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Edited by:
Tünde Alapi
Róbert Berkecz
István Ilisz

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DIELECTRIC MONITORING IN CERTAIN LIGNOCELLULOSIC BIOMASS UTILIZATION PROCESSES

Zoltán Péter Jákói[1], **Balázs Lemmer**[2], **Réka Dobozi**[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 Food Engineering, University of Szeged, H-6724 Szeged, Mars tér 7, Hungary*
e-mail: jakoiz@mk.u-szeged.hu

Abstract
In our present study, we wanted to investigate the applicability of dielectric properties measurement to monitor the progress of three distinct but connected subprocesses of lignocellulosic biomass utilization. These involved the enzymatic saccharification of corn-cob residues, followed by the bioethanol fermentation of the hydrolysates, and lastly, the anaerobic biogas co-digestion of industrial wastewater sludge and solid residues from the alcohol fermentation part. Our results revealed that a strong and established correlation can be found between the process indicators and the dielectric constant of the different fermentation media - namely, the absolute sugar concentration during the enzymatic hydrolysis, the ethanol concentration during the alcohol fermentation, and the biogas yield during the anaerobic co-digestion. The relationship between these parameters can be described via linear functions, and the coefficient of correlation *r* varied between 0.97-0.99, while the R^2-value between 0.96-0.99.

Introduction
The growing importance of sustainable growth and the need to lessen reliance on finite resources have increased attention on the use of biomass and the integration of eco-friendly technologies. Serving as a fundamental component in the circular economy framework, biomass utilization strives to eradicate waste and perpetually recycle resources within a self-sustaining system. Lignocellulosic biomass (LCB) stands as the planet's most plentiful biological raw material, accounting for an estimated 80% (or 450 gigatons of carbon) of all different kinds of biomass. Over the past several years, extensive research has been conducted on harnessing LCB for bioenergy generation, positioning it as a viable and eco-friendly substitute for finite energy resources [1]. There are numerous procedures that can be utilized for biomass processing, among which bioethanol production and biogas fermentation are the most commonly used ones. From an environmental and energetic perspective, combining these utilization methods is the most favorable solution, since it can "close" the whole lifecycle of a phytomass of any kind, and can be perfectly integrated into the circular economy concept [2]. The separated hydrolysis and fermentation process (SHF) is one of most frequently employed technologies for biomass saccharification and ethanol fermentation – the first two of the cycle -, since it grants usually great yields and efficiency, and can be precisely controlled. Although the feedstock loses a significant part of its organic matter during these processes, it still contains a significant number of utilizable compounds, making it a great secondary raw material for anaerobic biogas co-digestion [3]. However, the biochemical pathways and reactions that occur during these subprocesses are especially complicated, and the standard analytic methods for controlling are either time consuming, and/or expensive. Therefore, an alternative method for monitoring these utilization methods should be sought out. The applicability of dielectric behavior assessment has been widely investigated, and its reliable use in several fields of science and engineering - for example foresting, soil engineering or food science – has been proved [4]. The underlying principles governing dielectric behavior are established in

electrodynamic characteristics. Specifically, the presence of an electromagnetic field of any strength, denoted as E, induces what is known as a dielectric displacement, or D, in real materials. This can be interpreted as a form of phase shift, represented by δ. Consequently, the dielectric permittivity (ε), which describes how different materials react to the electromagnetic field, is considered a complex function dependent on the angular frequency ω of the field being applied:

$$D_0 e^{-i\omega t} = \varepsilon^*(f) E_0^{-i\omega t} \tag{1}$$

In the equation above, ε^* denotes the complex relative permittivity, ω is the (angular) frequency and $i = \sqrt{-1}$. In case E is not excessively high, the proportionality between E and D remains, and as the frequency increases the emerging δ phase difference becomes more prominent. The frequency at which the phase shift becomes prominent depends on the temperature, and the biological, chemical, and physical characteristics of the material, and can be defined as:

$$\varepsilon^* = \frac{D_0}{E_0} = |\varepsilon| e^{-i\delta} \tag{2}$$

$$\varepsilon^*(\omega) = \left|\frac{D_0}{E_0}\right| (cos\delta - isin\delta) = \varepsilon'(\omega) - i\varepsilon''(\omega) \tag{3}$$

