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Tünde Alapi
Róbert Berkecz
István Ilisz

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DETECTION OF THE BIODEGRADABILITY OF CELLULOSIC BIOMASS BY DIELECTRIC PARAMETERS

Laura Haranghy[1], Zoltán Jákói[1], Balázs Lemmer[2], Cecilia Hodúr[1], Sándor Beszédes[1]

[1]*Department of Biosystems Engineering, University of Szeged, H-6725 Szeged, Moszkvai krt. 9, Hungary*
[2]*Department of Mechanical Engineering, University of Szeged, H-6724 Szeged, Mars ter 7, Hungary*
e-mail: beszedes@mk.u-szeged.hu

Abstract
In biomass utilization technologies enzymatic hydrolysis and fermentation processes are widely used. Development of rapid and non-destructive measurement methods is needed to detect the enzymatic biodegradation of cellulosic biomass, for instance. In our researches the applicability of dielectric measurement methods was investigated for the monitoring the efficiency of enzymatic hydrolysis of Cobex (corn cob) biomass. The dielectric behavior of the Cobex suspensions and fermentation broth was characterized by the dielectric constant and dielectric loss factor, measured at a frequency range from 200 to 2400 MHz. The dielectric parameters were also determined during the ethanol fermentation process of the preliminary hydrolyzed biomass. Our results verified, that the dielectric parameters in the frequency range of 200-100 MHz are sensitive to the chemical changes occurred during the enzymatic hydrolysis of cellulose contented biomass, and, as well as to the presence of ethanol component in the fermentation broth. There was found good correlation of dielectric constant (at frequency range from 300 to 900 MHz) with the concentration of reducing sugars (produced in enzymatic hydrolysis), and the dielectric constant and dielectric loss factor (determined at the frequency of 300 MHz) with the ethanol concentration.

Introduction
The dielectric properties of biomaterials and biosystems have been investigated for decades to make possible the appropriate planning and design of processing equipment operating at microwave and radio frequencies. Dielectric constant (ε') measures the ability of materials for storage of energy in electric field; dielectric loss factor (ε'') corresponds with the energy dissipation of materials. Open-ended coaxial line sensors connected to vector network analysers are commonly used for the measurement of the dielectric properties and dielectric behaviour of materials at wide frequency ranges [1].
Dielectric properties are responsible for the materials-electromagnetic field, distribution of electromagnetic field inside of the materials [2]. In high water contented material the dielectric constant decreases with frequency, if polar molecules can follow the polarity change of the electromagnetic field. Nevertheless, over a critical frequency value, phase lag occurs between the dipole rotation and the change of the polarity of electric field. The two main dielectric loss mechanisms can be the dipole rotation and ionic conduction, depending on the applied frequency and the compounds of the system. Considering the frequency range of microwave heating should be noted that ionic conduction plays crucial role in heating efficiency at 915 MHz [3]. But, depending on the temperature and the components of the system, both the dipolar mechanisms and ionic conduction have also effect on the dielectric behaviour and thermal efficiency of microwave irradiation at the frequency of 2450 MHz [4].
In high water contented medium the dielectric parameters of water determined mainly the dielectric behaviour of the system [5]. The temperature has also effect on dielectric behaviour.

In polar dispersion region, the dielectric constant increases as temperature increases, but at other frequency ranges an opposite tendency can be found. In the frequency range of 300-3000 MHz the temperature increment cause the decreasing of dielectric constant, because the higher thermal energy makes difficult for dipolar components to align with the polarity change of electric field [1]. Considering the effects of water content on dielectric parameters, it can be concluded that the bound water contributed less in the dielectric behaviour of the materials, than the free water content. Free water molecules - as polar components - can orient easier in varying polarity electromagnetic field. Increase of the concentration of ionic components led to decrease of dielectric constant and increase of dielectric loss factor, because the dissolved salts are conductors in electric field [6]. The increase of water content in suspensions decrease the viscosity, and, therefore the 'binding' forces playing role in ionic conduction mechanisms are decreased [7].

