Wioleta ADAMCZAK, Mariusz ADAMSKI, Tomasz WOJCIECHOWSKI, Mirosław CZECHLOWSKI
Uniwersytet Przyrodniczy w Poznaniu, Instytut Inżynierii Biosystemów
ul. Wojska Polskiego 28, 60-637 Poznań, Poland
e-mail:mariusz.adamski1@wp.pl

CALIBRATION MODELS FOR EVALUATION OF ACETIC ACID AND DRY MATTER CONTENTS IN COMPOUND SUBSTRATES OF AGRICULTURAL ORIGIN

Summary

The paper presents the results of modeling an acetic acid and dry matter content carried out by the method of transforming spectral spectra VIS-NIR using software, Unscrambler X 10.1. To build the models reference data obtained applying the Nordman's method as well as dryer-weight one were used. In respect of of the proposed chemometric method the measuring range between 400 nm and 2170 nm was tested using a reflective method of obtaining spectral spectra. The studies indicated that the prepared calibration models of acetic acid and dry matter content in the fermenting mixture of corn silage and manure obtain the best fit factor (R^2) for the measuring range of 950 nm to 2170 nm. The best ratio of fit (R^2) for the model of dry matter content was 0.86, and for acetic acid content 0.68.
Key words: *model calibration, acetic acid, dry matter, spectral analysis*

MODELE KALIBRACYJNE DO OCENY ZAWARTOŚCI KWASU OCTOWEGO I MATERII SUCHEJ W MIESZANKACH SUBSTRATÓW POCHODZENIA ROLNICZEGO

Streszczenie

Przedstawiono efekty modelowania zawartości kwasu octowego i materii suchej zrealizowane przy wykorzystaniu metody transformacji widm spektralnych VIS-NIR przy zastosowaniu oprogramowania Unscrambler X 10.1. Do budowy modeli wykorzystano dane referencyjne uzyskane przy zastosowaniu metody Nordmana i metody suszarkowo-wagowej. W zakresie zaproponowanej metody chemometrycznej testowano zakres pomiarowy między 400 nm a 2170 nm przy zastosowaniu re-fleksyjnej metody pozyskiwania widm spektralnych. W wyniku przeprowadzonych badań wskazano, że wytworzone modele kalibracyjne zawartości materii suchej oraz kwasu octowego w fermentującej mieszance kiszonki kukurydzy i gnojowicy uzyskują najlepszy współczynnik dopasowania (R^2) dla zakresu pomiarowego od 950 nm do 2170 nm. Najlepszy współczynnik dopasowania (R^2) dla modelu zawartości materii suchej wyniósł 0,86, a dla modelu zawartości kwasu octowego 0,68.
Słowa kluczowe: *model kalibracyjny, kwas octowy, materia sucha, analiza widmowa*

1. Introduction

Noticeable in the last two decades the development of mass spectrometry technique in the agri-food sector, entails an increasing number of practical applications of spectrometric analysis of biomaterials, starting from monitoring soil variability, variability of crop yield and by monitoring the quality of raw materials for food production, as well as the food itself [1, 2, 3, 11, 14, 19]. This applies to both plant and animal manufacturing regardless of the production - consumption, feed raw materials, meat or milk [11]. Spectral methods have been successfully used to control the processes of aerobic and anaerobic stabilization of organic matter also for energy purposes [4, 10].

The spectral method has many advantages in conjunction with non-invasive means of obtaining information in relation to conventional methods and may reduce the time between studies while maintaining accuracy and reproducibility of results. The advantage of near infrared spectroscopy, compared to standard methods of analysis, it is possible to analyze a number of parameters using a modified calibration models [4, 5, 12, 18]. Near infrared spectroscopy (NIRS) is a suitable method for measuring the concentrations of constituent gases of biogas [5, 6, 7, 8].

