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Pre-treatment of bio fraction waste prior to fermentation processes

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Abstract

Current efforts are taken to increase resource efficiency, close material loops, and improve sustainable waste and by-products management. Thus, networking agro-food by-products and converting them into valuable products completely exhausting the potential of the raw material becomes significant. Model lignocellulosic and starch based biomass were subjected to pre-treatment with the application of acidic compounds, i.e. sulphuric (SA) and acetic (AA) acids. The response, i.e. total sugar content and derivative content was investigated depending on variables changed during hydrolysis: concentration of acid, process duration, temperature and the size of biomass particles. After saccharification, the hydrolysates were analysed via HPLC. Total reducing sugar concentration was in the range of 0.1–15.53 g/L. Among the substances present in the hydrolysates, protein, peptides, hydroxybenzyl acid (HA), 5-HMF, furfural (FF), vanillin (V), vanillic acid (VA), formic acid (FA) and levulinic acid (LA) were found in the range of 0.44–9.05 g/L and determined as total derivative concentration. The aim of the study was to evaluate the measurable effects of the research and deliver information about the statistically important parameters for the process course and relations between the variables.

Keywords

hydrolysates, saccharification, hydrogen, biowaste, anaerobic digestion

1. INTRODUCTION

Substrates used for production of biofuels allow to classify the biofuels as products of 1st, 2nd, 3rd and further generations. Organic food products, including starch, animal and vegetable fats, sugars (e.g. sucrose) play a superior role in the direct or indirect production of first-generation biofuels (Kucharska et al., 2018a). However, the use of such materials in the production of biofuels poses certain difficulties related to the competition of crops for energy and food purposes, and thus food security insurance affects a possible increase in food prices. Another negative impact of the production of 1st generation biofuels is the reduction of biodiversity. Biofuels are less resistant to biological and chemical decomposition, and more corrosive than fossil fuels (Watanabe et al., 1998). On the other hand, 2nd generation biofuels, have excellent qualitative properties and their production does not pose a threat to the quality of land and food production (Anwar et al., 2014). Developing biomass processing methods is crucial for the production of biofuels of the second and third generations. Examples of second generation biofuels include, lignocellulosic ethanol via alcohol fermentation, methane and biogas via methanation, biohydrogen via anaerobic digestion (AD) and syngas employing thermochemical methods (TM) or methods combining both AD and TM (Vijayaraghavan and Ahmad, 2006; Xing et al., 2010). Algae and marine biomass are used to obtain 3rd generation biofuels. Algae are characterized by fast growth. They allow for a very effective land usage, even 30 times higher than for terrestrial biomass and therefore, can produce more energy from a unit of area in comparison with energy obtained from the same area as 1st or 2nd generation biofuels (Bothe et al., 1974; Pointner et al., 2014; Sorokin and Krauss, 1958). Territorial deserts and wastelands are perfect for algae cultivation, since they provide unrestricted access to solar energy and as reported (Chisti et al., 2007; Dahmani et al., 2016; Ramli et al., 2023) although algae cultivation in the desert is feasible, it may not be practical. Algae are cultivated in Qatar deserts in salt (sea) water. Its cultivation does not require drinking water. It is possible to grow algae on wastewater from AD, i.e. from dark fermentation process, which is rich in organic acids and other substances used by algae as a sole carbon source in nitrogen deficit. Therefore, obtaining several biofuels with a possibility to create a closed circular loop seems possible when combining the 1st and 2nd generation biofuels followed by 3rd generation biofuel production. This approach is related strictly to circular economy, since it allows to carry management of post fermentation broths from anaerobic processes (Hay et al., 2013; Scholz et al., 2013).

In the literature, reports regarding non-homogeneous sources of raw materials for the production of biofuels from biomass appear relatively rarely. Most often, you can find model studies based on the analysis of a narrow case concerning a selected raw material, or possibly a comparison of several raw materials. In this paper, the authors present the results of pre-treatment, using a mixture of waste from the food sector and biomass waste as a raw material. This type of waste generated in real conditions is a direct fraction of municipal bio-waste, i.e. organic plant residues, obligatory collected selectively in the European Union (Keskin et al., 2011; Redondas et al., 2012). AD processes require a pre-treatment step in order to increase the availability of monosugars, i.e. the yield of sole carbon source in fermentation processes. Starch-containing solid wastes are relatively easy to process to produce carbohydrates and hydrogen gas compared to feedstocks containing complex carbohydrates such as cellulose and hemicellulose. Lignocellulosic biomass consists mainly of cellulose, hemicellulose, lignin and small amounts of extractives soluble in water and/or ethanol. The high resistance of lignocellulosic biomass is evidenced by the crystallinity of cellulose, its protective layer created by lignin, the heterogeneous nature of the particles, the content of syringyl, guaiacyl and the ratio of lignin to cellulose content (Kumar et al., 2016, Yokoi et al., 2001). Bio fraction waste delivers several difficulties in the matter of pre-treatment, since the main goal of the procedure is to enhance the saccharification process (Kucharska et al., 2018b). Structurally, cellulose is built of linear chains of D–glucose units connected by β–1,4–glycosidic bonds, therefore only glucose and its derivatives may be formed with exhausting saccharification. Hemicelluloses, i.e. glucuronoxylan, arabinogalactan, glucomannan, arabinoglucuronoxylan and galactoglucomannan deliver more diversified solutions of pentoses and hexoses (Kucharska et al., 2018b). The hemicellulose molecule also contains sugar acids such as uronic acids, i.e. α–D–glucuronic acid, α–D–galacturonic acid and α–D–4–O–methylgalacturonic acid. In the lignin present in wood, there are often ester and ether linkages between the hydroxyl groups of the polysaccharides and the phenylpropane α–carbanol subunits (Ravinder et al., 2001). In turn, in grasses between hemicellulose and lignin, the presence of feluric and p-coumaric acids was observed, probably forming intermolecular ester-ether bridges. The strong relationship between lignin, hemicellulose and cellulose may reduce access to sugars directly involved in fermentation. Therefore, pre-treatment of lignocellulosic materials should lead to the release of sugars contained in lignocellulosic fibers located in the heteromatrix of cell walls. At the same point starch saccharification is expected (Kucharska et al., 2021). To the best knowledge of the authors, based on a review of source literature in Scopus and Web of Science databases, the literature lacks information for diversified real samples of biomass and agri-food wastes, therefore this research is a contribution to the field.