Equation 3 is the separated form of the complex relative permittivity, where ε' – the dielectric constant – denotes the real part, and ε'' – the loss factor – the imaginary part. The dielectric constant quantifies the electric (or electromagnetic) absorbing and storing capabilities of a given material, while the loss factor shows how much of the absorbed energy is converted into other types of energy. As can be seen from the equation, both the dielectric constant and loss factor are frequency dependent, and the distinct frequency where the dielectric constant reaches its maximum (*i.e.* the inflexion point of the dielectric spectra) is called the relaxation frequency. For systems containing high amounts of water, this relaxation frequency occurs around 700-1000 MHz. These dielectric properties highly depend on the chemical – physical structure of a system as well – meaning, that a change in either the chemical or physical structure is reflected in the change of dielectric behavior as well. This makes the measurement of dielectric behavior a promising alternative in the controlling and monitoring of biotechnological processes, such as biomass utilization [5].

Experimental

Corn-cob residues served as raw material for the enzymatic saccharification; of which 10 m/m% aqueous suspensions were made by the addition of purified water. A mixture of different β-1,4-glucanase enzymes was added to the suspensions, and during the 6-days-long hydrolysis, the temperature was kept at 45±1°C, whilst the pH at 5.5±0.4. The sugar end-product of the hydrolysis was measured with dinitrosalicylic-acid based spectrophotometric method every 24 hours. Along with the sugar concentrations, the dielectric properties of the hydrolysates were also measured with a DAK-3.5 open-ended dielectric probe connected to a ZVL-3 vector network analyzer (bandwidth: 30 kHz – 3 GHz). The subsequent ethanol fermentation took place in automatic anaerobic fermenters, and standard *Saccharomyces cerevisiae* strains were used for the bioconversion of glucose to ethanol. The temperature was kept at 30°C, and the pH at 5.5. The ethanol concentration was measured with distillation, and the dielectric properties of the fermentation media were also measured with the aforementioned dielectric system. The solid residues after the fermentation were transferred to laboratory-grade glass fermenters, where they were mixed with 100 cm^3 of industrial wastewater sludge for mesophilic co-digestion. The optimal temperature condition (T=38°C) was achieved in a thermostatic cabinet.

The biogas yield was calculated based upon the absolute pressure inside the reactors, and along with it, the dielectric behavior of the fermentation media was also measured with the dielectric assessment kit.

Results and discussion

In order to evaluate the correlation between the nascent sugar concentration during the enzymatic hydrolysis and the dielectric properties of the medium, we plotted the values of the 900 MHz dielectric constant (i.e., the variable which is affected by the biochemical changes in the material) as a function of the corresponding sugar concentration (i.e., the variable which ultimately leads to the change in dielectric behavior). This can be observed in Figure 1.

