BIOETHANOL PRODUCTION FROM ABIES ALBA WOOD USING ADAPTIVE NEURAL FUZZY INTERFERENCE SYSTEM MATHEMATICAL MODELING

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This study investigates bioethanol production from Abies alba wood. The wood was first autohydrolysed, then delignified and the remaining cellulose was used as substrate for simultaneous saccharification and fermentation processes. The influence of temperature (180, 190 and 200 °C) and pretreatment time (5, 10 and 15 min) on the fermentation medium products was studied. The maximal bioethanol content (52.0 g L⁻¹) was obtained at a pretreatment temperature of 190 °C and pretreatment time of 10 min. The enzymatic hydrolysis and fermentation temperature was 38 °C for 72 h. The untreated, autohydrolysed and delignified wood was characterized by reflected light microscopy for morphological structure identification. The adaptive network-based fuzzy interference model (ANFIS) and the Gaussian membership function were used to reproduce the experimental results obtained for complete characterization of the wood fermentation broth. The proposed model uses two input variables (temperature and reaction time) and one output parameter based on two intelligent methods: back-propagation and a hybrid method. The hybrid intelligent method has good accuracy (99.2-100.0%) and correlation coefficient (0.998-1). The fermentation broth contains a mixture of bioethanol and secondary by-products, including acids, alcohols, aldehydes, ketones and esters. A maximum of 5.2 g bioethanol can be obtained from 100 g of woody biomass after autohydrolysis–delignification–SSF process.

Keywords: wood, autohydrolysis, bioethanol, ANFIS, SSF

INTRODUCTION

Woody biomass is the most abundant renewable resource worldwide. The increased greenhouse emission levels caused by pollution urge the identification of new renewable resources for biofuel production.¹,² One of the EU priorities is to reach 20% biofuel in the fuel composition by 2020, which brings into the focus the bioethanol from lignocellulosic biomass. Woody biomass has a great potential to be used as a raw material and can replace current fuels. It consists mainly of three structural polymers: cellulose, hemicellulose and lignin.³,⁴ The cellulose and hemicellulose are carbohydrates that can be converted into monosaccharides and then fermented into bioethanol.⁵,⁶ Bioethanol is produced by fermentation of sugars obtained from different feedstock.

Due to the different structure and composition of cellulose and hemicellulose, a special treatment of wood for their separation is necessary because the presence of lignin in the woody biomass composition can affect further processes and bioethanol yields.⁷ The lignin is a valuable resource for the production of value-added compounds, such as additives, etc. The separation
of lignin from wood before enzymatic hydrolysis can improve enzymatic hydrolysis yields. The reaction of substrates with solvents, such as acetic acid, acetone, ethanol, etc. represents processes applied to remove lignin from wood. In recent years, special attention has been given to the development of environmentally friendly methods for the production of bioethanol. In this regard, the autohydrolysis method using only water to extract the hemicellulose fraction as a mixture of oligosaccharides, monosaccharides and secondary by-products can be mentioned. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two methods used for the conversion of woody biomass into bioethanol. In SHF, enzymatic hydrolysis and fermentation processes are carried out separately, but these processes have a long reaction time. In SSF, enzymatic hydrolysis and fermentation are carried out in the same vessel and this method presents the advantages of short reaction time, low contamination risk and higher production rate.

In the literature, different types of models for composition prediction regarding the compounds resulting from different chemical processes have been reported. Statistical analysis, Artificial Neural Networks (ANNs) and Adaptive Neural Fuzzy Interference System (ANFIS) mathematical models have been used to predict the experimental results. Artificial intelligence models are currently widely used as an alternative to classical empirical models based on the statistical approach. The ANNs function like a human brain, which has the capability of learning the information required via a series of input (dependent variables) and output (dependent variables) data.

Akkaya used the ANFIS based prediction model for determining the biomass heating value (fixed carbon, ash and volatile matter) using proximate analysis components. In this study, three methods (sub-clustering, grid partition and fuzzy interference system) were used for the prediction model and the performance prediction of ANFIS indicated very good precision.

In a study conducted by Zamudio et al., ANFIS was used to predict the biomass chemical composition after the autohydrolysis process of *Paulownia* trihybrid (contents of glucose, xylose, arabinose, acetyl groups and xylo-oligomers). The model reproduced the experimental results with less than 6% error.