The process of biological decomposition of biomass occurs only in specific physico-chemical conditions and the most important are: no access to oxygen and light, the type of input materials, reaction of used substrates (pH), dry organic matter content, obtained degree of fermentation of organic matter, the temperature in which fermentation runs, scope of mechanical processing of the substrate and the process control parameters and factors related to the conditions of the continuous process of pumping biomass into/from reaction chamber. Control of biogas production process is related to the acceleration and deceleration of organic matter decomposition by bacteria [8]. Acceleration and slowing down the degradation of organic matter may be associated with inhibitors of the process. The alimentary substrate can have inhibitory nature if we assume the wrong dosage or dosage will vary due to the deterioration of its nutritional properties, spontaneous or forced [11]. Acetic acid is one of the transformation products of organic matter produced by a bacterial bed, belongs to the so-called group of volatile fatty acids produced in the digestion process of organic matter. Acetic acid is a monocarboxylic acid. The presence of acetic acid is an important indicator for quick assessment of the intensity of nutrient uptake by bacteria [8]. The concentration levels of volatile fatty acids expressed as acetic acid (abbreviated to VFAs) (Volatile Fatty Acids) suggesting the biological balance of the process are specific to a particular fermentation step and the substrates used [9, 13]. The large variation in fermentation generates the need for frequent testing. Therefore, it is necessary to frequently monitor the concentration of acetic acid in the fermenting substrate, which can be costly and time consuming when using a conventional detection method, e.g.,. the chromatographic method.

Real-time monitoring process of biogassing dynamics may allow its optimization, but the introduction of the use of new construction spectrometric probes must always be preceded by the development of high-quality calibration models. The quality of the models may depend on the used spectral range. The method of sample preparation and standardization of obtained spectral spectra is also an important factor influencing the quality of the calibration model [17, 18].

Aim of the study is to develop calibration models for determining the dry matter content and parameter of volatile fatty acids content based on a mixture of acetic acid reactants involved in the fermentation process using a spectrophotometric method. As part of the objectives in pursued work it was assumed the partial implementation of the tasks which include:
•   the classic methods of analysis of dry matter and concentration of acetic acid in mixtures of liquid and a plant substrate,
•   a proton spectra by the reflective method in the wavelength range of near infrared,
•   execution of calibration models that allow for detection of the assumed parameters using a spectrophotometric method in the wavelength range from 400 nm to 2170 nm.

2. Material and methods

The starting material for the study was a blend of corn silage and pig slurry, which was subjected to continuous fermentation in chamber of biogas plant with a capacity of 1 MW. The biogas plant based on the cooperation of the main and digestate chambers as regards wet fermentation allowed to operate the process of decomposition of organic matter under mesophilic conditions. The chamber was heated to intensify the production of biogas. The samples for testing were taken from the production of biogas from April to June 2016.

Purified samples obtained by segregation of solids using a centrifuge were used in spectrophotometric research and laboratory analyzes of the content of acetic acid and organic matter. Volume of each of the prepared samples was 50 mL (fig. 1).

Source: own research / Źródło: opracowanie własne

Fig. 1. Treated samples prepared for scanning
Rys. 1. Oczyszczone próbki przygotowane do skanowania

Then, on the bench the acquisition of the absorbance spectra was executed using reflective method. Acquisition of each sample was performed using a spectrometer tec5 AgroSpec equipped with a fiber optic probe NIRON. AgroSpec spectrometer is a device equipped with two optical sensors MMS 1 and PGS 2.2, allowing to conduct measurements in ranges from 370 to 1050 nm and 900-2200 nm. The acquisition trials have spectra with a spectral range of 400 to 2170 nm, the interpolated resolution of 2 nm.

The measuring head NIRON contact probe is active with the internal light source in the form of a halogen lamp with a power of 10 W. The test sample is illuminated with a beam angle of incidence equal to 90°. Acquisition of light scattered by the sample takes place via an optical system disposed at an angle of 45° relative to the observed surface of the sample. The optical system of the probe head NIRON is adapted to acquisition of optical signal for inhomogenous and liquid sample.

