi:10.2166/wst.2009.040.

[44] Holliger C, Alves M, Andrade D, Angelidaki I, Astals S, Baier U, et al. Towards a standardization of biomethane potential tests. Water Sci Technol 2016;74:2515–22. doi:10.2166/wst.2016.336.

[45] Jingura RM, Kamusoko R. Methods for determination of biomethane potential of feedstocks: A review. Biofuel Res J 2017;4:573–86. doi:10.18331/BRJ2017.4.2.3.

[46] Ohemeng-Ntiamoah J, Datta T. Perspectives on variabilities in biomethane potential test parameters and outcomes: A review of studies published between 2007 and 2018. Sci Total Environ 2019;664:1052–62. doi:10.1016/j.scitotenv.2019.02.088.

[47] Filer J, Ding HH, Chang S. Biochemical methane potential (BMP) assay method for anaerobic digestion research. Water (Switzerland) 2019;11:1–29. doi:10.3390/w11050921.

[48] Koch K, Lippert T, Drewes JE. The role of inoculum's origin on the methane yield of different substrates in biochemical methane potential (BMP) tests. Bioresour Technol 2017;243:457–63. doi:10.1016/j.biortech.2017.06.142.

[49] Hagen LH, Vivekanand V, Pope PB, Eijsink VGH, Horn SJ. The effect of storage conditions on microbial community composition and biomethane potential in a biogas starter culture. Appl Microbiol Biotechnol 2015;99:5749–61. doi:10.1007/s00253-015-6623-0.

[50] Nuchdang S, Khemkhao M, Techkarnjanaruk S, Phalakornkule C. Comparative biochemical methane potential of paragrass using an unacclimated and an acclimated microbial consortium. Bioresour Technol 2015;183:111–9. doi:10.1016/j.biortech.2015.02.049.

[51] Wang B, Strömberg S, Chao L, Nges IA, Nistor M, Liu J. Effects of substrate concentartion on methane potential and degradation kinetics in batch anaerobic digestion. Bioresour Technol 2015; 194:240–46. doi:10.1016/j.biortech.2015.07.034.

[52] Nizami AS, Orozco A, Groom E, Dieterich B, Murphy JD. How much gas can we get from grass? Appl. Energy 2012; 92:783–90. doi: 10.1016/j.apenergy.2011.08.033.

[53] Wang B, Björn A, Strömberg S, Nges IA, Nistor M, Liu J. Evaluating the influences of mixing strategies on the Biochemical Methane Potential test. J Environ Manage 2017;185:54–9. doi:10.1016/j.jenvman.2016.10.044.

[54] Koch K, Huber B, Bajon-Fernandez Y, Drewes JE. Methane from CO2: Influence of different CO2 concentrations in the flush gas on the methane production in BMP tests. Waste Manag 2016;49:36–39. doi:10.1016/j.wasman.2016.01.021.

[55] Parra-Orobio BA, Donoso-Bravo A, Ruiz-Sánchez JC, Valencia-Molina KJ, Torres-Lozada P. Effect of inoculum on the anaerobic digestion of food waste accounting for the concentration of trace elements. Waste Manag 2018;71:342–9. doi:10.1016/j.wasman.2017.09.040.

[56] Raposo F, Banks CJ, Siegert I, Heaven S, Borja R. Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. Process Biochem 2006;41:1444–50. doi:10.1016/j.procbio.2006.01.012.

[57] Hafner SD, Astals S. Systematic error in manometric measurement of biochemical methane potential: Sources and solutions. Waste Manag 2019;91:147–55. doi:10.1016/j.wasman.2019.05.001.

[58] Strömberg S, Nistor M, Liu J. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. Waste Manag 2014;34:1939–48. doi:10.1016/j.wasman.2014.07.018.

[59] Vassilev S V, Baxter D, Andersen LK, Vassileva CG. An overview of the chemical composition of biomass. Fuel 2010;89:913–33. doi:10.1016/j.fuel.2009.10.022.