Caparrós et al. used ANFIS modelling to analyse the influence of operational variables (viz., ethanol concentration, temperature and pulping time) on the yield, viscosity, kappa number, tensile index, burst index, tear index and brightness after autohydrolysis and organosolv pulping of *Paulownia fortunei* biomass. Rego et al. used ANN and ANFIS for the optimization of sugarcane bagasse pretreatment using alkaline hydrogen peroxide. The temperature (25-45 °C) and hydrogen peroxide concentration were used as independent variables, while the amount of insoluble lignin, glucose and xylose was used as a dependent variable. The studies suggested that the ANFIS model has better performance compared with the ANN model.

In our previous work, we have developed a mathematical model using ANFIS modelling for the prediction of yields and composition of liquid and solid fractions resulting after autohydrolysis pretreatment of fir wood.

The use of the ANFIS model to predict the composition of the fermentation broth obtained from fir wood after autohydrolysis, delignification and SSF process is yet to be explored.

The current study presents a method used for bioethanol production from *Abies alba* wood species. The influence of temperature (180, 190 and 200 °C) and pretreatment time (5, 10 and 15 min) on autohydrolysis and composition of pretreated and delignified wood (content of cellulose, hemicellulose and lignin, as well as yield) was studied. The ANFIS model was used for the prediction of the fermentation broth composition obtained after hydrolysis and fermentation of pretreated-delignified fir wood. A complete analysis of the fermentation broth was done.

**EXPERIMENTAL**

**Materials**

*Abies alba* wood was collected locally (Cluj county, Romania) and used as raw material. The dried material was stored in plastic bags at room temperature. All reagents were of analytical grade. Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Sodium chloride (80%) was purchased from Alfa Aesar GmbH & Co (Karlsruhe, Germany). The chemicals, such as acetic acid, sodium hydroxide, sulphuric acid (98%), acetone, sodium citrate, ethanol, methanol, n-propanol, 2-methyl-1-propanol, n-butanol, 2-methyl-1-butanol, pentanol, KH$_2$PO$_4$, MgSO$_4$, KH$_2$O, (NH$_2$)$_2$SO$_4$, MgSO$_4$, Mg$_3$(OH)$_2$PO$_4$, were purchased from Merck (Darmstadt, Germany). Enzyme *Accellerase 1500* was
donated by Genencor (derived from *Trichoderma reesei* (Genencor, Rochester, NY, USA). Peptone from animal tissue P5905 and yeast from *Saccharomyces cerevisiae* YSC2 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Abies alba** wood pretreatment and delignification

The biomass was pretreated by autohydrolysis using a steel pressure Parr reactor with a Parr 4523 temperature controller (Parr Instruments, Illinois, USA), equipped with a 1 L reaction vessel. The raw material and water were mixed at a 1:7 solid to liquid ratio. The mixture was heated to 180, 190 and 200 °C for 5, 10 and 15 min for each temperature. The solid fraction was separated by filtration and analyzed for cellulose, hemicellulose and lignin content. The range of investigated conditions for wood pretreatment was selected according to our previous results.25

The autohydrolysis pretreatment was quantified by severity factor. The severity factor was calculated by Equation (1):

\[
\text{Severity factor (S)}_0 = \log 10 \left[ 1 \times e^{\left( \frac{T_1 - T_0}{14.75} \right)} \right]
\]

where \( t_1 \) is the pretreatment time (min), \( T_1 \) – temperature (°C), and 14.75 is an empirical parameter related to temperature and activation energy. The nine conditions of severity factor include: \( S_0 = 3.05 \) (180 °C and 5 min), \( S_0 = 3.36 \) (180 °C and 10 min), \( S_0 = 3.53 \) (180 °C and 15 min), \( S_0 = 3.35 \) (190 °C and 5 min), \( S_0 = 3.65 \) (190 °C and 10 min), \( S_0 = 3.83 \) (190 °C and 15 min), \( S_0 = 3.64 \) (200 °C and 5 min), \( S_0 = 3.94 \) (200 °C and 10 min) and \( S_0 = 4.12 \) (200 °C and 15 min).

