Romanian Biotechnological Letters *Vol. 21, No. 5, 2016*

ORIGINAL PAPER

Influence of the hydrostatic pressure on biogas production in anaerobic digesters

Received for publication, November 11th, 2014
Accepted, December 12th, 2015

CARMEN MATEESCU
National Institute for Research and Development in Electrical Engineering ICPE-CA, Romania, e-mail: carmen.mateescu@icpe-ca.ro

Abstract

This paper aimed to investigate the influence of hydrostatic pressure on methanogens activity and consequently on biogas production in anaerobic digesters. The quality of biogas has been evaluated in terms of methane content in the gas resulting from fermentative processes in anoxic environments. A total of four experimental studies were carried out by using stainless steel pressure vessels, where an organic substrate, consisting of a mixture of cattle manure and unfiltered whey, has been conditioned in mesophylic temperature regime for pressures set at 0 bar, 2 bar, 4 bar and 6 bar, respectively. The activity of methanogens was determined by instrumental chemical analysis, using a gas chromatograph fitted with a flame ionization detector. Biogas generated in fermentative processes has been periodically sampled and analyzed during the period of the highest microbial activity. Furthermore, the number of methanogens before anaerobic digestion and after conditioning under various pressures has been determined by using the most probable number analytical method.

Keywords: waste, biogas, anaerobic digestion, microbial growth, hydrostatic pressure

1. Introduction

Some natural fermentative processes occurring in swamps or on the seabed made microbiologists presume that biogas production is not affected by pressure and that the cellular metabolism of methanogens runs with no any changes even in conditions of high pressure. Studies have shown that methanogens are active in deep sea waters and marine sediments but this is possible in the presence of some salts like sulphates and special environmental conditions where methanogens work together with sulphate-reducing bacteria which generate methane and hydrogen sulfide (R. M. MITTERER [1]). In some low-pressure metabolism experiments, Kral et al. have shown that methane production occurs at reduced pressures. Methanogens have been shown to produce methane at reduced pressures, in the presence of perchlorate salts, using carbonate as a sole carbon source, and to survive desiccation at both 1 bar and 6 mbar for extended periods of time (T.A. KRAL & al. [2]). However, results achieved for anaerobic digestion to methane production which took place in vertical reactors have proved a significant decrease in biogas production when, for various reasons, the pressure above the organic slurry in the digester got increased. Theoretical studies have shown that, in ordinary cylinder-conical shaped vertical digesters, generating biogas practically occurs preponderantly in the upper layer of the organic slurry for a maximum depth of 4 m (V. NICOLIC & al. [3]). The remaining volume of organic mass does not produce biogas since methanogenic microorganisms have been living latently in the lower layers of organic substrate. By homogenization, such inactive volumes will get lower pressure areas from the upper layers and the metabolic activity of methanogens will be resumed actively with biogas generation.

By fermentation of a large amount of biomass in tall fermentation tanks for producing biogas in industrial processes, an increased gas production could be provided if microbial activity was optimal, so that the biochemical processes to be carried out efficiently. In their research paper, Bal and Dhagat underline that in order to maintain the population of anaerobes, large reactor volumes or higher hydraulic retention times are required. But provision of larger reactor volumes or higher hydraulic retention times ultimately leads to higher capital cost. Among notable disadvantages, it has low synthesis/reaction rate hence long start up periods and difficulty in recovery from upset conditions. Special attention is, therefore, warranted towards, controlling the factors that affect process adversely; important among them being environmental factors such as temperature, pH and concentration of toxic substances (A. S. BAL & al. [4]). It can be stated that fermentation in large reactors could be more economically advantageous compared to those processes that occur in small vessels. These considerations have led the author to approach this topic and develop some laboratory experimental research aimed to emphasize the influence of hydrostatic pressure on metabolic activity of methanogenic microorganisms and consequently on biogas yield in anaerobic digesters. Knowledge of the fundamental processes involved in the anaerobic digestion is very important for the design, construction and operation of the biogas plants. Pressure effect on the growth of *Methanococcus thermolithotrophicus* in un-buffered and buffered minimal media has been studied by G. Bernhardt et al. for the elevated temperatures in the thermophylic range (G. BERNARDT & al. [5]). Preliminary experiments with the thermophilic, methanogenic archaebacterium *Methanococcus thermolithotrophicus* have shown that bacterial growth depends on both temperature and pressure. At the optimum temperature, increased hydrostatic pressure up to 50 MPa enhanced the growth yield; at a pressure above 75 MPa, cell lysis dominated. Changes in cell proliferation were accompanied by changes in morphology (G. BERNARDT & al. [6]). Despite very interesting results obtained for thermophylic methanogens, these cannot be extrapolated to mesophylic temperatures. Since anaerobic technologies have been largely developed for mesophylic range, studying the influence of pressure on biogas yield has been found of great interest for developers of anaerobic digesters (Y. CHEN & al. [7]). Based on these assumptions, the objective of the research was to show how pressure affects the metabolic activity of methanogens in mesophylic conditions. In this scope, four experimental investigations for anaerobic fermentation of organic slurry consisting of a mixture of cattle manure and unfiltered whey have been carried out, by using stainless steel pressure vessels. The monitoring of the fermentation process has been weekly performed over a period of three months, the operational parameters (temperature, pressure) being monitored daily. Biogas generated in fermentative processes has been periodically sampled and analyzed during the period of the highest microbial activity.