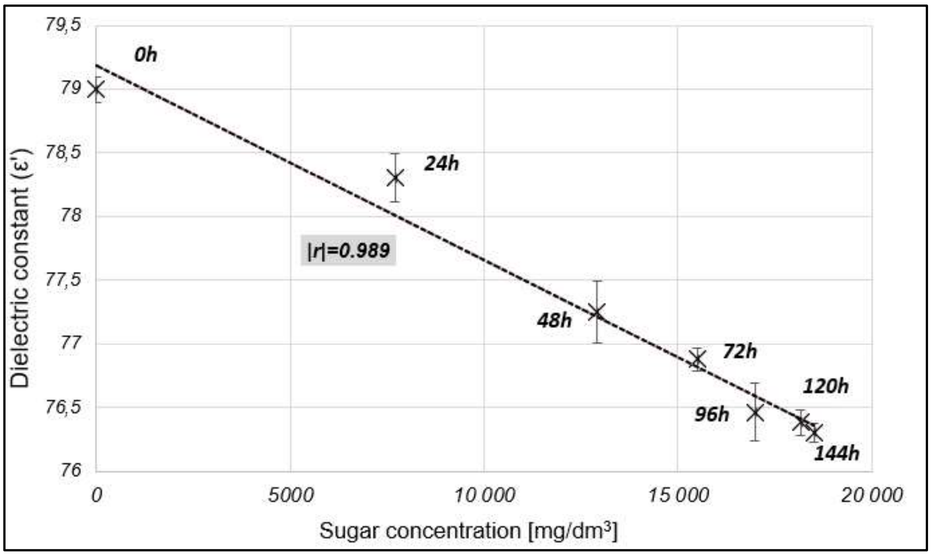

Figure 1. *The relationship between the sugar concentration and the 900MHz dielectric constant during the enzymatic saccharification*

The results obtained suggest that as the hydrolysis converges, the concentration of sugar shows saturation characteristics (i.e. the product concentration eventually reaches its maximum and does not go any further), which is a typical tendency of enzymatic reactions. The maximum value of sugar turned out to be around 18 g/dm^3, which means an approximately 76% bioconversion efficiency. In terms of the dielectric constant, it can be clearly stated that there is a strong and linear correlation between it and the sugar concentration (r = 0.989). It can also be seen that as the sugar concentration increases, the dielectric constant gradually decreases. This might be explained by the fact that the degradation of lignocellulose evidently produces smaller mono-, di- and oligosaccharides, the vast majority of which are soluble. This eventually leads to the reduction of free water molecules, which also implies a decrease in the dielectric constant. This strong relationship suggests that evaluating certain dielectric characteristics (in this case, the dielectric constant) can be a promising, fast, and accurate method for monitoring enzymatic processes.

During the second part of the SHF process, the hydrolysates underwent anaerobic alcohol fermentation, where the ethanol concentration was regularly measured every 24 hours, along with the dielectric characteristics. Figure 2 shows the results obtained for the fermentation process.

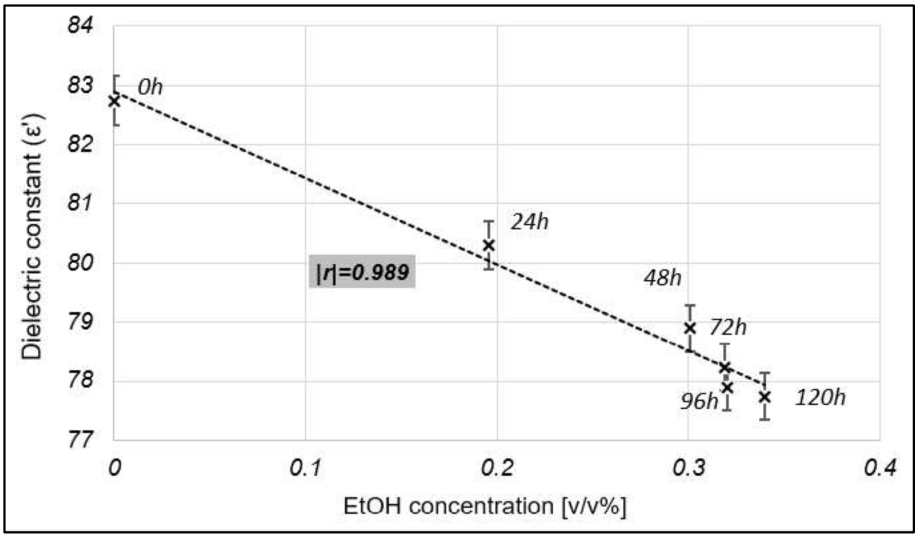

Figure 2. *The dielectric constant as the function of ethanol concentration during the fermentation process*