During measuring the sample from the bioreactor was placed in a Petri dish, and then the acquisition of spectral spectra was executed by contacting the probe measuring window with the sample surface. When registering, the spectra emitted by the optical radiation passe through the outer layer of the substrate which is absorbed by the analyzed compound causing vibrations. They disperse part of the radiation. Non-absorbed radiation is reflected back to the optical detector head NIRON. As a result of measuring the gain ratio of the intensity of radiation reflected from the sample in relation to the parameters obtained in the calibration standard. Acquired raw spectrum is a multidimensional data source. The spectra are subjected to spectral sequence of separation of noise and interference, the development of the mathematical course of changes in the intensity of radiation in the analyzed wave length emitted and delete unnecessary information related to the disappearance of changes in the spectra during the detection process. The spectra are classified to enable the quantification and quality of the test compounds. Followed by construction of the training set. For this purpose, each classified spectral spectra assigned the value of the analyzed parameter obtained by classical methods.

Following acquisition of spectra for all samples the content of volatile fatty acids was determined by automated Nordman' s method according to PN-75 / C-04616/04 and PN-74 / C-04540/00. Then, for all the samples the content of dry matter by the method of the dryer-weight according to DIN EN 12879.

The resulting classified spectral spectrum is subjected to a process of transformation with the use of various methods of interpolation and extrapolation. Knowledge indicates that the overlap line noise and spectral lines are considered as a serious obstacle to the interpretation of the spectra. So-called "anti-aliasing" spectra adjusted to improve the signal to noise ratio is reduced and the influence of non-linearity of variation of the baseline and minimizes the impact of light scattering by the sample to the intensity of the spectrum.

By building a model of the relationship between data and mathematically converted spectral image allows a quantitative and qualitative analysis of examined chemical compounds. To enable qualitative analysis using NIR spectroscopy a package of methods is used for solving problems of data classification and methods of the similarity analysis. The quantitative analysis allows the construction of a mathematical model of the relationship between a specific property of the sample (here, acetic acid content and the level of dry matter content) and the values of absorbance of radiation at specific wavelengths emitted by the radiation source. This relationship is determined by a linear calibration methods. For the construction of a quantitative model it shall be applied multiple linear regression, principal component regression and partial least squares regression.

For the construction of calibration models we used regression method of partial least squares (PLS called partial least squares regression) implemented in software

Unscrambler X 10.1. To assess the quality of developed models used for cross-validation method in which as indicators of correctness obtained model uses parameters such as the coefficient of determination R^2 and the average square error of validation RMSECV. The following rating scale models were adopted, depending on the value of R^2:

- <0.81, low quality models,
- 0.82÷0.9 for models with sufficient prediction,
- > 0.9 models fit well [2, 15, 17, 20].

3. Results

A total of 40 spectra were recorded, which were used to develop a specific operating range of calibration models. After a series of laboratory tests on the samples and spectrophotometric study, it was possible to create a learning sets needed to build calibration models.

The results of the training set for the LKT content and dry matter are shown in figures 2 and 3.

The reference data obtained with the use of laboratory analysis applying automated Nordman's methods and methods of the dryer-weight. During the study recorded 40 absorbance spectra for samples of the mixture of corn silage and pig slurry derived from the fermentation process. Absorbance spectra acquired by reflective method (fig. 4) were obtained in the laboratory, using the full spectral range which was used for testing, ie spectrometer. 400-2170 nm.