This study presents a new approach for pre-treatment, as bio fraction waste is used as raw material (Słupek et al., 2020). Methods of pre-treatment under acidic conditions are tested. Hydrolysates obtained during pre-treatment are subjected to fermentation processes in order to prove their potential in AD processes (Makoś et al., 2020). Therefore, the evaluation of the measurable effects of hydrolysis are believed to deliver information about the statistically important parameters for the process course and relations between the variables. The research has been planned to define whether it is possible to plan the course of hydrolysis to obtain saccharified biomass free from lignin derivatives and secondary derivatives of monosugars under acidic conditions. The assumed approach was verified applying two acid types – inorganic sulphuric acid and organic acetic acid. In both cases the hydrolysis process was carried with respect to Box-Behnken design. The aim of the study was to determine a criterion that could be used to identify whether the obtained hydrolysate has the potential for the production of liquid or gaseous biofuels or for biorefining processes.

2. MATERIALS AND METHODS

2.1. Materials

Glucose, xylose, arabinose, galactose, fructose, mannose, furfural, 5–hydroxymethylfurfural, 4–hydroxybenzoic acid, vanillin, acetonitrile, methanol, sulphuric acid, acetic acid, deionized water, and propionic acid of high purity ($\geq 98.0\%$) were purchased from Sigma Aldrich (St. Louis, MO, USA) and CHEMAT (Poland).

Real biomass and organic waste samples (bio fraction) were prepared as mixtures of potato pulp peelings, biomass i.e. residues of corn cobs and bakery wastes in proportions 1:1:1. To prepare waste samples moisture content was determined and the water and ethanol extractives were measured. To avoid mold outbreaks during storage, samples were kept as dry material and for the purposes of homogeneity and repeatability of the experiment only dry weight of samples has been applied for sample preparation. Further, the samples were pre-treated using combined mechanical pre-treatment and acidic hydrolysis.

Conditions of the pre-treatment process were defined in the range of four parameters, i.e. hydrolysis time 1 to 96 h; size of the biomass 0.25–4 mm, sulphuric and acetic acid concentration from 2 to 60%, temperature 20–80 °C. Sulphuric acid is broadly used for the hydrolysis of lignocellulose (Potumarthi et al., 2013; Ravichandran et al., 2012), but it may cause formulation of derivatives from hexoses (glucose) and pentoses (xylose). The authors applied acetic acid to compare the results obtained for stronger acid, i.e. sulphuric acid with milder acid. On the other hand acetic acid, is not reported to cause secondary changes in monosugar structure and it may be an advantageous hydrolysing agent, as its excess in the hydrolysate may be further metabolised by algae as a sole carbon source in photo fermentation.

2.2. Methods

2.2.1. Pre-treatment of the bio fraction

In the case of pre-treatment of bio fraction it is expected that large pieces of food residues and biomass would allow for a more favourable energy balance when processed. As this stage is the most energy-intensive part of the entire process, the aim is to keep the energy demand as low as possible while maintaining high product recovery efficiency (Arce and Kratky, 2022; Bhutto et al., 2017). In addition, the parameters of the pre-treatment process are selected so as to minimize the losses of sugars, in particular pentose sugars. Therefore, temperature during milling and mincing should not increase.

Meec Tools garden shredder 425 (MEEC Tools, Sweden) and RETSCH Ultra Centrifugal Mill ZM 200 (Retsch, Germany) were applied for milling and mincing of the bio fraction. The milled material was sieved throughout 0.25 mm, 0.75, 2 and 4 mm screens. Further, after grinding the material was dried at 105 °C, 4 h and stored in room temperature in containers. The containers allowed air movement to avoid mold outbreaks. Model lignocellulosic and starch based biomass were subjected to pre-treatment with the application of acidic compounds, i.e. sulphuric (SA) and acetic (AA) acids. The applied concentrations of acids were in the range from 2% up to 60%. The hydrolysis process duration (time) lasted from 1 h to 96 h and the process temperature (T) was in the range of 20–80 °C. The process was carried out with agitation (50–300 rpm). The proportion between biomass and hydrolysing agent amount (m/m) was in the range of 0.01 to 0.5 (by mass). The loss of biomass amount during pre-treatment was analysed with respect to the agitation.

The content of cellulose and hemicellulose in lignocellulosic part of the bio fraction was determined by HPLC with a Rezex Pb^{2+} column (300 × 7.8 mm, 8 µm) with refractometric detection (Knauer, Germany). A flow rate of 0.6 mL/min of water was used as the eluent.