One of the most important key compounds produced in enzymatic hydrolysis of lignocellulosic biomass is the glucose. Measurement of the glucose concentration enables to monitor the cellulose degradation process due to chemical, thermochemical and enzymatic processes, respectively. In glucose and sucrose contented solution has been verified, that relative permittivity decreased as the concentration increases. But, if salts are present in the solution generalized tendencies cannot be given at the frequencies higher than 200 MHz [4]. Beside the detection of chemical changes, the biological changes of materials can be monitored by dielectric measurements. Zhu et al. [8] verified that bacterial growth increases the capacitance of milk samples that led to increased dielectric constant. If microorganisms can decompose the macromolecules into lower molecular weight products the conductivity of the medium increases, therefore, at radio frequency ranges, the dielectric loss is influenced mainly by ionic conduction. Kouzai et al. [9] developed waveguide penetration method to determine the dielectric behaviour of fermentation broth. They verified, that the decrease of glucose concentration and the increase of ethanol concentration are correlated well with the complex permittivity. Arnoux et al. [10] used the permittivity to monitor the biomass production in lactic acid fermentation process. Olmi et al. [11] applied dielectric measurement method for on-line detection of the efficiency of sugar/alcohol conversion and carbon dioxide production in beer fermentation process.

The main objective of our work was to investigate the applicability of dielectric parameters for the detection of the efficiency of enzymatic hydrolysis and fermentation of cellulosic biomass.

Experimental

Dielectric constant (ε') and dielectric loss factor (ε") are measured at frequency range from 200 to 2400 MHz by open-ended coaxial-line probe (SPEAG DAK 3.5), connected to a vector network analyzer (ZVL-3 VNA, Rhode&Schwarz). Samples were measured in polytetrafluoroethylene (PTFE) tube container (diameter of 30 mm, volume of 35 mL). Immersion depth of DAK probe was 10 mm, temperature of samples was controlled at 25°C by water bath. The averaged dielectric parameters were calculated from 30 measuring points.

Corn cob residues (COBEX F12/30) was used as raw material for the enzymatic hydrolysis tests, which has an average particle size of 840 μm, moisture content of 7.3 w%, and cellulose and hemicellulose content of 32.1% and 37.3%, respectively. For the 7 days enzymatic hydrolysis tests Cellic CTEC2 (Novozymes) industrial enzyme blend (with cellulase, β-glucosidase and hemicellulose activities) was used at the temperature of 40°C and pH of 4.8 using 3.5 w% TS contented Cobex suspensions. For the ethanolic fermentation stage commercial *Saccharomyces cerevisie* was applied in 0.5 w% concentration. For the enzymatic hydrolysis and fermentation experiments Labfors Minifors (Infors) bioreactors were used.

The cellulose degradation was characterized by the concentration of reducing sugars measured by DNSA method. Ethanol concentration of the fermentation broth was measured based on refraction index, after distillation. Beside the non treated Cobex samples (Cont.), alkaline (Alk.) pre-treatment (dosage of 50 mg/g_{TS} NaOH) and microwave (MW) pretreatment (100 mL of suspension with MW power of 500 W for 4 minutes at 2450 MHz frequency) were also applied before the enzymatic hydrolysis to increase the biodegradability of the biomass.

Results and discussion

In the first series of our experiments, the dielectric behavior of non-treated (Cont.) alkaline (Alk.) and microwave (MW) pretreated biomass was investigated as the change of dielectric constant as the function of measuring frequencies. Our results show, that partial decomposing of cellulose fraction due to microwave pre-treatment resulted in the decreased dielectric constant, compare to the control sample. However, the dosage of NaOH increased the dielectric constant of Cobex suspension (Figure 1.). This increment is caused mainly by the strengthening of ionic conduction mechanisms [4]. For a given sample, the dielectric constant decreases as the hydrolysis time increased. These establishments are in a good agreement with the literature: in the biomass pre-treatment step, and/or the enzymatic hydrolysis process the macromolecules decompose to smaller molecular weight components, which can be polarized easier in the electric field [9]. This effect can be manifested in the decrease of dielectric constant.