Spectra acquired using a probe NIRON are characterized by a large variance in absorbance to a wavelength close to 1900 nm, and exhibit a rapid decrease in this parameter. It could be observed a significant noise signal in the border areas of operation of the spectrophotometer, ie. above 1900 nm.
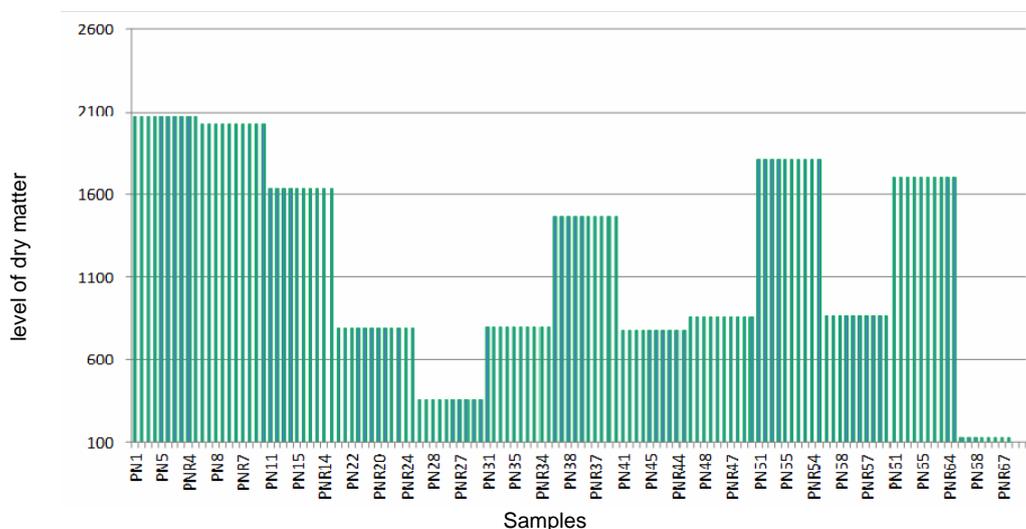
Source: own research / Źródło: opracowanie własne

Fig. 2. Change in the level of acetic acid and alkalinity in the samples in time
Rys. 2. Zmiana poziomu zawartości kwasu octowego i zasadowości w próbkach w czasie
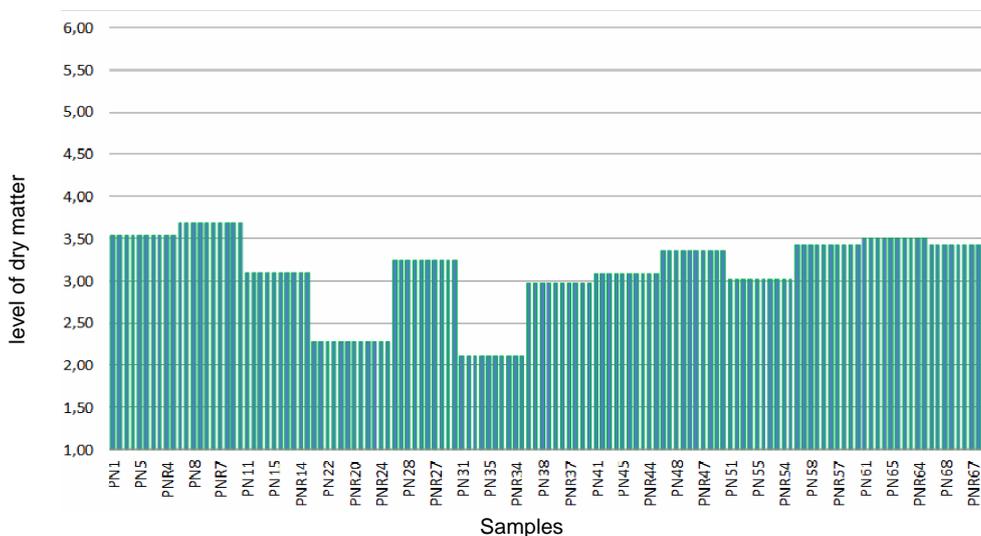
Source: own research / Źródło: opracowanie własne