2.2.2. Determination of monosugar concentration

The analysis of the concentrations of reducing sugars in the samples started with the preparation reagent A (1% 3,5–dinitrosalicylic acid, DNS). In the first stage 20 mL of a 2M NaOH solution was prepared: 1.6 g of sodium base in the form of microgranules, and then 20 mL of demineralized water was added. A 100 mL volumetric flask with an aqueous acid solution of 3,5–dinitrosalicylic acid (dissolved 1 g of acid in 50 mL of demineralized water) and then a previously prepared NaOH solution, 30 g were added. The calibration curve necessary for the subsequent determination of the concentration of reducing sugars in the tested hydrolysates was prepared. Further, after closing and mixing the probe contents, the tubes were placed in the water bath for 15 minutes (100 °C). After removal and cooling under a stream of cold water, the solution absorbance was measured at 565 nm using a blind sample as reference. Glucose exhibited two absorption bands – at wavelengths of 500 nm and 565 nm (Razola-Díaz et al., 2020). Linear results were obtained at 565 nm.

2.2.3. Observation of changes in the structure of the bio fraction during pre-treatment

The samples of hydrolysates were measured for occurring changes. Sample were centrifuged for 7 minutes using a Hettich EBA 8S centrifuge (Hettich, Germany). Part of the supernatant solution was poured into Eppendorf centrifuge tubes with a capacity of 2 mL using Omnifix Solo 20 mL Luer Lock syringes and PES membrane syringe filters. Then the precipitate was subjected to vacuum filtration using a laboratory set consisting of: a glass funnel, aluminium buckle, sintered caps for filters, filter flask. Acetone (pure for analysis), was used to wash the precipitate. The filtered precipitate was collected with metal spatulas and dried at 80 °C. After drying, the samples were weighed on analytical scale. The filtrates and sediments were examined on the Bruker FT-IR tensor 27 spectrophotometer (Bruker, Netherlands). The software used for reading, processing and evaluating the spectra was OPUS. Measurements were made with the following parameters:

- resolution: 4 cm^{-1},
- number of background scans: 256 scans,
- number of sample scans: 256 scans
- data recording in the range: 6300 cm^{-1} to 500 cm^{-1},
- spectrum of results: absorbance.

During the measurements, distilled water was used as a reference system for filtrates, and air for sediments.

2.2.4. Determination of lignin and sugar derivatives in biomass

Analysis for identification and determination of lignin derivatives was performed using high-performance liquid chromatography (HPLC) (Beckman, Germany) coupled with a single-beam spectrophotometric detector at a wavelength of 284 nm (Beckman 166, Germany) RID detector was used (RID-RI Detector 2100, Knauer, Germany), and a Kinetex 2.6 µm thermostated at 35 °C column, 100 × 4.6 mm, Polar C18 100 Å (Phenomenex, Torrance, CA, USA). The LpChrom software was used to record and process the results. Prior to each analysis, the model and real hydrolysate samples were filtered through a 0.45 µm hydrophilic syringe filter. The volume of the test sample was 50 µL.

2.2.5. Statistical methods

In order to determine the best conditions for the pre-treatment process, the experiment was planned according to the BBD design (table 1). The study examined the influence of four main factors: biomass particle size (X1), temperature (X2), sulphuric or acetic acid concentration (X3a, X3b, respectively), time (X4).

These variables are coded in a range of levels from –1 to +1, and the precise values for the coding levels are shown in Table 1.

Table 1. The values of process parameters applied during the experiment, by coding level.

Coding level	X1	X2	X3a	X3b	X4
–1	0.25	20	2	2	1
0	2	50	31	31	48
1	4	80	60	60	96

The results of the analysis were processed by multiple regression in Minitab 10.2. Based on the results, the advantageous conditions for maximum and minimum sugar yield were defined.

3. RESULTS AND DISCUSSION

3.1. The loss of biomass during pre-treatment

During the pre-treatment of bio fraction, a decrease in the biomass amount is observed. With the progress of saccharification, monosugars are released to the solution while the solid bio fraction waste residues drop to the bottom of the flask. The agitation favours the increase of the contact surface between the hydrolysed biomass and the acid solution, and thus further saccharification is obtained. Consequently, the total yield of monosugars in the hydrolysate increases. The obtained results are presented in Figure 1.

It was found that for the average concentrations of acids, a decrease in the content of solid biomass residues during hydrolysis was observed (Fig. 1), corresponding to the increase in the applied agitation. Stirring during hydrolysis has a significant impact on the size of the contact area, which in the case of suspensions of solid particles decreases drastically when the process is carried out without shaking. It can be observed that as the biomass and the solid part of the bio fraction decrease, the concentration of reducing sugars passing into the solution increases, which is directly related to the decrease in the degree of complexity of the solid structures. At the same time, a difference between results obtained for SA and AA was observed. SA is a stronger acid than AA, therefore stronger saccharification effect occurs. Further, the effect of the type of acid on the composition of hydrolysates shall be defined, since the same secondary derivatives may be formed due to the presence of strong hydrolysing agents. Loss of biomass during pre-treatment is a typical and desirable phenomenon (Han et al., 2020; Sahare et al., 2012), because the heteromatrix of bio fraction waste contains polymerized sugars, which may be a sole carbon source after depolymerization. Further, in the liquid phase, monosugars are then available for anaerobic digestion. Therefore, the higher biomass loss is synonymous to the higher hydrolysis efficiency.