2. Materials and Methods

The experiments of anaerobic digestion under conditions of high hydrostatic pressure were carried out in stainless steel laboratory vessels, each container having a capacity of 5 liters. The pressure vessels were manufactured by the German company Krautzberger and have been fitted with sealed removable lids which are able to keep tightly closed the air or various other inert gases such as argon, nitrogen used to pressurize the liquid or other materials with low viscosity. These laboratory recipients are equipped with output for the material, air pressure pipe fittings, pressure relief valve and pressure gauge. For the experiments of anaerobic digestion under high pressure conditions, there have been used a total of four such containers, which operated under the following conditions of pressure: 0 bar, 2 bar, 4 bar and 6 bar, respectively. For pressurization, Nitrogen type 5.0 of 9.999%

purity has been used. To create and maintain a constant temperature under a mesophylic temperature range, a 190-liter capacity temperature and climate test chamber type Vötsch Industrietechnik VC 4018, with digital control unit display of temperature and humidity, has been used for conditioning of the pressure vessels during the fermentative processes of the organic substrate with temperature deviation lower than ±0.5K.

Figure 1 shows the experimental set-up diagram, with indication of materials, equipments and technological steps involved in the anaerobic digestion experiments.

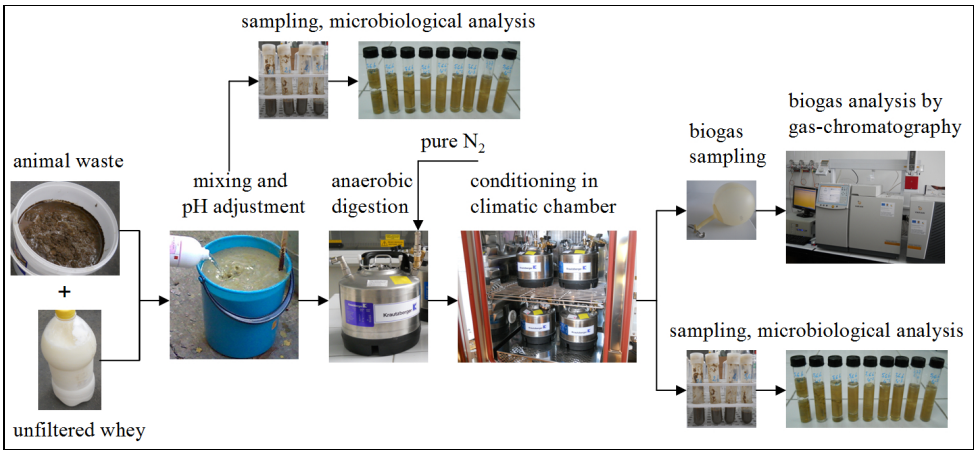

Fig. 1. Experimental set-up diagram for the anaerobic digestion experiments

The quality of the organic material used as biomass for the anaerobic digestion represents the main factor determining the biogas production. This is the case when it is necessary to obtain biogas as a significant energy surplus to the energy self-consumption of the biogas required for biomass heating in the biogas plant.