Pretreated wood was delignified using sodium chlorite in acetic acid solution in accordance with previous reports.26 Sodium chlorite (NaClO₂, 0.6 g g⁻¹ biomass) was reacted at 75 °C with woody biomass in an acetate buffer solution (pH 4.5). After delignification, the wood was carefully washed with deionized water and acetone, and the yields of chlorite delignification were determined. The delignified wood was analyzed for cellulose, hemicellulose and lignin content. The cellulose and hemicellulose content were determined as holocellulose content by treating delignified wood with NaClO₂ in acetic acid solution (10%) (repeated for 4 times). The cellulose was determined by treating the above obtained holocellulose with 17.5% NaOH solution at 20 °C for 40 min. The hemicellulose content was calculated as the difference between holocellulose and cellulose contents. The lignin content from delignified wood was determined by treating delignified wood with 72% sulphuric acid at 20 °C for 4 h.

**Simultaneous saccharification and fermentation (SSF)**

SSF experiments on the solid residue recovered after wood delignification were carried out in a 2 L bioreactor (Lambda Minifor, Lambda Laboratory Instruments), equipped with dissolved oxygen, pH and temperature sensors. SSF media were prepared by mixing nutrient solution (150 mL), *S. cerevisiae* inoculum solution (150 mL) with 8% (w/v) solid loadings in citrate buffer (0.05 M) and 0.7 mL g⁻¹ glucan of Accellerase 1500 (Genencor, Rochester, NY, USA) at pH 5. The nutrient solution contained per liter: 5 g of yeast extract, 20 g of KH₂PO₄, 10 g of MgSO₄·7H₂O, 20 g of (NH₄)₂SO₄ and 1 g of MgSO₄·7H₂O and the inoculum solution contained 10 g of yeast extract, 20 g of peptone and 50 g of glucose. All the SSF experiments were performed at 38 °C for 72 h. A complete analysis of the fermentation broth was done.

**Characterization of untreated, autohydrolysed and delignified wood by reflected light microscopy**

Before and after pretreatment and delignification, the wood was characterized by reflected light microscopy (Kern OKN-1, Germany). The microscope is an Infinity Optical System, provided with a 50 W halogen incident illumination unit. The samples were analyzed at up to 40x magnification.

**Analytical methods for fermentation broth**

Ethanol, methanol, n-propanol, 2-methyl-1-propanol, n-butanol, 3-methyl-1-butanol, 2-methyl-1-butanol and pentanol were analysed using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a CTC Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and a flame ionization detector (Agilent Technologies, 6890N GC). The method used for the analysis of alcohols is the full evaporation headspace gas chromatographic method (FE-HS-GC), according to our publication.27 The acids, aldehydes, ketones, esters and ethers were extracted from the fermentation medium in 20 mL of chloroform. The extract was concentrated by an evaporator and then dried using a stream of filtered nitrogen gas. A 7890N gas chromatograph (Agilent Technologies) and a capillary column DB-WAX (30 m x 320 µm x 0.25 µm) were used to analyse the extracts of the fermentation medium, with a split ratio of 50:1. The carrier gas was helium. The GC column temperature program applied was as follows: the initial oven temperature was set to 40 °C, held for 10 min, with temperature increases of 4 °C min⁻¹ to 220 °C for 10 min.

**Statistical analysis by Adaptive Neural Fuzzy Interference System (ANFIS)**

The ANFIS model proposed by Jang, based on first order Sugeno-fuzzy modeling was used for prediction of experimental results.28 Artificial neural network models consist of three layers: input, hidden and output. Each layer is formed from neurons that operate the information required. The input information is given to the input layer, which is then transferred to the hidden layer. The
The temperature and time parameters are independent variables. In this study, two methods were used: back-propagation and a hybrid method. The hybrid method is described as a back-propagation for the parameters associated with the input membership functions and least squares estimation for the parameters associated with the output membership functions. Figure 1 shows the architecture of the proposed ANFIS model.

\[
Y_e = \frac{\sum_{i=1}^{m} y_i^l R_i}{\sum_{i=1}^{m} R_i}
\]

where \( Y_e \) is the estimate value of the output variable, \( m \) – the number of rules, \( y_i^l \) – difuzzifier, and \( R_i \) – the product of the selected membership functions.