[60] Cai J, He Y, Yu X, Banks SW, Yang Y, Zhang X, et al. Review of physicochemical properties and analytical characterization of lignocellulosic biomass. Renew Sustain Energy Rev 2017;76:309–22. doi:10.1016/j.rser.2017.03.072.

[61] Matheri AN, Ntulia F, Ngila JC, Seodigeng T, Zvinowanda C, Njenga CK. Quantitative characterization of carbonaceous and lignocellulosic biomass for anaerobic digestion. Renew Sustain Energy Rev 2018;92:9–16. doi:10.1016/j.rser.2018.04.070.

[62] Ohemeng-Ntiamoah J, Datta T. Evaluating analytical methods for the characterization of lipids , proteins and carbohydrates in organic substrates for anaerobic co-digestion. Bioresour Technol 2018;247:697–704. doi:10.1016/j.biortech.2017.09.154.

[63] Isengard HD. Rapid water determination in foodstuffs. Trends Food Sci Technol 1995;6:155–62. doi:10.1016/S0924-2244(00)89024-X.

[64] Ahn JY, Kil DY, Kong C, Kim BG. Comparison of oven-drying methods for determination of moisture content in feed ingredients. Asian-Australasian J Anim Sci 2014;27:1615–22. doi:10.5713/ajas.2014.14305.

[65] Novotny L, King J, Phillips K, Thiex N. Recommendations and Critical Factors In Determining Moisture in Animal Feeds 2018:1–16.

[66] Thiex N, Richardson CR. Challenges in measuring moisture content of feeds. J Anim Sci 2003;81:3255–66. doi:10.2527/2003.81123255x.

[67] Agger JW, Nilsen PJ, Eijsink VGH, Horn SJ. On the Determination of Water Content in Biomass Processing. Bioenergy Res 2014;7:442–9. doi:10.1007/s12155-013-9388-2.

[68] Kreuger E, Nges I, Björnsson L. Ensiling of crops for biogas production: Effects on methane yield and total solids determination. Biotechnol Biofuels 2011;4:1–8. doi:10.1186/1754-6834-4-44.

[69] Begum S, Anupoju GR, Sridhar S, Bhargava SK, Jegatheesan V, Eshtiaghi N. Evaluation of single and two stage anaerobic digestion of landfill leachate: Effect of pH and initial organic loading rate on volatile fatty acid (VFA) and biogas production. Bioresour Technol 2018;251:364–73. doi:10.1016/j.biortech.2017.12.069.

[70] Mukengele M, Oechsner H. Effectof ensiling on the specific metahne yield of maize. Landtechnik 2007;62:20–1.

[71] Schievano A, Tenca A, Lonati S, Manzini E, Adani F. Can two-stage instead of one-stage anaerobic digestion really increase energy recovery from biomass? Appl Energy 2014;124:335–42. doi:10.1016/j.apenergy.2014.03.024.

[72] Porter MG, Murray RS. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. Grass Forage Sci 2001;56:405–11. doi:10.1046/j.1365-2494.2001.00292.x.

[73] APHA, AWWA and WPCF (2012) Standard Methods for the Examination of Water and Wastewater. 22st Edition, American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington DC.

[74] Raposo F, de la Rubia MA, Borja R, Alaiz M, Beltrán J, Cavinato C, et al. An interlaboratory study as useful tool for proficiency testing of chemical oxygen demand measurements using solid substrates and liquid samples with high suspended solid content. Talanta 2009;80:329–37. doi:10.1016/j.talanta.2009.06.071.

[75] Raposo F, Fernández-Cegrí V, De la Rubia MA, Borja R, Beltrán J, Cavinato C, et al. Quality improvement in determination of chemical oxygen demand in samples considered difficult to analyze, through participation in proficiency-testing schemes. TrAC - Trends Anal Chem 2010;29:1082–91. doi:10.1016/j.trac.2010.06.005.