Like in the case of enzymatic hydrolysis, the ethanol concentration also showed a saturation tendency during the fermentation, which is related to both the concentration of free sugars available and the alcohol tolerance of the microbes. Since the maximum v/v% achieved was around 0.35%, the conversion efficiency did not exceed 40%. A possible explanation for this is that during the degradation of lignocellulose, a huge variety of compounds can be generated (especially from the lignin) that can limit the microbial metabolism. Although the ethanol conversion efficiency was relatively low, the change in dielectric behavior showed strong linear correlation (r = 0.989) with the ethanol concentration as well. Since ethanol has a lower dielectric constant than water, even a small amount of it can influence the dielectric behavior of the medium, which can be observed in Figure 2.

During the anaerobic co-digestion, the solid residues from the alcohol fermentation (after drying) were mixed with 100 cm^3 of industrial sludge. The produced gas volume was measured on every seventh day of the process, along with the dielectric constant of the fermentation media. Figure 3 shows the results obtained.

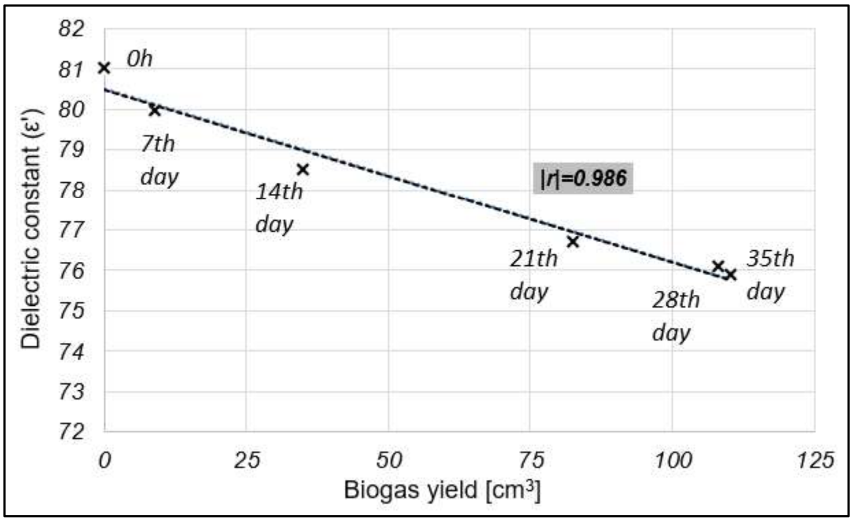

Figure 3. *The connection between the dielectric constant and bigos yield during the anaerobic co-digestion process*

It can be seen from the results that the digestion process reached the steady-state (or "stagnating") phase around the 28th day, after which no detectable difference was experienced in the biogas volume. During this stage the biochemical reactions slow down and eventually no recognizable chemical / physical structural change occurs. This is in great alignment with the dielectric characteristics, namely that the decreasing tendency stops after the 28th day. Like the previous processes, the dielectric constant shows a strong linear correlation with the actual phase of the fermentation media ($r = 0.986$), meaning that it might be used as a fast and accurate monitoring tool for anaerobic biogas fermentation processes tool.

Conclusion

In the present study, we investigated whether certain biomass utilization processes, namely enzymatic hydrolysis of lignocellulose, alcoholic fermentation of the hydrolysates and the biogas co-digestion of fermentation residues, can be monitored by measuring dielectric parameters. Our results revealed that a strong a linear correlation could be established between the dielectric constant, and the indicator parameters of these biomass utilization processes, namely the sugar, ethanol and biogas yield or concentration (r = 0.989, 0.989 and 0.986, respectively). This suggests that – after proper optimization – dielectric measurement can be utilized as a fast, reliable, and relatively cheap technique in monitoring and controlling of these, or similar biomass processing technologies.

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