Thus, the biomass must ensure the proper environment for growth and metabolic activity of the anaerobic microorganisms that contribute to digestion of the organic substrate and finally to production of biogas. This environment must meet the following conditions: contain biodegradable organic matter; have a high water content, over 90%; have a neutral or near neutral pH (6.8 to 7.3); contain carbon and nitrogen in a proper ratio (C:N ratio = 15-25); not contain substances which are inhibitory to microorganisms (heavy metals, detergents, antibiotics, high concentrations of sulfate, formaldehyde, phenols and poly-phenols etc.). In order to produce biogas, organic materials of various sources can be used. The most suitable substrates are mainly farm waste and wastewaters, vegetable waste, household leavings, residuals generated by food and beverages industries, algae and aquatic waste etc. For these experiments of anaerobic digestion to be performed in the four pressure vessels, an organic mixture of cattle manure and unfiltered whey which were diluted with water has been used. The volumetric ratio of the components in the total feedstock volume of 7 liters was the following: 2 liters unfiltered whey (28.57%), 4.5 liters cow manure (64.28%) and dilution water 0.5 liters (7.14%). It is well known that anaerobic digestion processes are carried out optimally in acidity between 6.8 and 8. Any pH values below 6.5 significantly slow the conversion of carbon dioxide and of acetate ion, resulting even in the complete stop of the methane production. Given the particular sensitivity of methanogenic bacteria to small variations in pH, to control the proper operation of the fermentative process implies to keep

pH parameter within a relatively narrow range and allow very slight variations (N. S. SUNADA & al. [8]). Likewise, for a proper start of the fermentative processes it is important that the acidity of the organic mass fed into the digester be not less than 7.2, being preferably in the range of 7.2 - 8. In this respect, before feeding the mixture into the anaerobic vessels it is important to adjust the acidity of the organic substrate by adding an alkali (lime, sodium hydroxide etc.). Before starting the experiments of anaerobic digestion, the acidity of the organic slurry has been checked by using a Hanna Instruments pH-meter, in accordance with standard SR ISO 10523:2009. The initial pH of the mixture was found to be 6.7, fairly lower than the recommended pH range. Therefore, a progressive addition under continuous stirring of a 0.1N sodium hydroxide solution to the mixture of biomass was required in order to adjust the acidity. Finally, the pH value was increased up to 7.4, which complied with the requirements for an efficient anaerobic digestion. The water content and respectively the total solid content of the biomass have been determined after pH adjustment, according to the standard SR EN 14346:2008. Given the heterogeneity of the mixture, as well as the presence of some floaters and suspended solids, the water level has been determined for a total number of three samples taken randomly after the slurry homogenization. As a valid result, the average value of the three measurements has been considered. Table 1 presents the values of the water content as well as the total solids content for the three biomass samples used for analysis.

Table 1. Water content and total solids content of the biomass

Sample No.	Sample mass, [g]	Water level, [weigh %]	Total solids, [weigh %]
1	24.761	96.433	3.566
2	20.326	91.965	8.034
3	31.327	96.606	3.393
Average value		95.002	4.497

Equal volumes of 1200 ml biomass mixture were taken for experiments in each of the four pressure vessels. Achieving pressure conditions inside the vessels was done by introducing nitrogen until reaching the established pressure values indicated by the pressure gauge attached to the container. The four containers ready for experiments were placed into the climate chamber for conditioning at 39 ± 0.5ºC for a period of 4 weeks. The influence of hydrostatic pressure on the bacterial activity in the organic slurry and implicitly on biogas production has been assessed by a qualitative analysis of the fermentative gases in terms of methane content, by using a gas chromatograph type Varian 450-GC. The gas chromatograph is fitted with both a FID (Flame Ionization Detector) and an ECD (Electron Capture Detector), coupled to a Mass Spectrometer type MS-240, by which complex mixtures of hydrocarbons may be separated, identified and quantified. This instrument is widely used in applicative research for determination of the chemical composition in a gaseous or liquid mixture. The gases mixture has weekly been sampled from the conditioned pressure vessels using a natural latex rubber balloon special designed for hydrocarbons sampling, which has been attached to the chromatograph.

3. Results and Discussions

The physiological and ecological investigation of methanogens has often involved monitoring of gases released under the complex metabolic processes. In general, the metabolism of the biological species producing biogas implies multiple biochemical reactions related to the oxidation of hydrogen, concomitantly with reduction of carbon dioxide. Assessment of the microbial activity by means of the biogas production has been achieved in the current experimental research by instrumental chemical analysis. The qualitative

assessment of biogas has been performed for a total experimental period of two months after the pressure vessels had been initially conditioned for four weeks in a mesophilic temperature range of 39 ± 0.5 ºC. It is supposed that this conditioning period of four weeks could be assimilated with time of both the initial lag phase and exponential phase in bacterial growth, being recorded the top of the microbial activity for biogas production at the end of these two phases of bacterial population growth. Volumes of biogas were sampled from the pressure vessels using the rubber sampler. The flask was attached to the gas chromatograph through a suction pipe from the gas chromatograph configuration. The level of methane in biogas was determined for all biomass samples corresponding to the four established conditioning pressures of 0 bar, 2 bar, 4 bar and 6 bar, based on the recorded chromatograms. The experimental values of the methane content are shown in the Table 2.