Fig. 3. Change in the level of dry matter content in the samples in time
Rys. 3. Zmiana poziomu zawartości materii suchej w próbkach w czasie
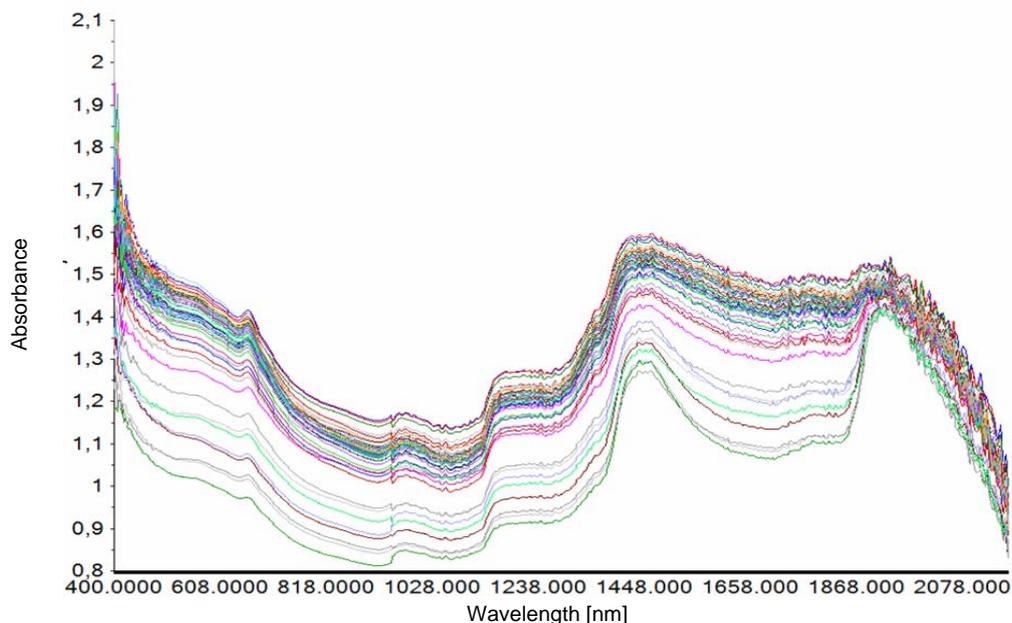
Source: own research / *Źródło: opracowanie własne*

Fig. 4. The spectral absorbance spectra of samples obtained by the probe NIRON
Rys. 4. Widma spektralne absorbancji dla próbek uzyskane za pomocą sondy NIRON

Obtained in the process of parameterization of spectra models were characterized by varying degrees of fit and quality designations depending on the spectral range. Detailed statistics for created regression equations are summarized in table 1.

Table 1. Statistics of obtained calibration model for designation of the dry matter content of volatile fatty acids
Tab. 1. Statystyki uzyskanych modeli kalibracyjnych oznaczenia zawartość materii suchej oraz lotnych kwasów tłuszczowych

Parameter	Wavelength [nm]	R^2	RMSECV
Dry matter	400-2170	0.75	0.23
	400-950	0.55	0.31
	950-2170	0.86*	0.17
Volatile fatty acids	400-2170	0.66	360.00
	400-950	0.38	488.94
	950-2170	0.68*	352.16

*The bestmodels

Source: own research / *Źródło: opracowanie własne*

Match factor R^2 ranged from 0.55 to 0.86 for the determination of dry matter, and for volatile fatty acids from 0.38 to 0.68 indicating different potential models obtained for qualitative analysis and screening . Least fitting model was obtained for a range of 400÷950 nm, while the best corresponded to waveband 950÷2170 nm.

Comparing the values shown in the table for the analyzed bands it can be concluded that the most powerful models are comprised in the range of 950÷2170 nm for the content of dry matter and volatile fatty acids. The best model was generated for the dry matter with fitting model error R^2 at the level of 0.86, and the error of determination was 0.17 mg · dm⁻³.

The worst matching coefficients of the models from among analyzed methods were acquired for volatile fatty

acids. The coefficients of model fiting in this case were $R^2 = 0.38$,
and error in determining RMSECV = 488.94 mg · dm⁻³.

4. Conclusions

1. Methodology of the research, involving the development of a set of training data based on samples of substrate from the process of fermentation, allows to acquire variable spectral spectra with the assigned values of the actual studied parameters.
2. Laboratory application of the reflective probe NIRON allows for efficient acquisition of spectral data referring to the spectra of acetic acid and the level of dry matter in the test digestate.
3. Appropriate spectral range (from 950 nm to 2170 nm) allows for the efficient use of calibration models in conditions of real technological process in agricultural biogas plant.
4. The effect of the application of chemometric methods based on spectrophotometric VIS-NIR enables permanent monitoring of examined parameters' values which determine a quality of produced biogas.
5. Application of the proposed methodology to build the prediction models enables significantly lower costs of individual analysis and reduces a time-consuming preparation of the samples for tests.

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