The parameters were estimated using the ANFIS Edit tool in the MATLAB 7.0 software. The mathematical equation, which responds to the Gaussian membership function, is:

\[
\mu_{low} = \exp \left( -0.5 \left( \frac{x_{low} - \mu_{low}}{L} \right)^2 \right)
\]

where \( \mu_{low} \) is the membership function; \( x_{low} \) is a low value for temperature and time, respectively; and \( L \) – the width of Gaussian distribution of temperature and time.

Nine fuzzy rules were used for modelling a combination of the membership function depending on two independent variables, function of the extreme (high, medium and low) values, namely:

- **Rule 1:** Low T (180), Low t (5)
- **Rule 2:** Low T (180), medium t (10)
- **Rule 3:** Low T (180), high t (15)
- **Rule 4:** Medium T (190), low t (5)
- **Rule 5:** Medium T (190), medium t (10)
- **Rule 6:** Medium T (190), high t (15)
- **Rule 7:** High T (200), low t (5)
- **Rule 8:** High T (200), medium t (10)
- **Rule 9:** High T (200), high t (15)

The correlation coefficient (R), root mean square error (RMSE) and accuracy are used for evaluating the performance of the ANFIS – the efficiency of the model between the predicted and the measured values. The correlation coefficient is calculated based on Equation (4), RMSE is calculated based on Equation (5) and accuracy is calculated based on Equation (6):

\[
R = \frac{\sum_{i=1}^{N} (t_i - \bar{t})(o_i - \bar{t})}{\sqrt{\sum_{i=1}^{N} (t_i - \bar{t})^2 \sum_{i=1}^{N} (o_i - \bar{t})^2}}
\]

\[
\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (t_i - o_i)^2}
\]

\[
\text{Accuracy} = \frac{1}{2} \left( 1 - \frac{\text{RMSE}}{\bar{t}} \right) \times 100
\]

where \( N \) – the amount of data, \( t_i \) – the experimental value, \( o_i \) – the predicted value, \( \bar{t} \) – the average of the experimental value and \( \bar{o} \) – the average of the predicted value.

**RESULTS AND DISCUSSION**

**Autohydrolysis pretreatment of wood**

The raw material composition of the *Abies alba* wood was analyzed and the contents of cellulose, lignin and hemicellulose were 42.0%, 27.0% and 23.0%, respectively.

The autohydrolysis pretreatment used only water for the hemicellulose solubilization; the hydronium ions generated from water in the first stage and the acetic acid formed during the reaction in the second stage contributed to hydrolysis as catalysts. Moreover, other studies
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reported that the woody biomass itself has a buffering effect due to the presence of mineral salts in the structure of wood. Based on our previous publications, the experiments were carried out at three temperatures: 180, 190 and 200 °C for 5, 10 and 15 min residence time for each temperature. Figure 2 presents the composition of the solid fraction recovered after autohydrolysis pretreatments as a function of the severity factor. Besides the solid fraction, the liquid fractions were also analyzed and the results were published in our recent papers. The analysis of the solid fraction showed that the cellulose content was significantly higher at the severity factor of 3.94. This suggests that, during the autohydrolysis pretreatment, the hemicellulose is degraded at a high severity factor value. The results presented in Figure 2 show that approximately 90% of cellulose remains unaltered under a low severity factor. The content of cellulose recovered after pretreatment was calculated based on the content of cellulose in pretreated wood (48.6 g/100 g pretreated wood) related to the initial cellulose content in the raw material (42%). The content of hemicellulose recovered from the solid fraction is low.

The optimum severity factor of 3.94 could be used to ensure the elimination of hemicellulose by water extraction. The solid yield decreases from 81.5 g/100 g raw material (on dry basis) obtained at the severity factor of 3.05, to 71.9 g/100 g raw material obtained at the severity factor of 4.12. The decrease in the solid yields could be the result of the elimination of the hemicellulose in the liquid fraction and a small part of lignin. The lignin content undergoes no significant modification during the autohydrolysis experiments.

Chlorite delignification

The autohydrolyzed wood was delignified with sodium chlorite in acetic acid solution for lignin removal before the SSF process. Within each experiment, the solid phase obtained after the delignification process was analysed in order to determine the content of cellulose, hemicellulose and lignin. In Figure 3, the solid yields and the composition of the solids resulting after delignification are highlighted. In recent years, various delignification methods have been applied before enzymatic hydrolysis, having in view the increase of both enzymatic hydrolysis yield and the available surface area for enzymes. The application of wood delignification aims to destroy the hydrogen bond between cellulose and lignin. After the delignification process, the wood contains individual microsized cellulose fibres.