Table 2. Methane content in biogas for various conditioning pressure

Date	Methane content in biogas, [volume %]			
	0 bar	2 bar	4 bar	6 bar
26.07.2012	72.91	7.66	3.80	2.85
31.07.2012	90.89	10.55	4.01	3.08
06.08.2012	71.82	11.89	4.49	2.96
11.08.2012	78.58	14.42	4.50	3.35
17.08.2012	71.69	14.76	5.06	3.38
23.08.2012	69.75	16.13	5.12	3.71
28.08.2012	69.81	16.97	5.44	3.94
03.09.2012	83.55	21.59	7.04	4.31
08.09.2012	96.35	47.50	17.91	10.66
14.09.2012	90.00	43.66	18.73	14.70
20.09.2012	86.33	46.88	21.58	13.79

Figure 2 indicates the graphic representation of variation of methane concentration in time, for the selected working pressures used in the four experiments. Analyzing the data presented in Table 2, it can be noticed that the methane content in biogas is significantly superior for the biomass sample exposed for anaerobic digestion in standard pressure of 0 bar, compared to other biomass samples which have been fermented under various hydrostatic pressure up to 6 bar. It is clearly proved that the samples conditioned for fermentation at pressures of 2, 4 and 6 bars have produced a definitely lower quality biogas, which confirms the theory that the hydrostatic pressure has an adversely negative influence on methanogenesis.

Moreover, the graphic of Figure 2 indicates the oscillations of methane concentration in time which have been found to be more evident for the pressure of 0 bar. A proper interpretation of these oscillations in methane concentration for each work pressure can be drawn up if look for a thorough and deeply understanding of the biochemical processes which address the fermentative processes in absence of oxygen. Anaerobic digestion is a very complex biochemical process that involves several different types of hydrolytic, acidogenic and methanogenic bacteria. Complex polymeric materials such as polysaccharides, proteins, and lipids (fat and grease) are firstly hydrolyzed to soluble products by extracellular enzymes, secreted by microorganisms, so as to facilitate their transport or diffusion across the cell membrane.

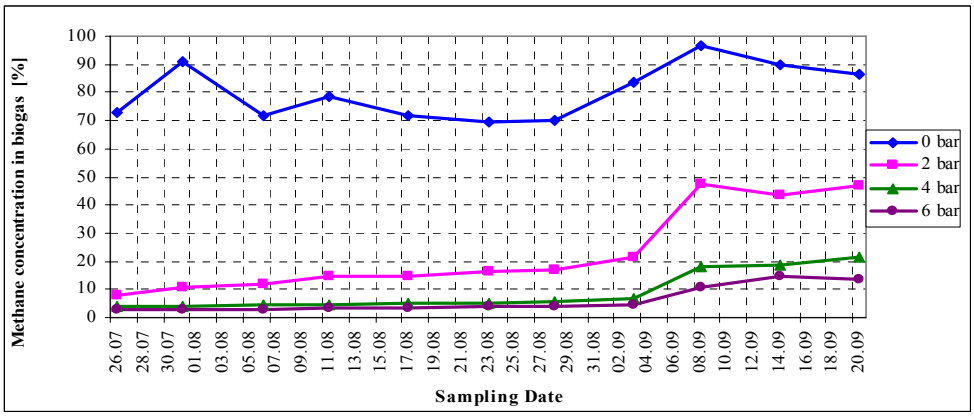

Fig. 2. Variation of methane content in biogas for different conditioning pressures