[76] Raposo F, de la Rubia MA, Borja R, Alaiz M. Assessment of a modified and optimised method for determining chemical oxygen demand of solid substrates and solutions with high suspended solid content. Talanta 2008;76:448–53. doi:10.1016/j.talanta.2008.03.030.

[77] Noguerol-Arias J, Rodríguez-Abalde A, Romero-Merino E, Flotats X. Determination of chemical oxygen demand in heterogeneous solid or semisolid samples using a novel method combining solid dilutions as a preparation step followed by optimized closed reflux and colorimetric measurement. Anal Chem 2012;84:5548–55. doi:10.1021/ac3003566.

[78] Andre L, Pauss A, Ribeiro T. A modified method for COD determination of solid waste , using a commercial COD kit and an adapted disposable weighing support. Bioprocess Biosyst Eng 2017;40:473–8. doi:10.1007/s00449-016-1704-8.

[79] Cazaudehore G, Schraauwers B, Peyrelasse C, Lagnet C, Monlau F. Determination of chemical oxygen demand of agricultural wastes by combining acid hydrolysis and commercial COD kit analysis. J Environ Manage 2019;250:109464. doi:10.1016/j.jenvman.2019.109464.

[80] Rasit N, Idris A, Harun R, Wan Ab Karim Ghani WA. Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: An overview. Renew Sustain Energy Rev 2015;45:351–8. doi:10.1016/j.rser.2015.01.066.

[81] Luque de Castro MD, Priego-Capote F. Soxhlet extraction: Past and present panacea. J Chromatogr A 2010;1217:2383–9. doi:10.1016/j.chroma.2009.11.027.

[82] Ramluckan K, Moodley KG, Bux F. An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method. Fuel 2014;116:103–8. doi:10.1016/j.fuel.2013.07.118.

[83] Picard S, Beline F, Girault R, Bridoux G, Cambert C, Davenel A. Determination of lipid fraction from organic wastes using Nuclear Magnetic Resonance (NMR): Comparison to the soxhlet method, IWA (Ed.), 13th World Congress on Anaerobic Digestion, Santiago de Compostela, Spain.; 2014.

[84] Yenigün O, Demirel B. Ammonia inhibition in anaerobic digestion: A review. Process Biochem 2013;48:901–11. doi:10.1016/j.procbio.2013.04.012.

[85] Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen IJ. Protein determination—method matters. Foods 2018;7. doi:10.3390/foods7010005.

[86] Dumas A. (1826): Annales de chimie, 33,342.

[87] Kjeldahl J. A New Method for the Determination of Nitrogen in Organic Matter. Zeitschrift Für Anal Chemie 1883;22:366–82. doi:http://dx.doi.org/10.1007/BF01338151.

[88] Sweeney RA, Rexroad PR. Comparison of LECO FP-228 “nitrogen determinator” with AOAC copper catalyst Kjeldahl method for crude protein. J Assoc Off Anal Chem 1987;70:1028–30.

[89] Thompson M, Owen L, Wilkinson K, Wood R, Damant A. A comparison of the Kjeldahl and Dumas methods for the determination of protein in foods, using data from a proficiency testing scheme. Analyst 2002;127:1666–8. doi:10.1039/B208973B.

[90] Jones DB. Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Protein. USDA (US Department of Agriculture) Circular Series, No. 183, 1931:1–21.

[91] Sosulski FW, Imafidon GI. Amino Acid Composition and Nitrogen-to-Protein Conversion Factors for Animal and Plant Foods. J Agric Food Chem 1990;38:1351–6. doi:10.1021/jf00096a011.

[92] Lourenço SO, Barbarino E, Lavín PL, Marquez UML, Aidar E. Distribution of intracellular nitrogen in marine microalgae : Calculation of new nitrogen-to-protein conversion factors. Eur J Phycol 2004;39:17–32. doi:10.1080/0967026032000157156.

[93] Mariotti F, Tomé D, Mirand PP. Converting Nitrogen into Protein - Beyond 6.25 and Jones’ Factors. Crit Rev Food Sci Nutr 2008;48:177–84. doi:10.1080/10408390701279749.