The results presented in Figure 3 show that the solid yields of delignification decreased with the increase in the temperature of the pretreatment performed before delignification. The delignified solid yields varied between 38.2-48.5 g of delignified wood/100 g raw material. The highest cellulose content predicted by the model was 93.0 g/100 g delignified wood (on dry basis), operating at a severity factor of 3.94. The highest cellulose content was obtained for wood samples treated at high temperature and medium reaction time. The hemicellulose and lignin contents decreased with the increase in the pretreatment temperature. Delignification eliminates approximately 89.0% of lignin. The high recovery of cellulose after delignification was calculated based on the content of cellulose in pretreated wood (48.6 g/100 g pretreated wood) related to the initial cellulose content in the raw material (42%). The content of hemicellulose recovered from the solid fraction is low.

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applying delignification and the elimination of hemicellulose and lignin from the solid phase suggests that the substrate is ready to be used for the SSF process.

Figure 3: Composition of pretreated and delignified wood as a function of severity factor

Reflected light microscopy characterization of untreated, autohydrolysed and delignified wood

Reflected light micrographs for the untreated, autohydrolysed and delignified wood (rule 5-190 °C, 10 min) are shown in Figure 4 (a, b, c). The texture of the autohydrolysed wood is different from that of the untreated wood.

The structure of the untreated wood (Fig. 4a1 and 4a2) shows the formation of resistance, whereas the autohydrolysed one (Fig. 4b1 and 4b2) clearly indicates how the wood structure was degraded after the autohydrolysis pretreatment.

The observed microstructure of the untreated wood has a longitudinal section and fibrous structure, whereas the microstructure of the autohydrolyzed wood presents spherical particles. Also, in Figure 4b1 and 4b2, the crystallinity of cellulose can be observed, which confirms that the applied pretreatment increased the crystallinity of cellulose. The lignin particles (black color) and the non-uniformity of the morphological structure of pretreated wood confirm the recovery of cellulose and lignin. The results show that the pretreatment plays an important role in the separation of wood components. Figure 4c1 and 4c2 present the reflected light micrographs of delignified wood. The surface of delignified wood presents particles of cellulose and small thin ones of lignin, which confirms the experimental results highlighted in Figure 3.
Figure 4: Reflected light micrographs of (a) untreated, (b) autohydrolyzed *Abies alba* wood (190 °C, 10 min) and (c) delignified wood (190 °C, 10 min) at 20x magnification and 40x magnification

**SSF process by Accellerase 1500 enzymes and *S. cerevisiae***

The SSF process was performed on pretreated and delignified wood by using a Minifor bioreactor. It is known that the composition of the fermentation medium is significant for bioethanol production.\(^{35,36}\) Also, it is necessary to find the optimal temperature of SSF due to the fact that the temperature of the SSF process is one of the most important factors influencing bioethanol productivity.\(^{16,35}\) One must mention that the SSF process combines both the enzymatic hydrolysis (the optimum temperature of enzyme efficiency is between 40 to 60 °C) and the fermentation (the optimal temperature is of about 35 °C). The presence of nutrient sources (nitrogen, high amount of carbon), enzymes, and yeast influences the production of bioethanol and secondary fermentation by-products as well. The ideal temperature for *S. cerevisiae* is 30 °C and 50 °C for *Accellerase 1500* enzymes.

In this study, a temperature of 38 °C was chosen for all the performed experiments. The fermentation medium was analysed in order to identify and determine the existing components. The yeast *S. cerevisiae* has the ability to produce ethanol only from glucose, but the presence of nutrients, carbon source and minerals led to the formation of other by-products as well, including acids, esters, aldehydes *etc.*