These relatively simple, soluble compounds are fermented or anaerobically oxidized, further to short-chain fatty acids, alcohols, carbon dioxide, hydrogen, and ammonia. The short-chain fatty acids (other than acetate) are converted to acetate, hydrogen gas, and carbon dioxide. Methanogenesis finally occurs from the reduction of carbon dioxide and acetate by hydrogen to produce methane (Bal & al. [4]). The acidogenic bacteria multiply and grow up very fast. They are not very sensitive to any variations in the physical parameters of the environment (temperature, acidity) compared to methanogenic bacteria that can multiply and grow up more slowly, while being very sensitive to such variations. The acid fermentation stage is much faster as compared to the methane fermentation stage. This means that a sharp increase in the easily degradable organics will result in a faster acid production with a subsequent accumulation of acids in the organic slurry. This process definitely inhibits the methanogenesis step. Methanogenic bacteria are slow growers and are considered the rate-limiting component in the anaerobic digestion process (Bal & al. [4]). To conclude with, the methane content oscillations can be explained by the heterogeneous nature of the organic mass to be fermented, on the one hand, correlated with the multitude of microbial species working together and living in antibiosis, on the other hand. Dependencies of the hydrostatic pressure on dynamics of average methane concentration for the all experiments carried out over a total experimental period of two months is shown in figure 3. Such mixture of various microbial consortiums must co-exist in the same space of the fermentation mass, although they disturb each other in terms of optimal acidity for a healthy growth. The methanogens slow down their enzymatic activity or even die if the environment conditions in the fermentation reactor are not very appropriate. When methanogens become not active or die, the acidogenic bacteria continue to multiply and grow up quickly. As an effect, an increase of the volatile acids concentration occurs, thus lowering the pH of the organic mass in the reactor and stopping the biogas production. In this way, the system faces constant variations of the organic mass acidity but also slight variations of the biochemical reactions heat. As a result of a complex combination of different types of bacteria, the anaerobic digestion is a very unstable biochemical process, with the risk of slowing or stopping the production of biogas from any slight variations of the environmental parameters. Such variations occur during fermentation even in case if the value of the temperature is kept constant.

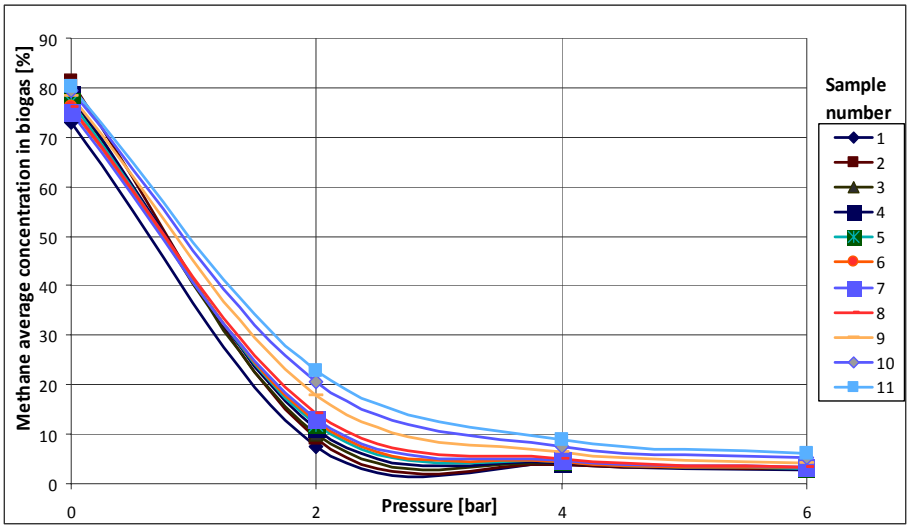

Fig. 3. Pressure dependencies on dynamics of methane average concentration

The complexity of the organic compounds coupled with the multitude of microbial species that require different growth conditions have as effect significant oscillations of the methane concentrations, since methane is produced by the metabolism of methanogenic microorganisms that live in antibiosis with other microbial species in the system. It is interesting to notice that this variation of the methane concentration in time is fairly less evident at high pressures. This is probably due to the precisely inhibitory effect of hydrostatic pressure on the activity of microorganisms responsible for decomposition of organic mass. With the increasing pressure in the system, the microbial activity is clearly reduced, the level of decomposition of organic matter is significantly diminished and therefore the bacterial populations generate several specific metabolites (organic acids, methane, carbon dioxide, etc.) in much smaller quantities which make the oscillations in methane production be also reduced. It is well known that anaerobic digestion is a quite slow biochemical process which requires weeks or even months to complete the decomposition of the organic matter to the final stage of biogas. The more appropriate conditions for an effective microbial activity are, the time required to finalize the fermentation process is shorter. As shown in the present experiments, the hydrostatic pressure has clearly an inhibitory effect on fermentative processes, so that the biochemical processes are much slowed. It is supposed that for longer experimentation period, the methane concentrations will increase gradually but slowly, also for cases where the system pressure is high. In these conditions, the methane production is not stopped, but the biochemical processes take place more slowly. The methane concentration will increase slowly but progressively until the complete decomposition of the organic compounds. The exceptional quality of biogas obtained in the anaerobic digestion experiments proved that anaerobic technologies for waste to biogas represent a very feasible option to produce energy simultaneously with wastewater treatment. From experimental studies, it can be underlined that a maximum efficiency of the biochemical processes can be reached if the hydrostatic pressure stress is minimal.