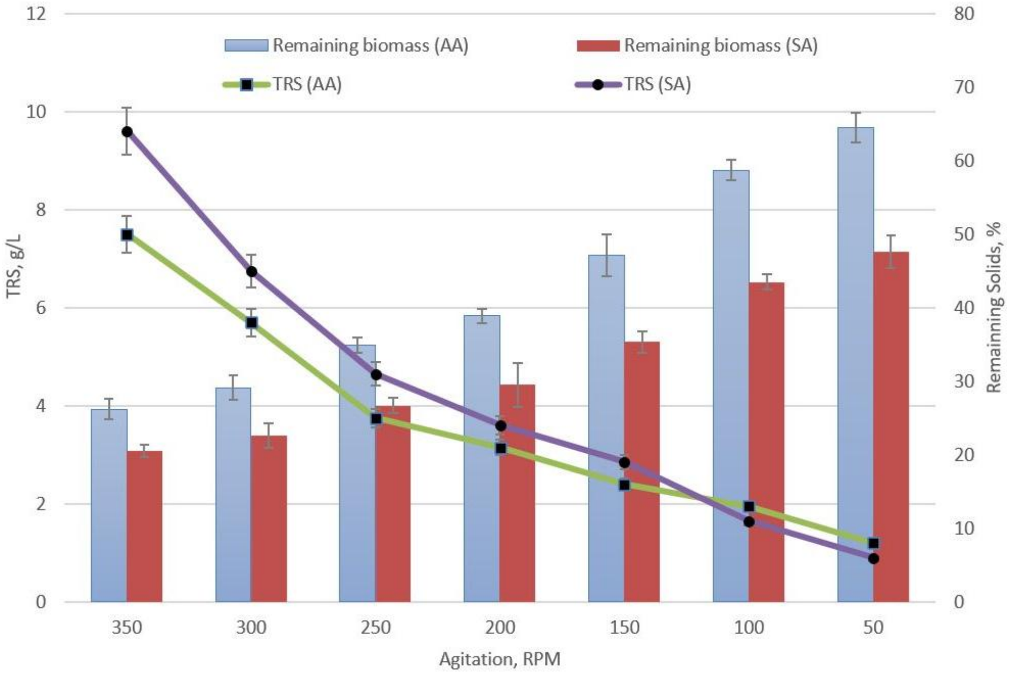

Figure 1. The dependence of remaining solids and total reducing sugar concentration (TRS) obtained during pre-treatment with respect to agitation, for average sulphuric acid (SA) and acetic acid (AA) concentrations. The samples were analysed in triplicates. The standard deviation for triplicate samples is provided in the diagram. The presented TRS values for AA and SA hydrolysis show a similar trend depending on agitation, although they differ statistically depending on the hydrolysis catalyst used. The TRS values correspond to the biomass loss with respect to remaining solids.

3.2. The composition of hydrolysates

Before proceeding to the analysis of mutual interactions between variable process parameters during hydrolysis, the typical compositions of hydrolysates were examined. Average concentrations of acids used for hydrolysis were used. In the hydrolysates obtained after 48 hours, substances with the character of saccharides, as well as lignin derivatives and secondary products of monosaccharide transformations in the presence of acids of various strengths (SA, AA) were determined. The results were summarized as a pie chart showing how the shares of individual groups of chemical substances change during hydrolysis and how they differ when different hydrolysing agents are used (Fig. 2).

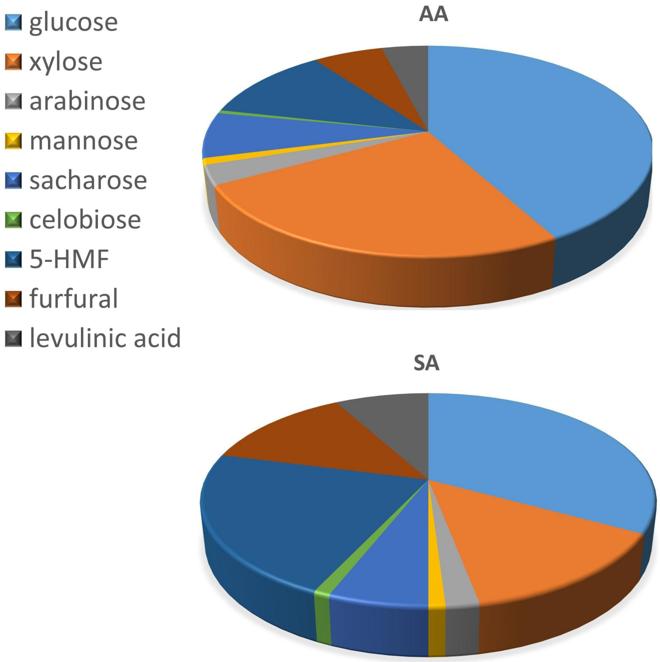

Figure 2. Composition of hydrolysates obtained during hydrolysis with average concentrations of acids. Acidic Acid, AA); Sulphuric Acid, SA.