[94] Le C, Stuckey DC. Colorimetric measurement of carbohydrates in biological wastewater treatment systems : A critical evaluation. Water Res 2016;94:280–7. doi:10.1016/j.watres.2016.03.008.

[95] Dreywood R. Qualitative test for carbohydrate material. Ind Eng Chem Anal 1946. doi:10.1021/i560156a015.

[96] Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. A colorimetric method for the determination of sugars. Nature 1951.

[97] Piccolo A, Zena A, Conte P. A comparison of acid hydrolyses for the determination of carbohydrate content in soils. Commun Soil Sci Plant Anal 1996;27:2909–15. doi:https://doi.org/10.1080/00103629609369749.

[98] Van Soest PJ. Development of a Comprehensive System of Feed Analyses and its Application to Forages. J Anim Sci 1967;26:119–28. doi:doi:10.2527/jas1967.261119x.

[99] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polyssacarides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97. doi:10.3168/jds.S0022-0302(91)78551-2.

[100] Giger-Reverdin S. Review of the main methods of cell wall estimation : interest and limits for ruminants. Anim Feed Sci Technol 1995;55:295–334. doi:https://doi.org/10.1016/0377-8401(95)00791-K.

[101] D'heer BG, Boever JL De, Vanacker JM, Boucqué C V. The filter bag versus the conventional filtration technique for the determination of crude fibre and Van Soest cell wall constituents. J Anim Feed Sci 2000;9:513–26. doi:https://doi.org/10.22358/jafs/68072/2000.

[102] Ferreira G, Mertens DR. Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. Anim Feed Sci Technol 2007;133:335–40. doi:10.1016/j.anifeedsci.2006.04.010.

[103] Krueger CG, Albrecht KA, Reed JD, Bures EJ, Owens VN. Sodium sulphite effects on recovery and composition of detergent fibre and lignin from forage legumes varying in levels of proanthocyanidins. J Sci Food Agric 1999;79:1351–6.doi:https://doi.org/10.1002/(SICI)1097-0010(199908)79:11.

[104] Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. J AOAC Int 2002;85:1217–40.

[105] Cassida KA, Turner KE, Foster JG, Hesterman OB. Comparison of detergent fiber analysis methods for forages high in pectin. Anim Feed Sci Technol 2007;135:283–95. doi:10.1016/j.anifeedsci.2006.07.004.

[106] Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. J Agric Food Chem 2010;58:9043–53. doi:10.1021/jf1008023.

[107] Templeton DW, Scarlata CJ, Sluiter JB, Wolfrum EJ. Compositional Analysis of Lignocellulosic Feedstocks. 2. Method Uncertainties. J Agric Food Chem 2010;58:9054–62. doi:10.1021/jf100807b.

[108] Hatfield R, Fukushima RS. Can Lignin Be Accurately Measured? Crop Sci 2005;45:832–9. doi:10.2135/cropsci2004.0238.

[109] Rowland AP, Roberts JD. Lignin and cellulose fractionation in decomposition studies using acid-detergent fibre methods. Commun Soil Sci Plant Anal 1994;25:269–77. doi:10.1080/00103629409369035.

[110] Klason P. Beitrag zur Kenntnis der Konstitution des Fichtenholz-Lignins (II). Ber Dtsch Chem Ges 1922;55:448–55. doi:https://doi.org/10.1002/cber.19220550219.

[111] TAPPI. T 222 om-02. Acid-insoluble lignin in wood and pulp. 2002 n.d.

[112] Hatfield RD, Jung HG, Ralph J, Buxtond DR, Weimer PJ. A Comparison of the Insoluble Residues Produced by the Klason Lignin and Acid Detergent Lignin Procedures. J Sci Food Agric 1994;65:51–8. doi:https://doi.org/10.1002/jsfa.2740650109.