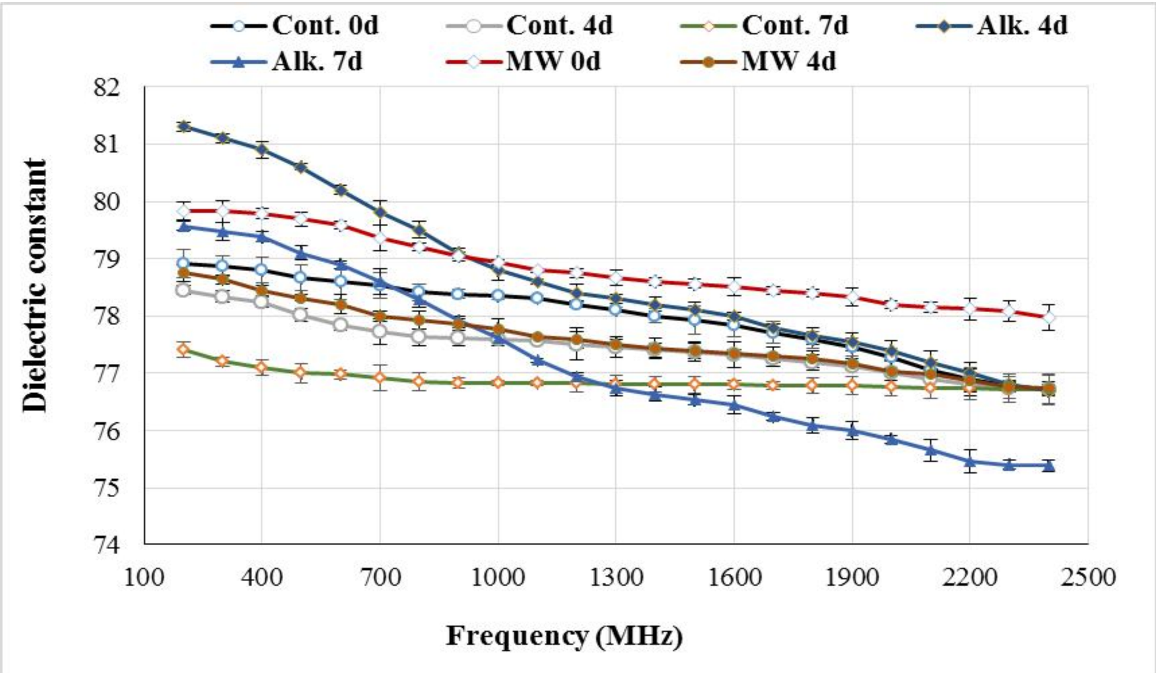

Figure 1. Dielectric constant of enzimatically hydrolized samples in the frequency range of 200-2400 MHz (t=25°C) (‚d' denotes the days of enzymatic hidrolysis)

Our research aimed the investigation of the relationship between the dielectric parameteres and the degree of celluose hydrolysis. The cellulose degradation was characterized by the change of the concentration of reducing sugars (RS). The experimental results show a good correlation of dielectric constant at lower frequencies with the concentration of reducing sugars. At frequencies of 300 MHz and 900 MHz the relationship can be given by quadratic or linear equation for alkaline pre-treated (Alk.), or control (Cont.) sample, respectively (Figure 2.).