3.3. Box Behnken design

Box Behnken design and the results obtained for total reducing sugars TRS and total derivatives TD are given in Table 2 (for AA and for SA). The symbols X1, X2, X3 and X4 represent the relevant factors, i.e. granulation, process temperature, concentration of acetic acid (or sulphuric acid) solution and hydrolysis time. Analysis of the interactions between variables allowed to estimate the efficiency of the acid hydrolysis step (understood as the concentration of reducing sugars in the hydrolysate) and the trend to undergo secondary transformation (understood as the concentration of derivatives) based on the given values of process parameters and outcomes of the research. Analysis of variance was carried out to select the most significant parameters and their interactions affecting the pretreatment process. Response Surface Methodology with BBD is widely used in experiment optimisation (He et al., 2018; Raheem et al., 2022a; Raheem et al., 2022b). Statistical *p*-values and *F*-values were adopted as criteria at a 95% confidence level. A *p*-value above 0.05 was defined as a statistically insignificant factor in the Box Behnken design. Results confirmed that the calculated regression equation is statistically significant with respect to p-value. The obtained equations showed a high determination coefficients R^2, predicted determination coefficient $R^2_{\text{(pred)}}$ and adjusted determination coefficient $R^2_{\text{(adj)}}$. The correlation between the experimental results allowed to predict new data in the tested range. Equations and statistical parameters are shown in Table 3.

The authors observed that in the experimental pattern there are samples for which higher TD concentrations are obtained and those for which higher TRS concentrations are obtained. Since both products may be interesting from the point of view of the process implementation, the authors decided to perform calculations for both model responses. In the case of TD, an experimental path is obtained that justifies the process for extraction into green solvents, and with high concentrations of TRS. It is worth implementing the process for anaerobic digestion and photo fermentation. The development of the criterion for the selection of the operating route is crucial from the point of view of the efficiency of both processes, since it is not possible to obtain high concentrations of TRS and TD simultaneously, and obtaining average concentrations of both components will be undesirable. Maximum size of the biomass, concentration of the acetic and sulphuric acids, temperature and time promotes obtaining the highest TRS concentration. Unfortunately, also maximum TD concentrations are obtained when maximum temperature and acid concentration is applied. To avoid this situation, the lowest size of particles must be used and the process parameters may then be milder, i.e. AA concentration of 22% in the temperature equal to 55 °C and 16% of SA in 43 °C is accurate to obtain high TRS concentration, while Td is low.

In Figure 3 selected response surface areas and the results of the interaction between two factors in a configuration where the other two factors remain constant are presented. Based on results in Table 2 and data in Figure 3 a positive correlation between the concentration of reducing sugars and size of the biomass particles was identified for both hydrolysing agents. In the range of low temperatures, an increase in biomass granulation caused a slight decrease in saccharification efficiency. As the temperature rose, the efficiency of acid hydrolysis increased. However, in lower temperatures the secondary transformations of monosugars leading to higher TD values were limited. Beside acid type and concentration, also temperature led to derivative formulation.

The analysis of the results presented in Table 2 showed that the highest TRS values were obtained during the 96-hour process. Unfortunately, the extension of time is also favoured by the partial formation of monosaccharide derivatives, the more pronounced the higher the acid concentration. Longer processes usually provided higher efficiency (Raheem et al., 2022a; Tamoradi et al., 2021), although at the same time, additional derivatives were formed (Akobi et al., 2016). To verify the obtained models a diagram comparing the model-predicted and obtained values was prepared and is presented in Figure 4.

The correlation between the data is satisfactory. For hydrolysis carried out with sulfuric acid, this effect is more pronounced than for acetic acid, but in both cases the relationship between TRS and TD remained proportional over the entire variability range. Despite achieving slightly lower saccharification yields, it seemed to be advantageous to carry out the process with the use of acetic acid. The environmental aspect is of great importance here, i.e. the potential use of hydrolysates. High proportions of reducing

Table 2. Box Behnken design obtained for hydrolysis of bio fraction.

Size [mm]	Temp [°C]	C [%]	Time [h]				
X1	X2	X3	X4	TRS(AA) [g/L]	TRS(SA) [g/L]	TD(AA) [g/L]	TD(SA) [g/L]
2.00	50	31	48.5	3.70	5.93	2.41	2.89
2.00	50	31	48.5	3.00	5.27	2.07	2.56
2.00	80	31	1.0	0.85	2.69	0.88	1.13
2.00	20	2	48.5	0.10	1.78	0.47	0.62
0.25	50	60	48.5	0.89	2.92	0.95	1.30
4.00	50	31	96.0	9.84	15.53	6.34	8.88
2.00	50	2	1.0	0.00	1.51	0.38	0.45
0.25	20	31	48.5	0.18	1.78	0.49	0.61
0.25	50	31	96.0	0.91	2.77	0.92	1.18
2.00	20	31	1.0	0.03	1.69	0.43	0.57
2.00	80	2	48.5	0.38	2.22	0.65	0.88
0.25	50	31	1.0	0.01	1.76	0.44	0.62
4.00	80	31	48.5	8.00	11.10	4.78	5.73
2.00	50	60	96.0	9.32	13.11	5.61	6.95
2.00	80	31	96.0	11.70	16.58	7.07	9.05
2.00	50	60	1.0	0.15	1.84	0.50	0.66
2.00	50	2	96.0	0.47	2.34	0.70	0.96
4.00	50	60	48.5	7.58	12.36	4.99	6.98
4.00	20	31	48.5	2.96	5.05	2.00	2.40
4.00	50	31	1.0	0.15	1.74	0.47	0.59
2.00	50	31	48.5	3.70	6.33	2.51	3.21
2.00	20	60	48.5	2.86	5.43	2.07	2.73
2.00	20	31	96.0	2.93	5.68	2.15	2.93
0.25	50	2	48.5	0.03	1.79	0.46	0.64
2.00	80	60	48.5	11.44	15.23	2.55	3.06
0.25	80	31	48.5	0.74	2.47	0.80	0.99