In the literature, many methods for bioethanol production from different types of lignocellulosic biomass are presented, but information regarding the entire composition of the fermentation medium is not available.\(^{7,9}\) Khattake\_et_al.\(^{37}\) reported analyses regarding the content of ethanol, acetic acid and acetaldehyde in the fermentation broth during the SSF process of waste from beer fermentation broth (WBFB). In our previous studies, Dan\_et_al.\(^{31}\) and Lazar\_et_al.\(^{38}\) detailed two methods for bioethanol production from fir wood (acid hydrolysis and SSF method after autohydrolysis and chlorite delignification). Based on the two methods, a concentration of 43.69 g L\(^{-1}\) for SSF hydrolysis (96 h fermentation time) and 37.53 g L\(^{-1}\) for acid hydrolysis was reported. In the present study, a higher concentration of bioethanol was obtained. The differences of bioethanol concentrations between this work and those presented above could be attributed to the equipment used for the SSF process (bioreactor, compared to classical
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equipment) and temperature. These results showed that the use of a bioreactor, in contrast to classical equipment, led to enhanced bioethanol content and reduced the fermentation time to 72 h.

In order to give a more accurate analysis of the fermentation medium obtained in this study, a complete analysis was done. The composition of bioethanol and acetic acid (compounds found in high concentration) and methanol from the fermentation medium obtained after the SSF process was modelled with the ANFIS model. The back-propagation and hybrid models were applied in order to determine the optimum value of all the dependent variables. The hybrid method used a learning algorithm based on back-propagation and a least square estimator. The model proposed has one dependent variable and two independent variables. The width of the Gaussian distribution (L) of temperature was 4.21 and 2.12 for time.

The content of bioethanol, acetic acid and methanol obtained (experimental values) and the values recorded after processing the experimental data with the ANFIS model using both back-propagation and the hybrid method are presented in Table 1. For this study, the number of rules has been selected as nine. The data presented in parentheses represent the values predicted using Gaussian member’s functions and operational variables. The performance of the model is given by R, RMSE and accuracy parameters.

The correlation coefficients calculated according to Equation 4 were between 0.998-1 for the hybrid method, and between 0.24-0.9995 for the back-propagation model. The prediction accuracy values (Eq. 6) were in the range of 100-100.2 for the hybrid method and of 34.0-99.2% for the back-propagation method (low accuracy was obtained for the prediction of methanol content by using the back-propagation model). By comparing the experimental values with the ones predicted by the ANFIS model, the RMSE values are 0.33-1.3.

Table 1
Values of independent variables and bioethanol, acetic acid and methanol content predicted by the neural fuzzy model for dependent variables, using nine rules

| Rules | Experimental results | Back-propagation (hybrid) | Experimental results | Back-propagation (hybrid) | Experimental results | Back-propagation (hybrid) |
|-------|----------------------|---------------------------|----------------------|---------------------------|----------------------|---------------------------|
| Rule 1 | 29.3 | 29.2 (29.3) | 6.0 | 6.2 (6.0) | 0.1 | 0.3 (0.1) |
| Rule 2 | 35.1 | 35.0 (35.1) | 7.5 | 7.7 (7.5) | 0.2 | 0.3 (0.2) |
| Rule 3 | 38.9 | 38.8 (39.9) | 8.0 | 8.2 (8.0) | 0.2 | 0.4 (0.2) |
| Rule 4 | 42.1 | 41.8 (42.1) | 9.5 | 9.9 (9.5) | 0.3 | 0.6 (0.3) |
| Rule 5 | 52.0 | 51.7 (52.0) | 10.0 | 10.4 (10.0) | 0.3 | 0.7 (0.3) |
| Rule 6 | 46.8 | 46.5 (46.8) | 9.0 | 9.4 (9.0) | 0.2 | 0.5 (0.2) |
| Rule 7 | 37.5 | 37.0 (37.5) | 7.6 | 8.3 (7.6) | 0.1 | 0.8 (0.1) |
| Rule 8 | 40.6 | 40.0 (40.6) | 8.2 | 9.0 (8.2) | 0.2 | 0.8 (0.2) |
| Rule 9 | 39.1 | 38.5 (39.1) | 7.8 | 8.6 (7.8) | 0.2 | 0.9 (0.2) |

Based on the obtained results, the performance of the hybrid method for the prediction of bioethanol, methanol and acetic acid contents was very good. The results presented in Table 1 indicate that the modelling of experimental results by the hybrid method has good reproducibility.

In Figure 5, the bioethanol content variations are presented, as a function of autohydrolysis temperature and time. The highest bioethanol content was obtained for rule 5 (medium temperature and time). The content of bioethanol varied in the range of 29.3-52.0 g L⁻¹.