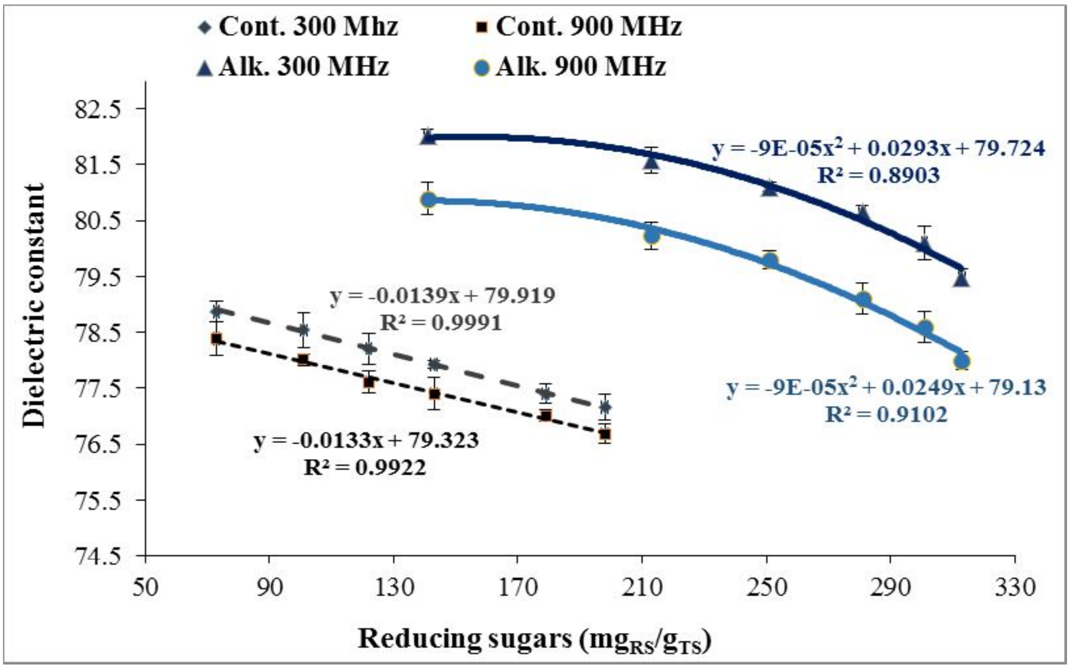

Figure 2. Correlation between dielectric constant and the concentration of reducing sugars

Olmi et al. [11] found a good correlation between the dielectric loss factor and ethanol concentration in a four steps beer fermentation process. Therefore, measurements were conducted to examine the dielectric parameters during the ethanol fermentation of hydrolized corn cob biomass.

It was verified that the sugar/ethanol conversion (production of ethanol from sugary by yeast fermentation) can be detected by the dielectric parameters. At the measuring frequency of 300 MHz a good correlation was found between the ethanol concentration and dielectric constant and dielectric loss factor, as well (Figure 3.).

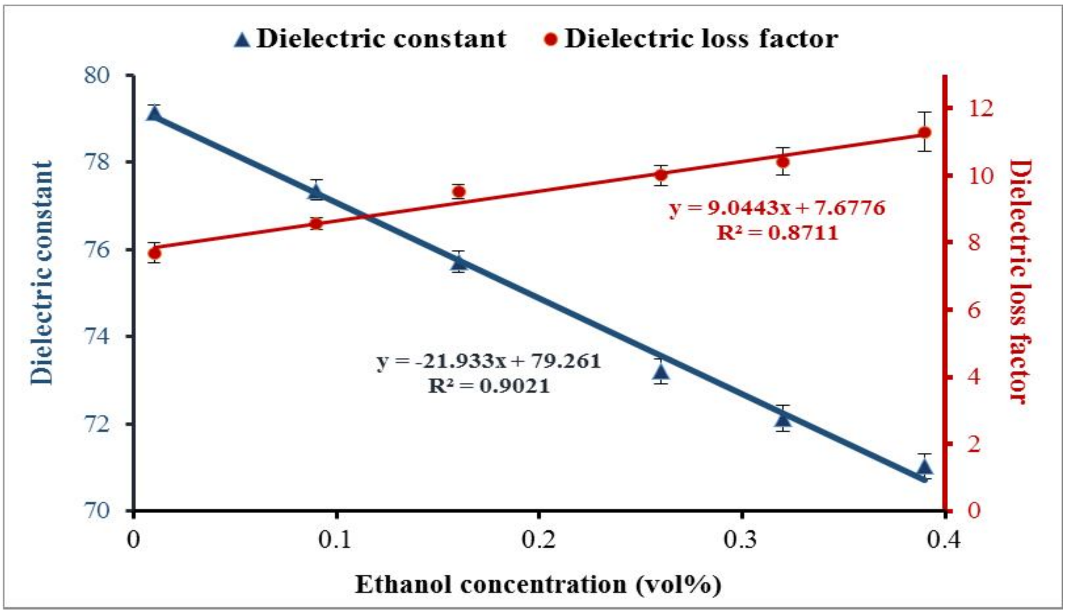

Figure 3.Change of dielectric constant and dielectric loss factor as a function of ethanol concentration (f=300 MHz, t=25°C)

Conclusion

The main aim of our research was to investigate the relationship between the dielectric parameters and conventionally used analytical parameters in the biological utilization of cellulosic biomass.

Our results show that the reducing sugar yield in the enzymatic hydrolysis process was in a good correlation with the dielectric constant determined at lower frequency range.

In fermentation process, the sugar/ethanol conversion rate can be detected by the measurement of dielectric constant and dielectric loss factor, as well.

These results verified the applicability and usability of dielectric measurements, as a non-destructive, chemical-free and rapid method, for the monitoring of enzymatic hydrolysis of cellulosic biomass and ethanol fermentation process, as well.

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