LOD = 0.003 g/L (total derivatives; TD); 0.002 g/L (total reducing sugars; TRS) sugars gave hope for the possibility of using hydrolysates in fermentation processes, e.g. alcoholic fermentation or dark fermentation. When it comes to the subsequent management of fermentation broths, the choice of acid can be of great importance (Raheem et al., 2015; Sorokin and Krauss, 1958). If we use acetic acid, the following broths can become a charge for photo fermentation processes. Conducting photo fermentation as a process that can occur after other fermentation processes seems to be an interesting solution, as it allows for greater efficiency. The general concept uses the metabolisation of hydrogen by photosynthetic bacteria, so organic acids resulting from dark or alcohol fermentation will be a source for them to produce additional hydrogen for photosynthetic bacteria (Ji et al., 2011; Mabutyana and Pott, 2021). The authors have previous successes in carrying out the procedure of subsequent dark and photo fermentations (Kucharska et al., 2019; Kucharska et al., 2021; Słupek et al., 2019; Słupek et al., 2020). The former processes were carried for single type of biomass, while in this paper, the approach is broad and directed to bio fraction as a mixture of several polyccharidic polymer types.

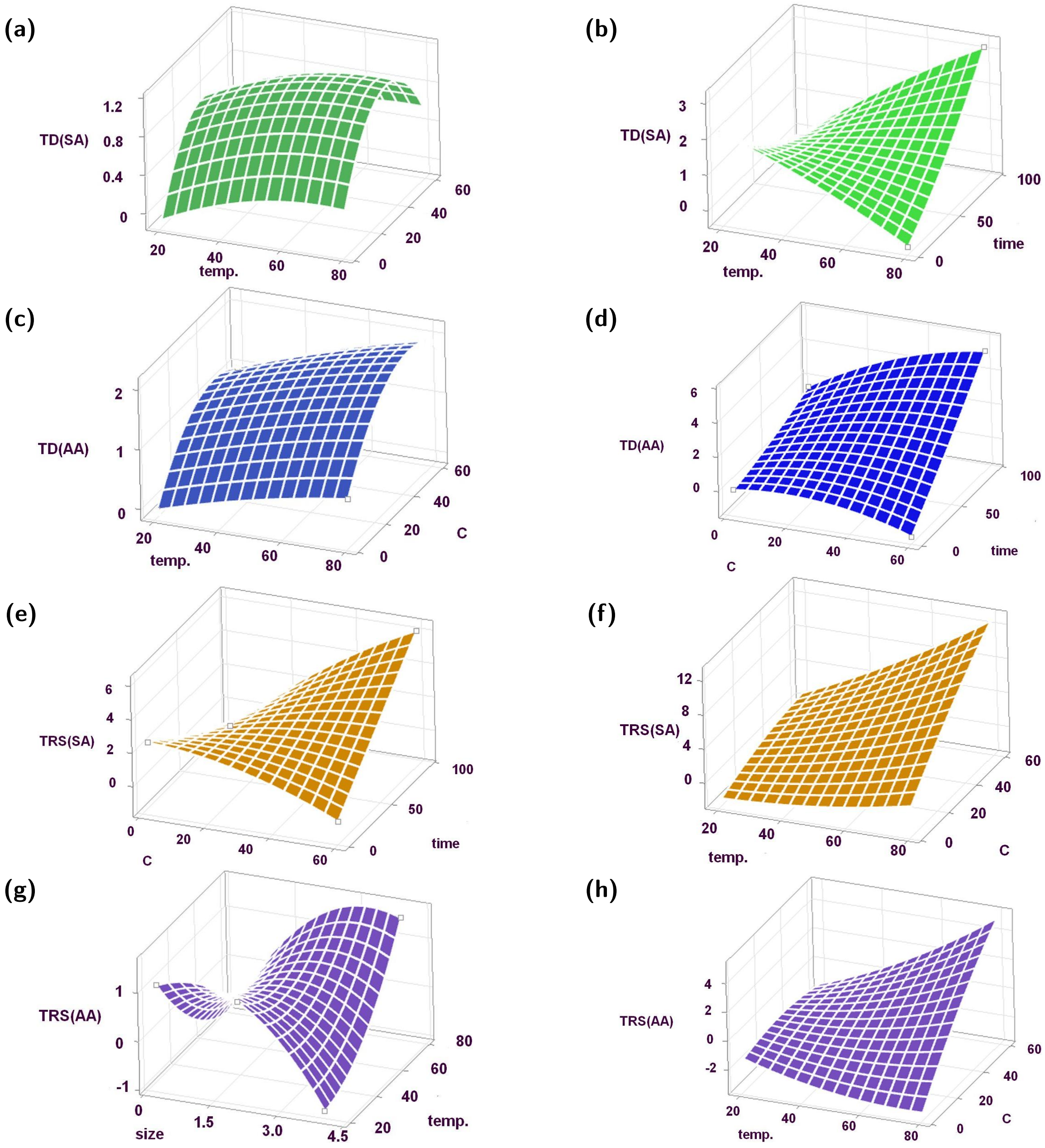

Figure 3. Selected response surface plots for model outcomes. Surface area dependence on a) temperature and concentration for TD (SA) optimisation, b) temperature and time for TD (SA) c) temperature and concentration for TD (AA) optimisation d) concentration and time for TD (AA) optimisation; e) concentration and time for TRS (SA) optimisation; f) temperature and concentration for TRS (SA) optimisation; g) temperature and size for TRS (AA) optimisation; h) temperature and concentration for TRS (AA) optimisation.

Table 3. Results of the BBD with statistical parameters.