[113] Jung HG, Varel VH, Weimer PJ, Ralph J. Accuracy of Klason Lignin and Acid Detergent Lignin Methods As Assessed by Bomb Calorimetry. J Agric Food Chem 1999;47:2005–8. doi:10.1021/jf981250q.

[114] Godin B, Agneessens R, Gerin P, Delcarte J. Structural Carbohydrates in a Plant Biomass: Correlations between the Detergent Fiber and Dietary Fiber Methods. J Agric Food Chem 2014;62:5609–16. doi:dx.doi.org/10.1021/jf500924q.

[115] Boruff CS, Buswell AM. The Anaerobic Fermentation of Lignin. J Am Chem Soc 1934;56:886–8. doi:10.1021/ja01319a037.

[116] Colberg PJ, Young LY. Anaerobic Degradation of Soluble Fractions of [14C-Lignin]Lignocellulose. Appl Environ Microbiol 1985:345–9.

[117] Susmel P, Stefanon B. Aspects of lignin degradation by rumen microorganisms. J Biotechnol 1993;30:141–8. doi:https://doi.org/10.1016/0168-1656(93)90035-L.

[118] Den Camp HJMO, Verhagen FJM, Kivaisi AK, Windt FE De, Lubberding HJ, Gijzen HJ, et al. Effects of lignin on the anaerobic degradation of (ligno) cellulosic wastes by rumen microorganisms. Appl Microbiol Biotechnol 1988;29:408–12.

[119] Grabber JH. How Do Lignin Composition, Structure, and Cross-Linking Affect Degradability? A Review of Cell Wall Model Studies. Crop Sci 2005;45:820–31. doi:10.2135/cropsci2004.0191.

[120] Stinson JA, Ham RK. Effect of Lignin on the Anaerobic Decomposition of Cellulose As Determined through the Use of a Biochemical Methane Potential Method. Environ Sci Technol 1995;29:2305–10. doi:10.1021/es00009a023.

[121] Khan MU, Ahring BK. Lignin degradation under anaerobic digestion: Influence of lignin modifications - A review. Biomass and Bioenergy 2019;128. doi:10.1016/j.biombioe.2019.105325.

[122] Montgomery H. Preventing the progression of climate change: One drug or polypill? Biofuel Res J 2017;4:536–536. doi:10.18331/BRJ2017.4.1.2.

[123] Tabatabaei M, Aghbashlo M, Valijanian E, Panahi HKS, Nizami AS, Ghanavati H, et al. A comprehensive review on recent biological innovations to improve biogas production, Part 1: Upstream strategies. Renew Energy 2020;146:1204–20. doi:10.1016/j.renene.2019.07.037.

[124] Tabatabaei M, Aghbashlo M, Valijanian E, Panahi HKS, Nizami AS, Ghanavati H, et al. A comprehensive review on recent biological innovations to improve biogas production, Part 2: Mainstream and downstream strategies. Renew Energy 2020;146:1392–407. doi:10.1016/j.renene.2019.07.047.

[125] Aghbashlo M, Tabatabaei M, Soltanian S, Ghanavati H, Dadak A. Comprehensive exergoeconomic analysis of a municipal solid waste digestion plant equipped with a biogas genset. Waste Manag 2019;87:485–98. doi:10.1016/j.wasman.2019.02.029.

[126] Barati MR, Aghbashlo M, Ghanavati H, Tabatabaei M, Sharifi M, Javadirad G, et al. Comprehensive exergy analysis of a gas engine-equipped anaerobic digestion plant producing electricity and biofertilizer from organic fraction of municipal solid waste. Energy Convers Manag 2017;151:753–63. doi:10.1016/j.enconman.2017.09.017.

[127] Almasi F, Soltanian S, Hosseinpour S, Aghbashlo M, Tabatabaei M. Advanced Soft Computing Techniques in Biogas Production Technology. In: Tabatabaei M, Ghanavati H, editors. Biogas. Biofuel Biorefinery Technol., Springer International Publishing; 2018, p. 333–49. doi:10.1007/978-3-319-77335-3.