4. Conclusions

The selected mixture of organic waste and wastewaters possesses an important methanogenic potential compared to other organic materials commonly recovered to biogas production. For fermentation under no any additional pressure stress, the organic slurry has generated a very qualitative biogas that reached a methane content of 96.350 % in weigh, which is much above the average of 60% methane in biogas as defined in literature. This level of methane reached in biogas produced in laboratory experiments is nearly as high as the hydrocarbons level in natural gases. On the other hand, the organic samples exposed at pressures of 2, 4 and 6 bar respectively, have generated biogas of a much lower quality, which confirm in this way some theoretical suppositions that the hydrostatic pressure negatively influences the methanogenesis in anaerobic digesters. The assessment of biogas quality by instrumental analysis along with the determination of methanogens cell numbers per unit volume for the raw material and fermented slurry, has demonstrated a definitely higher efficiency of anaerobic digestion for the organic mixture exposed in anaerobic condition and lower hydrostatic pressure. These laboratory results lead to conclusion that efficiency of biogas production in vertical reactors is diminished with the height of the digester due to the negative influence of the hydrostatic pressure on the biogas producing microorganisms.

5. Acknowledgments

The author thanks Ministry of Education and Research and particularly National Authority for Scientific Research who funded these experimental studies under the National Research Program Nucleu, Project No. 0935/2009.

References

1.0 - R. M. MITTERER, Methanogenesis and sulfate reduction in marine sediments: A new model, *Earth and Planetary Science Letters*, **295**, Issue 3-4, pp. 358-366 (2010).

2.0 - T. A. KRAL, , T. S. ALTHEIDE, A. E. LUEDERS, T. H. GOODHART, B. T. VIRDEN, W. BIRCH, K. L. HOWE, P. GAVIN, Methanogens: a model for life on Mars, Astrobiology Science Conference 2010: Evolution and Life: Surviving Catastrophes and Extremes on Earth and Beyond, held April 26-20, 2010 in League City, Texas. LPI Contribution No. 1538, pp.5084 (2010).

3.0 - V. NIKOLIC, T. VINTILĂ *Producerea şi utilizarea biogazului pentru obţinerea de energie,* Editura Mirton Timişoara, Romania (2009).

4.0 - A. S. BAL, N. N. DHAGAT, Upflow anaerobic sludge blanket reactor – a review., *Indian Journal of Environment Health*, **43**(2),1-82 (2001).

5.0 - G. BERNHARDT, A. DISTCHE, R. JAENICKE, B. KOCH, H. LÜDEMANN, K. STETTER, Effect of carbon dioxide and hydrostatic pressure on the pH of culture media and the growth of methanogens at elevated temperature, *Applied Microbiology and Biotechnology* **28**,176-181 (1988).

6.0 - G. BERNHARDT, R. JAENICKE, H. LÜDEMANN, High-Pressure Equipment for Growing Methanogenic Microorganisms on Gaseous Substrates at High Temperature, *Applied and Environmental Microbiology*, **53**(8) (1987).

7.0 - Y. CHEN, J. J. CHENG, K. S. Creamer, Inhibition of anaerobic digestion process: A review, *Bioresource Technology* **99**, 4044-4064 (2008).

8.0 - N. S. SUNADA, A. C. ORRICO, M. A. PREVIDELLI ORRICO, F. MIRANDA DE VARGAS, R. G. GARCIA, A. R. MENDES FERNANDES, Potential of biogas and methane production from anaerobic digestion of poultry slaughterhouse effluent, *Revista Brasileira de Zootecnia,* **41** (11), On-line version ISSN 1806-92902012 (2001).

Romanian Standard SR ISO 10523:2009 Water quality – Determination of pH

Romanian Standard SR EN 14346:2008 Characterization of waste. Calculation of dry matter by determination of dry residue or water content