Modelled outcome	Obtained equation	R^2/ $R^2_{\text{(adj)}}$ $R^2_{\text{(pred)}}$	*p*-value
TRS(AA) [g/L]	$= 7.30 - 0.743 \cdot X1 - 0.1738 \cdot X2 - 0.1261 \cdot X3 - 0.1011 \cdot X4 - 0.259 \cdot X1 \cdot X1 + 0.000599 \cdot X2 \cdot X2 - 0.000617 \cdot X3 \cdot X3 - 0.000082 \cdot X4 \cdot X4 + 0.01918 \cdot X1 \cdot X2 + 0.02790 \cdot X1 \cdot X3 + 0.02444 \cdot X1 \cdot X4 + 0.002384 \cdot X2 \cdot X3 + 0.001396 \cdot X2 \cdot X4 + 0.001580 \cdot X3 \cdot X4$	96.64% 92.71% 81.05%	< 0.05
TRS(SA) [g/L]	$= 10.90 - 1.51 \cdot X1 - 0.1960 \cdot X2 - 0.1560 \cdot X3 - 0.1400 \cdot X4 - 0.261 \cdot X1 \cdot X1 + 0.000618 \cdot X2 \cdot X2 - 0.000669 \cdot X3 \cdot X3 + 0.000000 \cdot X4 \cdot X4 + 0.0229 \cdot X1 \cdot X2 + 0.0409 \cdot X1 \cdot X3 + 0.03574 \cdot X1 \cdot X4 + 0.002691 \cdot X2 \cdot X3 + 0.001737 \cdot X2 \cdot X4 + 0.001892 \cdot X3 \cdot X4$	96.88% 93.25% 82.40%	< 0.05
TD(AA) [g/L]	$= 2.78 - 0.804 \cdot X1 - 0.0299 \cdot X2 + 0.0013 \cdot X3 - 0.0676 \cdot X4 - 0.0808 \cdot X1 \cdot X1 - 0.000077 \cdot X2 \cdot X2 - 0.000730 \cdot X3 \cdot X3 + 0.000055 \cdot X4 \cdot X4 + 0.01093 \cdot X1 \cdot X2 + 0.01761 \cdot X1 \cdot X3 + 0.01505 \cdot X1 \cdot X4 + 0.000085 \cdot X2 \cdot X3 + 0.000783 \cdot X2 \cdot X4 + 0.000868 \cdot X3 \cdot X4$	95.06% 89.31% 89.31%	< 0.05
TD(SA) [g/L]	$= 3.66 - 1.305 \cdot X1 - 0.0287 \cdot X2 - 0.0013 \cdot X3 - 0.0917 \cdot X4 - 0.069 \cdot X1 \cdot X1 - 0.000152 \cdot X2 \cdot X2 - 0.000849 \cdot X3 \cdot X3 + 0.000120 \cdot X4 \cdot X4 + 0.01306 \cdot X1 \cdot X2 + 0.02535 \cdot X1 \cdot X3 + 0.02168 \cdot X1 \cdot X4 + 0.000017 \cdot X2 \cdot X3 + 0.000976 \cdot X2 \cdot X4 + 0.001051 \cdot X3 \cdot X4$	94.77% 88.66% 70.55%	< 0.05

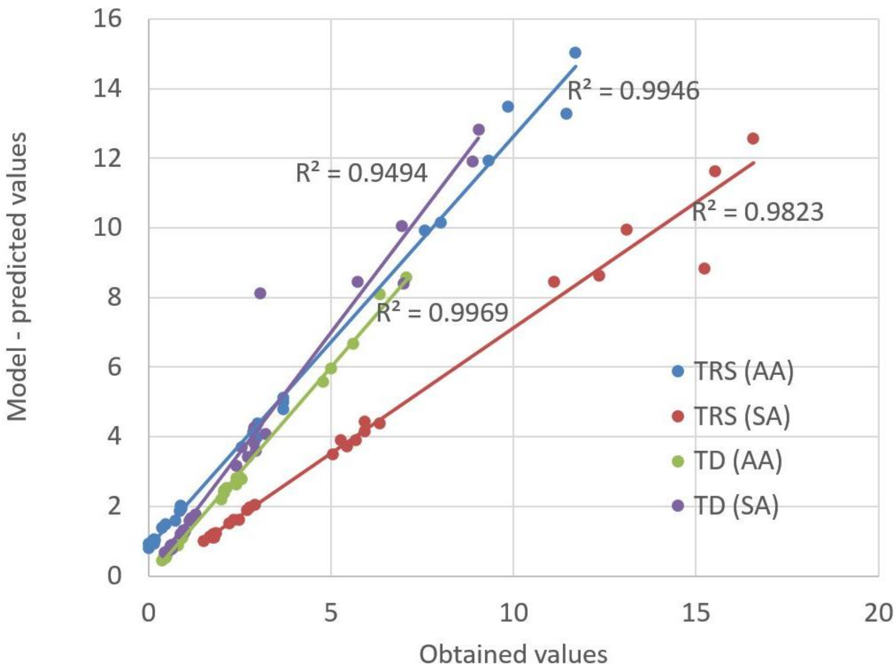

Figure 4. Correlation between model-predicted and measured data. TD – total derivative concentration, TRS – total reducing sugar concentration, SA – sulphuric acid, AA – aceticacid.

3.4. Changes in biomass structure

Changes in the biomass during the hydrolysis process were studied using infrared spectroscopy. The most interesting spectrum is presented in Figure 5.