The ethanol content obtained after SSF fermentation with *S. cerevisiae* was strongly affected by the autohydrolysis pretreatment conditions. It was reported that by increasing the temperature, the sugar conversion increased and the ethanol concentration in the SSF process decreased. The ethanol concentration after 72 h was 52.0 g L⁻¹ (rule 5). The production of high
bioethanol content is favored by medium temperature and medium time.

The production of acetic acid can be attributed to the presence of minor amounts of hemicellulose in the pretreated and delignified wood. The content of acetic acid as a function of the independent variables is shown in Figure 6. A high negative influence of the temperature on the acetic acid content has been observed.

The content of methanol is slightly affected by the reaction time. A high influence of the methanol content was noted at medium temperature, similarly to the bioethanol content. At low temperature, low values of acetic and methanol content have been observed.
The content of methanol as a function of the independent variables is presented in Figure 7. The simulation model by using the back-propagation algorithm shows slightly reduced efficiency, the prediction of the methanol content is no more accurate than that of the hybrid model due to the self-learning ability. The methanol content, in all the cases, is little influenced by the pretreatment conditions.

To the best of our knowledge, there is no previous report on the use of ANFIS to predict the concentration of the fermentation broth products after autohydrolysis–delignification–SSF process of wood.

At the end of the fermentation process, the fermentation medium samples were analysed in terms of their content of other by-products. The fermentation broth obtained from the biomass wood is a dark brown mixture of liquid and solid fraction (which contains raw materials, inoculum and nutrients). The liquid fraction was separated from the solid part and analysed with regard to other by-products.

The analysis of higher alcohols, aldehydes, esters and acids is presented in Figure 8 (rule 5). Higher alcohols are formed during fermentation due to the presence of nitrogen in yeast fermentation media. The concentration of these by-products is much lower than that of bioethanol and acetic acid. The results presented in Figure 8 indicate that, in the fermentation medium, alcohols, such as pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, n-butanol, 2-methyl-1-propanol and n-propanol, are identified. With regard to acetic acid, generally, the lowest amount of alcohols is found. Among higher alcohols, the compound present in the highest amount was represented by n-propanol (0.4 g L\(^{-1}\)). Other compounds present in high concentrations were propanoic acid (0.62 g L\(^{-1}\)), 2-methyl-1-butanal (0.51 g L\(^{-1}\)) and ethyl hexanoate (0.32 g L\(^{-1}\)).

Figure 8: Content of secondary by-products in experimental bioethanol produced by \textit{S. cerevisiae} strains

Figure 9: Mass balance for bioethanol production from \textit{Abies alba} wood
The mass balance for bioethanol production from *Abies alba* wood is presented in Figure 9.

To sum up our findings, the content of cellulose in 100 g of *Abies alba* wood was reduced from 42.0 g to 39.2-41.0 g after the autohydrolysis pretreatment. After delignification, the obtained content of cellulose was of 33.6-37.0 g, which was subsequently hydrolyzed and fermented to bioethanol. A maximum of 5.2 g bioethanol was obtained under the following conditions: 10 min pretreatment autohydrolysis at 190 °C, followed by 72-hour hydrolysis and fermentation period.

**CONCLUSION**

*Abies alba* wood was used as raw material for the production of bioethanol by autohydrolysis–delignification–SSF method, followed by the SSF process. The chlorite delignification method eliminated 89.0% of lignin (medium temperature and medium reaction time) and enhanced the SSF process for bioethanol production.

According to the obtained results, the optimum conditions to be applied to produce bioethanol from wood, using the autohydrolysis–delignification–SSF method, are as follows: a medium temperature of 190 °C, and medium reaction time of 10 min. The mass balance suggests that, from 42 g cellulose content in *Abies alba* wood, a maximum of 5.2 g of bioethanol can be obtained after autohydrolysis–delignification–SSF process.

In this study, the adaptive neural fuzzy interference system mathematical model (using back-propagation and hybrid modeling methods) has been used to estimate the bioethanol and secondary by-products concentration that can be obtained by the described method and compared to the experimental results. The findings indicate that the proposed ANFIS model using the hybrid method can predict more precisely the experimental results (accuracy of 100.0-100.2%) than the back-propagation method (34.0-99.2%). The ANFIS model can also be applied for modelling other chemical processes.

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