The red line was recorded before the hydrolysis process. As a result of hydrolysis, the FTIR spectrum of sludge suspension changed significantly, which should be attributed to a large share of liquefied biomass. Carrying out the process in conditions for which the highest shares of TRS and TD were obtained, i.e. for hydrolysis with sulphuric acid, size 0.25 mm, time 96 h, temperature 60 °C, allowed to register the spectrum on which vibrations characteristic of saccharides were observed. The signal in the range of 1000–1200 cm^{-1} indicated the presence of stretching vibrations for ether groups (C–O–C). Stretching vibrations for secondary alcohols (R2–CH–OH) were also visible in the range of approx. 1080–1160 cm^{-1}. The appearance of the peak at 1800 cm^{-1} was associated with the participation of carbonyl groups (C=O). Stretching

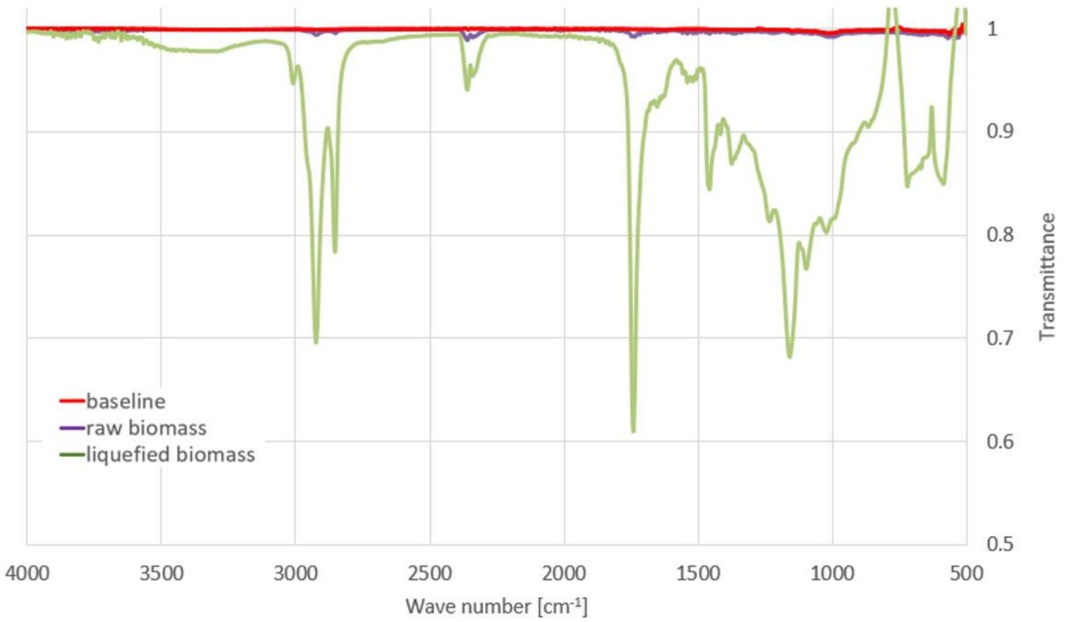

Figure 5. FT-IR spectrum of biomass samples recorded before (red line) and after hydrolysis with sulphuric acid, 0.25 mm, time 96 h, temperature 60 °C (green line).

vibrations representing C–H bonds for the wavenumber from 2800–3200 cm^{-1} were also noticeable. In the region of 3200–3600 cm^{-1}, a signal of low intensity was observed, which may result from the presence of a hydroxyl group. All these components are present in sugars. The vibrations visible on it are related to the presence of ether, alcohol, alkyl and carbonyl groups in the analysed solutions, which appeared in the structure of glucose. Similar structures were identified on FTIR spectrum of other biomass related samples (Matwijczuk et al., 2019). Acidic conditions are reported to promote complete liquefaction of biomass (Razola-Díaz et. al., 2020).

4. CONCLUSIONS

This study presented a comparative approach for acid hydrolysis of bio fraction waste prior to fermentative processes. The obtained results allow to form two main conclusions. Firstly, the saccharification based on concentrated acid leads to decrystallisation of cellulose followed by secondary transformations of monosugars. In this approach, the separation of acids from sugars and acid recovery are critical unit operations, unless, the acid may become a source of carbon for further fermentation. Secondly, diluted acid hydrolysis allows milder conditions to hydrolyse hemicellulose, while the second stage is optimised to hydrolyse the more resistant cellulose fraction. This approach represents better efficiency, when the process duration is prolonged. Liquid hydrolysates from both processes require dilution, although both are fermentable. It is possible to test the acid hydrolysis procedure for bio fraction of waste by acid hydrolysis. However, the obtained results clearly indicate that it is not possible to conduct the process in such a way as to avoid obtaining derivatives of their disintegration at the same time as the high concentration of sugars. With regard to this type of mixture, it is not possible to apply the previously known procedures directly. The complicated matrix, which is the bio fraction, should not be treated only as a source of monosaccharides, and when planning the experiment, attempts should be made to plan and obtain by-products, which are lignin derivatives and secondary monosaccharide derivatives. Future experiments should take into account the optimisation of separation of the useful stream in fermentation processes from the one that can provide substrates for the production of biochemicals.

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SYMBOLS

AA	Acetic acid
AD	Anaerobic digestion
BBD	Box Behnken Design
DES	Deep Eutectic Solvents
FTIR	Infrared spectroscopy with Fourier Transformation
RSM	Response surface Methodology
SA	Sulphuric Acid
TRS	total reducing sugar
TD	total derivatives

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